P2.001

HYponatremia AFTER TRANSSIphenoidal SURGERY FOR Pituitary Tumors.
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We observed postoperative hypotremia (<135 mmol/l) in 32 (35%) of 91 consecutive patients (44 males, 47 females, age 45 (12-76) yrs.) operated transsphenoidally for pituitary tumors. Hyponatremia was symptomatic in half of the patients. Neither the size nor the operability of the tumor or transient postoperative polyuria predicted development of hyponatremia. Hyponatremia was first observed on the sixth or seventh postoperative day. Treatment with water restriction and increase of substitution dose of glucocorticoids resulted in uneventful recovery within five days. High urine osmolality and plasma vasopressin concentration during hyponatremia in a subgroup of study patients with these measurements indicated that inappropriate vasopressin secretion was involved in the pathogenesis of hyponatremia. In conclusion, hyponatremia is common after transsphenoidal surgery for pituitary tumors. Therefore all the patients should be screened for serum electrolytes for one week after transsphenoidal surgery.

P2.002

HYPOTONURISM IN PATIENTS WITH PITUITARY ADENOMAS
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Twenty-three consecutive patients with pituitary adenomas (24 PRL-, 2 TSH-, 3 FSH-/LH-, 2 GH- and 12 "nonfunctioning" adenomas) were examined 1) to analyze the frequency of their sexual disorders and 2) to determine the strategy which must be taken for to exclude or to confirm a pituitary adenoma in an impotent patient.

Ad 1) Only 3 patients in our group (13 X) had consulted for impotence, but the questionnaire concerning their sex life had shown impotence in 10 of them (43 X). All patients with impotence described a loss of libido. Pubic hair was reduced in 9 and testes atrophy was present in 8 of them. The plasma testosterone in 10 impotent patients was significantly lower (4,2±3,0 nmol/l) than in 13 non-impotent patients (20,2±6,6 nmol/l), p < 0,01.

Ad 2) For exclude or confirm a pituitary adenoma in an impotent patient it is recommended firstly to measure the plasma testosterone and plasma prolactin levels and to control all pathological results and secondary to the hyperprolactinemia is above 35 μg/l and/or the hypotestosteronemia is below 7 nmol/l, to undergo a complete endocrine investigation: a pituitary hormonal examination, a CT scan of sella turcica and if sellar mass suspicion exists, to complete an MRI scan.

P2.003

IMMUNOBIOCHEMICAL (ICC) DETECTION OF PITUARY GLYCOPROTEIN HORMONE A SUBUNIT (α SU) IN A SERIES OF 17 SOMATOTROPHACTINIC (SP) PITUITARY ADENOMAS (PA).

The expression of α SU is mainly constant in thyrotropin-secreting PA and common in those secreting gonadotropins, whereas the presence of this α SU in plurihormonal PA is less documented. Therefore, the aim of this study was to systematically research an α SU by ICC as an unmonitored model of mixed PA. Seventeen surgically removed PA (15-53 years of age; 15 women) were selected, based on confrontations of clinical and biological data with results of histological and immunohistochemical findings.

Immunohistology or IHC demonstrated 1 micro and 16 macro-PA (9 with extracellular expansion) all the patients suffered from endocrine disturbances accordingly (N = 15, 5 males, polycorias (11 females, 9 with amenorrhea), average growth hormone (GH) mean ± SD = 33 ± 44 ng/ml; normal <5) was elevated in 13 cases, 9 of them showing also high prolactin levels (PRL, mean 40.2 ± 56.2 ng/ml; normal <20, PRL was below 100, mostly in 1 male case with impotency. One male only exhibited normal GH and PRL levels. Thirteen patients were subjected to a stimulation test of PRL by TRH + 4 had prior normal basal PRL levels. 2 of the latter above and 2 others with elevated basal PRL levels responded greater than 100% basis.

In all these adenomas ICC showed immunoreactivity for PRL and GH polyclonal antisera (NIBDK), located in different cells, and, in 13 of them, additional immunoreaction for the α SU exclusively detected in some somatotroph cells (BIOPRODUCT). Of these 13 patients, 11 had elevated plasma GH and 12 presented macro-PA. Therefore, 95% of these 17 SPA were positive for an α SU. The significance of these findings remains to be determined.

P2.004

INHIBITORY EFFECT OF ANGIOGENESIS INHIBITORS FUMAGILIN AND ITS ANALOG AGM-1470 ON PROLACTIN SECRETION IN STILBOESTROL-INDUCED PITUITARY PROLACTINOMA IN RATS.
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Formation of new blood vessels is a key factor determining solid tumor growth and metastasis progression. An abnormal arterial vascularization is one of the factors that predisposes also Fischer 344 rats to develop prolactin-secreting pituitary tumors in response to prolonged treatment with oestrogens. Fumagillin, which is an antibiotic isolated from Aspergillus fumigatus, has been extensively studied (1) and its synthetic derivatives AGM-1470 (O-chloroacetylcaramoyl fumagillin) are known to selectively inhibit endothelial cell proliferation, but their effect on prolactin cells secretory function and pituitary prolactinoma formation has not been described yet. To investigate this problem, we have examined the effects of fumagillin and AGM-1470 (gift from Dr. Katsuchi Sudo, Biology Research Laboratories, Takeda Chemical Industries, Osaka, Japan) administration on prolactin secretion in long-term diethylstilboestrol (DES)-treated male Fischer 344 rats. As expected, subcutaneous implantation of silastic tubes containing 10 mg of stilboestrol (Stilboestrol, Polfa, Poland) induced a dramatic rise in serum prolactin levels, seven weeks after hormone implantation. Both angiogenesis inhibitors injected in s.c. doses of 10 mg per kg body weight attenuated the stimulatory effect of stilboestrol on serum prolactin concentration, but the effect of AGM-1470 was weaker in comparison to fumagillin. This is the first report of the antiprolactin efficacy of angiogenesis inhibitors in experimentally DES-induced pituitary adenomas, which is a suitable model for studying clinical application of various drugs in the treatment of human prolactinomas.

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**P2.005**

**EPIDEMIOLOGY OF PITUITARY ADENOMAS: A SURVEY OF 1200 PATIENTS**


In order to evaluate the epidemiological profile of pituitary adenomas, a consecutive series of 1200 pituitary adenomas, operated on in 2 centers during a 6-year period, was studied. Patients included 771 females and 429 males, aged 7 to 89 years (mean = 41 years). Based on clinical and immunocytochemical data, patients were classified as having prolactin (n = 422; 338 females, 84 males; mean age = 33 years), growth hormone (n = 159; 97 females, 62 males; mean age = 45 years), growth hormone and prolactin (n = 153; 85 females, 68 males; mean age = 42 years), corticotropin (n = 161; 117 females, 44 males; mean age = 39 years), gonadotropin (n = 55; 17 females, 38 males; mean age = 55 years), thyrotropin (n = 22; 10 females, 12 males; mean age = 52 years), alpha-subunit (n = 38; 16 females, 22 males; mean age = 53 years) and non-secreting (n = 190; 91 females, 99 males; mean age = 52 years), adenomas. Thyrotropin, alpha-subunit and gonadotropin adenomas are the least common, and thyrotropin, alpha-subunit and gonadotropin adenomas are mainly observed in females, while gonadotropin and alpha-subunit adenomas are predominant in males. Prolactin and corticotropin adenomas are found in younger, and gonadotropin, alpha-subunit, thyrotropin and non-secreting adenomas in older patients.

**P2.006**

**PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE INCREASES INTRACELLULAR cAMP LEVELS IN HUMAN NONFUNCTIONING PITUITARY ADEMOMA**


The hypothalamic factor pituitary adenylate cyclase activating polypeptide (PACAP) has been demonstrated to induce adenylyl cyclase (cAMP) accumulation in pituitary cells, although its role in regulating pituitary function is not completely known. The activation of cAMP pathway in pituitary cells is not only mediated by hormones but also by extracellular stimulations. We studied the effect of PACAP on intracellular cAMP levels in cultured pituitary cells from human clonally nonfunctioning pituitary adenomas. Cells were enzymatically dispersed, after surgery, and grown in Dulbecco’s Modified Eagle Medium. Immunocytochemical analysis performed on a fragment of the tumor excluded the presence of any adrenocorticohormone and of the alpha subunit of glycoprotein hormones and revealed pituitary cells to be of oncocytic type. The cells were treated with PACAP 27 dissolved in serum free medium at different concentrations: 1, 10, 100, 1000, 10000, 100000, 1000000 ng/ml. Twenty-four hours later the cells were harvested with 1 M EDTA solution and extracted with 1 M HCl. The samples were analyzed by radioimmunoassay for the presence of cAMP. Significant cAMP accumulation was observed in PACAP treated cells at 1000000 ng/ml. These results suggest that PACAP could be a good substitute for other trophic factors in stimulating the growth of pituitary cells.

**P2.007**

**FRACTIONATED RADIOTHERAPY OF PITUITARY ADENOMAS UNDER STEREOELECTRIC CONDITIONS: EARLY MORPHOLOGICAL RESULTS**


Conventional external photon beam irradiation has been recently improved by the use of non invasive stereotactic frame (Laitinen stereodraper) allowing very precise irradiation of small brain lesions (as reported by Delannes et al, Int. J. Radiation Oncology Biol. Phys., 21, 749-755). Multimean irradiation is still delivered in fractioned doses in a volume reduced from 125 cm³ (conventional radiotherapy) to 2 cm³. We report the results of this new technique in the treatment of pituitary adenomas (50 Gy delivered in 5 days, 1.8 Gy per day). Patients: 18 patients, 11 women, 7 men, age 47 yrs (19-78 yrs). Adenomas: 6 gonadotropin or non functional, 3 ACTH, 7 GH, 2 PRL, as defined by immunohistochemistry; mean size (estimated on pre-radiotherapy 3D coronal sections): 23 x 23 mm. Pituitary irradiation has been indicated either immediately after incomplete surgical cure (n=11), either after long term post surgical recurrence (n=7). In 5 cases there were visual defects. After treatment, patients have been followed during a mean of 19 months (3 - 36 months). Early results (1): Mean size of the tumors decreased to 13 x 17 mm. This regression was obtained in 12 patients over 18 (10 x 13 mm after versus 23 x 21 mm before radiotherapy in this group). This effect began after a mean of 21 months (8 - 28 months). Visual defects were improved in 4/5 patients. 2) The adverse effects observed in the first year were one inflammation of cavernous sinus spontaneously resolutive and one panhypopituitarism in a patient with hemorrhagic signs on IVM. Cerebral radioendocrinosis was never observed nor aggravation of optic damage.

The results indicate that this new technique of radiotherapy is well tolerated and rapidly effective on morphological regression of pituitary adenoma.

**P2.008**

**THE RESULTS OF TRANSSPHENOIDAL SURGERY AND RADIOTHERAPY OF PITUITARY MICROADENOMAS**

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This report included the data on about 380 patients treated with proton-beam therapy (PBT), first sample, 1976 and 200 patients treated with MS, second sample, since 1990. From the first sample 280 patients were treated with Cushing’s disease, 80 with acromegaly and 40 with prolactinomas. Second sample consisted of 40 with Cushing’s disease, 20 with acromegaly and 70 with prolactinomas. The following methods within the aim of diagnostic were performed, the basic rate of pituitary microadenomas was obtained on blood and in conditions of stimulation (functional tests), CT and MRI. Besides that traditional diagnostic methods we utilized a new valid diagnostic test - bilateral intravascular petromalin minus sampling (IPS) for hormones measurement. There was performed a long-term study after both methods of treatment (follow-up period 3-5 years). The data obtained showed that clinical and biochemical remission was noticed in the first sample in 80-85% of investigated cases of Cushing’s disease, 75-80% cases of acromegaly and 50-54% cases of prolactinomas. In the second sample the corresponding results were 95% of cases of Cushing’s disease, 78-80% with acromegaly and 70% with prolactinomas. Thus the results of long-term observation of patients treated by PBT or MS confirm the suggestion that MS is more preferable in patients with more acute course of the disease, in cases with lower intensity and greater duration of the disease with the same effect. Chronification PBT could achieve the significant improvement of patient’s status.
BRAIN NECROSIS DUE TO RADIOThERAPY FOR PITUITARY TUMORS

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Patients with pituitary tumors who are irradiated often have pituitary dysfunction, but other endocrinological abnormalities are rarely described. In order to elucidate the potential risk of the radiotherapy, we analyzed, retrospectively, 108 patients who were previously operated because of pituitary tumors. Forty cases had adenocarcinoma, 18 - prolactinomas, 16 - Cushing's disease, 25 - non-functioning pituitary tumors and 9 - craniopharyngiomas. Ninety patients were treated with an external 30Co unit and 18 with linear accelerator 9 MV. The total radiation dose ranged from 3600 to 5600 cGy (rads). In 18 of 20 patients, examined with MRI (1.5 T Magnetom-Simens) because of side effects of irradiation the focal brain necrosis was found. In 4 patients who died because of postirradiation necrosis of hypothalamus, the diagnosis was confirmed on pathomorphological examination. Clinical signs of postirradiation brain damages were as follows: visual field defects in 10 cases (9%), blindness in 4 (3.7%), deafness in 5 (4.6%), mental disorders in 4 (3.7%), and fatigue (5.4%). These signs were previously commonly connected with natural history of pituitary tumors. We conclude that the brain necrosis is dependent mainly on the individual sensitivity and on the dose of radiation but less on the type of the pituitary tumor. Therefore, conventional radiotherapy for pituitary tumors should be avoided or limited to the invasive tumors which have been reoperated unsuccessfully.

INCIDENTALOMAS OF THE PITUITARY GLAND

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A high frequency of pituitary adenomas is found in autopsies but only a little number of cases with incidentalomas of the pituitary gland is reported (1). We examined 31 patients with intrasellar masses, incidentally diagnosed by computed tomography or magnetic resonance imaging. All patients underwent endocrine testing and ophthalmologic examination. Patient's age ranges from 20 to 73 years (mean 47). Average diameter of the mass at time of diagnosis was 1.5 cm. Endocrine testing revealed 10 patients with partial hypopituitarism. 8 patients showed hypogonadotropic hypogonadism. 2 patients had inadequate low response to TRH. Twenty patients with intrasellar masses showed no hormone excess, while 6 tumors were prolactinomas. Inadequate high values for growth hormone were discovered in one patient, indicating acromegaly. Bitemporal hemianopsie was diagnosed in one patient suffering from macroprolactinoma. 4 patients underwent neurosurgery. Within 6 months 2 tumors enlarged from 1.5 to 1.7 and 0.6 to 0.8 cm, respectively. We conclude that patients with incidentally diagnosed intrasellar masses should undergo endocrine testing to identify adenomas with hormone excess (which we found more frequent than other authors) to prevent our pituitary dysfunction or to start hormone replacement therapy early. Pituitary masses without hormone excess of any size should be followed up with magnetic resonance imaging, endocrine testing and ophthalmologic examination after 6 and 12 months.


GROWTH PARAMETERS AND ENDOCRINE FUNCTIONS IN CHILDREN AND ADULTS WITH EMPTY SELLA SYNDROME

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Empty sella syndrome (ESS) is reported to be associated with different endocrine abnormalities. We studied the anthropometric parameters, serum growth hormone (GH) response to high dose clomiphene (0.15mg/kg), cortisol response to ACTH (synacthen) and glucose response to an oral load of glucose (1.75g/kg), and estimated the circulating concentrations of free thyroxine (FT4), thyroid stimulating hormone (TSH) in 20 children and 12 adults with ESS. 8 children with ESS and sickle cell disease (SCD) and 15 children with normal variant short stature (NVSS). In the adult group we investigated the response of gonadotropins (LH and FSH) to luteinizing hormone releasing hormone (LH/RR) and TRH response to TRF. Out of the 20 children with ESS, two had panhypopituitarism (10%), six had isolated GH deficiency (6%) and two children were normal (10%). In the sickle cell group with ESS, (90%) of the children did not mount an appropriate GH response to clomiphene; and their thyroid and adrenal functions were normal. Serum insulin-like growth factor (IGF-I) concentrations were significantly lower in the two groups of children with ESS compared to those with NVSS. The Hitzig standard deviation scores (HSDSS) were significantly lower and the annual growth velocity (GV) were slower in children with ESS compared to those with NVSS and those with ESS+SCD. The bone age delay (yrs) did not differ among the 3 groups of children. All children with ESS had normal body mass indices (BMI), while all the children with SCD+ESS had BMI below the 5th percentile for corresponding age and gender . None of the children had hypertension or glucose intolerance. In the adult group (10 women and 2 men) the following disorders were noted: 3 patients had obesity (body mass index (BMI) >27.1) (66.6%) two of them had glucose intolerance, 7 had height below 5th percentile for age and gender (58.3%), 3 had hypertension (25%), 3 had a subnormal GH response to clomiphene provocation and insulin hypoglycemia (25%), 4 had an abnormal TSH response to TRH testing (33.3%) (one primary and 3 secondary hypothyroidism) secreting thyroid replacement, 2 patients had secondary adrenarche identified by insulin tolerance test and ACTH test, one male and one female had secondary hypogonadism, and one patient had hyperprolactinemia (84±9±1mIU/ml). In summary growth retardation and abnormal hypothalamic pituitary functions are common in children and adults with ESS.

EFFECT OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDES IN HUMAN PITUITARY ADENOMAS

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It is well known that Pituitary Adenylate Cyclase Activating Peptide (PACAP) has a stimulatory effect on adenylate cyclase (AC) activity in rat pituitary cultured cells and in clonal pituitary cell lines (A1T-20 and GH3). The aim of the present study was to evaluate the effect of PACAP-38 on AC activity and cAMP accumulation in different human pituitary tumor. The series include 10 nonfunctioning pituitary adenomas (NFPAs), 5 GH-secreting adenomas (GHomas) and 2 PRL-secreting adenomas (PRLomas). Tumors were obtained after adenectomy by transpharyngeal route and then enzymatically dispersed to obtain cell cultures or frozen at -20°C. In all NFPAs studied, PACAP (0.1µM) induced a marked increase of AC activity, the percent stimulation ranging from 140 to 170%. In these tumors, Vasopressin Inertial Peptide (VIP, 1µM) had a similar effect on AC activity (% stimulation: from 102 to 1259). Although the maximal effect induced by the two peptides was similar, PACAP resulted to be more potent than VIP, since PACAP was effective at concentrations 100-1000-fold lower than VIP (0.1µM vs 0.1µM). The effect of PACAP was due to the activation of specific receptors since the addition of a VIP antagonist (1µM) did not inhibit cAMP formation induced by PACAP while completely abolished the effect of VIP. In NFPAs cells, in which TRH (0.1µM) caused an important (Ca2+)-i rise (% increase: from 55 to 350), PACAP (0.1µM) was ineffective in increasing (Ca2+)-i levels. The effect of PACAP was also investigated in secreting adenomas. While in PRLomas PACAP (0.1µM) induced a poor AC stimulation (about 40%), in GHomas it caused an increase of cAMP, (mean±SD: 1083±1144) which was not significantly different from that obtained by the addition of GHRH at the same dose (967±1305). By contrast, in these tumors the response to PRL (100µg) was still potent (200/540). The only GH-oma in which PACAP was ineffective had a constitutive activation of AC due to gsp oncogene. In conclusion, PACAP causes a marked stimulation of AC activity in NFPAs and GHomas, while its effect on PRLomas seems to be poor. Taking into account the crucial role of cAMP in pituitary function, it is tempting to hypothesize that PACAP may exert a stimulatory action on pituitary cell function and proliferation.

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P2.013
PATHOMORPHOLOGY OF PITUITARY ADENOMAS
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There was conducted the clinico-morphological study of 335 cases of pituitary adenomas on the basis of light optical and electron microscopy techniques, immunohistochemical analysis. In 54 cases we used cytometry to study the nuclear DNA content and radiography to analyze the proliferation rates. We also analyzed 32 autopsy cases and studied block-specimen of the skull base. The revealed difference in the structural cellular differentiations is correlated with the character of pituitary adenomas growth and the degree of sensitivity towards radio- and medicamentous therapy. In our study invasive tumor growth was associated with aneuploid nuclear DNA content, higher proportion of cells in the growth factor and low-differentiated cells. Aneuploid adenomas more often consisted of low-differentiated cells and possessed the locally invasive growth, outgrew into the orbit, thus causing exophthalmus; along cranial nerves route and basilar venous plexus and occupied clivus. Well vascularized adenomas recur more often. In rare cases one could observe hormone activity decrease as a result of the oncotication of the tumor cells or due to low-differentiated adenocytes increase in the tumor.

P2.014
STIMULATORY EFFECT OF FSH AND LH ON INTERLEUKIN-1ß (IL-1ß), INTERLEUKIN-6 (IL-6) RELEASE FROM HUMAN PERIPHERAL BLOOD MONOCYTES AND INTERLEUKIN-2 (IL-2) RELEASE FROM LYMPHOCYTES IN VITRO: A DOSE-RESPONSE STUDY.
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Gonadotrophins have been demonstrated to modulate biological activities of immune system. The effects of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on LPS-stimulated IL-1ß, IL-6 release from human peripheral blood monocytes and PHA-stimulated IL-2 release from lymphocytes cultured in vitro were studied. The interleukines were measured in supernatants of 24 hrs. cell cultures by radioimmunoassay (RIA) method. It was found that LH could stimulate the release of IL-1ß and IL-6 from monocytes in concentrations ranged from 0.5 to 30.0 mIU/ml and FSH in dilutions from 5.0 to 25.0 mIU/ml for IL-1ß and from 5.0 to 50.0 mIU/ml for IL-6. The positive correlation was noted between FSH concentrations and IL-6 levels only ($r = 0.9092$, $p < 0.01$). Administration of LH in dilutions from 25.0 to 50.0 mIU/ml and FSH in dilutions from 5.0 to 30.0 mIU/ml augmented the release of IL-2 from cultured lymphocytes as well. The positive correlations between LH ($r = 0.733$, $p < 0.01$) and FSH ($r = 0.8909$, $p < 0.01$) concentrations and IL-2 secretions were revealed in cultured lymphocytes. These results suggest that both gonadotrophins exert a stimulatory effect on the immune system in humans by monocyte and lymphocyte activations to IL-1ß, IL-6 and IL-2 release.

P2.015
C-TYPE NATRIURETIC PEPTIDE (CNP) IN THE PITUITARY: LOCALIZATION AND ACTION IN GONADOTROPES.
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Natriuretic peptides influence neuroendocrine systems, acting both centrally and at the pituitary level. The highest tissue concentrations of C-type natriuretic peptide (CNP) are found in the anterior pituitary and CNP is a potent stimulator of cGMP accumulation in rat pituitary cells and in a gonadotrope-derived cell line (1). Here we report that Northern blotting revealed pituitaries of rats and mice to contain abundant CNP mRNA, but not ANP or BNP mRNA. Using RT-PCR both A-type and B-type natriuretic peptide receptor (GC-A and GC-B respectively) transcripts were detected in pituitaries although only GC-B mRNA was measurable by Northern blotting. Immunohistochemically, CNP positive cells were identified in the anterior (but not posterior) pituitary lobes of rats and the vast majority of the CNP positive cells were gonadotropes as judged by co-expression of follicle-stimulating hormone. Targeted toxicity, achieved by culturing pituitary cells with gonadotropin-releasing hormone conjugated to ricin A chains, caused comparable reductions in cGMP-stimulated cGMP accumulation and, GnRH-stimulated LH release. We conclude that CNP is synthesized in the pituitary where it is predominantly located in gonadotropes and that GC-B receptors likely mediate direct stimulatory effects of CNP on gonadotropes. Accordingly CNP appears to be a novel autocrine regulator of these cells.


P2.016
C-TYPE NATRIURETIC PEPTIDE (CNP) IN αT3-1 CELLS: PRODUCTION, ACTION AND MODULATION BY GONADOTROPIN RELEASING HORMONE (GnRH).
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Natriuretic peptides act via plasma membrane receptors with intrinsic guanylate cyclase activity to stimulate cGMP production. Pituitary gonadotropes are suggested to be sites of natriuretic peptide action and CNP, which occurs at its highest tissue concentrations in the anterior pituitary, is a potent stimulator of cGMP production in the gonadotrope-derived αT3-1 cell line (1). Here we report that three natriuretic peptides stimulate cGMP accumulation in αT3-1 cells with a rank order of potency suggesting action via the GC-B receptor subtype (CNP >> BNP > ANP) and that, consistent with this profile, GC-B receptor transcripts were detected in these cells by RT-PCR and Northern blotting. αT3-1 cells also contain CNP (but not BNP or ANP) mRNA and immunoreactive CNP which co-localises with autocrine CNP on reversed phase HPLC. CNP-stimulated cGMP production in αT3-1 cells was found to be inhibited by GnRH whereas no such inhibition was seen when sodium nitroprusside was used to stimulate soluble guanylate cyclase. The stimulatory effect of CNP on intracellular cGMP was rapidly reversed by GnRH and the inhibitory effect of GnRH was rapidly reversed by a GnRH antagonist. We suggest that CNP, acting via GC-B receptors, is a likely autocrine regulator of αT3-1 cells, and that GnRH might affect cGMP signalling in gonadotropes by inhibition of GC-B receptor mediated guanylate cyclase activation.

P2.017

**DOPAMIN AGONISTS REDUCE SERUM GH IN ACREMEGALY FOR 15 YEARS**

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Dopaminergic treatment of 26 acromegalic patients with persistent or recurrent hypersomatotropism after surgical therapy resulted in long-lasting reduction of serum GH in 20 cases. The follow-up period of treatment with bromocriptine (7.5 - 25.0 mg/d) or lisurid (0.6 - 2.0 mg/d) was 3 to 10 years in 16, and 11 to 15 years in 10 patients.

In 20 patients the mean values of all GH measurements during the last year of treatment were found to be lower than 60% compared to the pretreatment period, 11 of them showing values lower than 40%. 4 of 5 patients treated for 15 years presented normal GH values. The mean values of GH in 20 responders were 75.6 ± 55.0 mU/L before therapy, 17.6 ± 13.4 mU/L after 5 years of treatment (17 patients), 14.6 ± 10.6 mU/L after 10 years (11), and 7.2 ± 5.7 mU/L after 15 years (5), respectively. A short-term drug withdrawal performed in most patients led to re-increase of serum GH which exceeded pretreatment levels in 11 cases.

In contrast to other authors, combined treatment with cyproheptadine for 3 to 12 months in 9 patients did not result in further improvement.

Conclusion: Dopamin agonists can reduce serum GH in acromegaly for 15 years and can be recommended for long-term therapy in selected patients.

P2.018

**CROSS-TALK BETWEEN Ca²⁺ AND PROTEIN KINASE C IN THE ACTION OF GnRH UPON GONADOTROPIN α-SUBUNIT GENE EXPRESSION IN cT3-1 CELLS**

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[Δ-Trp]²⁶⁷ GnrH (GnRHa) induces a rapid elevation (30 min) of α-subunit mRNA levels which is abolished by actinomycin D. The effect is mimicked by the protein kinase C (PKC) activator TPα or the Ca²⁺ ionophore, ionomycin. No additivity is found upon the combined addition of GnRHa and TPα, GnRHa and ionomycin, or TPα and ionomycin. The effect of GnRHa is blocked by the PKC inhibitors staurosporine or G109203X. Furthermore, down regulation of PKC activity by TPα resulted in inhibition of the effect of GnRHa, TPα and ionomycin. Removal of Ca²⁺ abolished the response to GnRHa and TPα. GnRHa also stimulates a secondary rise in α-RNA levels detected at 12-24 h of incubation. Unlike the first phase, TPα and ionomycin had no effect, on their own, on α-RNA levels at 24 h of incubation, but together they mimicked the late phase of the neurohormone action. Thus differential cross-talk between Ca²⁺ and PKC is involved in the two phases of GnRHa-induced α-subunit gene expression. Furthermore, the site of Ca²⁺ action is both up- and downstream to PKC activation.

P2.019

**LONG-TERM MORTALITY IN PATIENTS WITH HYPOTHYROIDISM**

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A single study has shown increased mortality from cardiovascular disease in patients with hypothyroidism due to a variety of causes. A significant reduction in the number of deaths caused by malignancy was also seen.

We have reviewed hospital case records and death certificates of 107 patients with non-functional pituitary tumours and evidence of hypoglycaemia affecting one or more axes, in order to confirm these findings. Our cohort consisted of 64 males (mean age 52 years) and 43 females (mean age 52 years). 68% were hypothyroid. 68% were hypopituitary and 68% hypogonadal. GH deficiency was confirmed on insulin stress testing in 53% while 33% had no formal assessment of GH status.

Death rates were compared to the general population using previously published data. After excluding smoking related events mortality was increased in the cohort as a whole (P<0.05) but this was confined almost exclusively to the female group (Observed/Expected death ratio 15.3:1 P<0.01). Males did not have a significant increase in mortality (Observed/Expected death ratio 15.13:1 P<0.06). 9/27 deaths were due to cardiovascular disease but interestingly an equal number were due to malignant causes. This appears to contradict the findings of the previous study.

In conclusion our findings have reduced life expectancy but this appears to be limited to females. In addition the cause of this excess mortality is different and requires further study.

P2.020

**CLINICALLY NONFUNCTIONING PITUITARY ADENOMAS: RELATION TO LEVELS OF PITUITARY HORMONE IN VIVO AND ITS RESPONSES TO THE TSH, LHRH, GNRH AND TRH TESTS**

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We studied the hormonal levels in vivo in group of 12 patients, 9 of 12 were operated because of CNFPA and in each such the presence of a pituitary tumour was verified by histological examination of the removed tissue. The mean age of 2 men was 36,5 years and that of the 10 women was 38,5 years (range 25-53). All 12 patients had macroadenoma on CT scan and MRI. Some were due to mass effect - visual disturbances, headache, hypocortisolism, etc. 5 patients had slightly elevated serum prolactin (PRL). 2 patients had elevated plasma GH, without any clinical signs of acromegaly. In remained 11 patients serum GH levels were low (mean 0,8 mg/ml). The responses to TRH, LHRH and GnRHa were studied on separate days. In 12 patients the response to TRH levels after TRH administration were normal, the main increase of PRL was significantly low in patients with CNFPA (74% of basal levels) than in control group (37%±25%). The percentage increase was independent of the baseline hormone levels. 10 of 12 patients had elevated in serum gonadotrophin in response to TRH. Serum LH levels increased from 11.7 to 83.3 IU/L, FSH levels from 32.4 to 301.5% in 6 patients in the GnRHa-test. Parasite administration in 7 patients led to a significant decrease in gonadotrophin levels in 5 patients. We conclude in response to TRH, patients with CNFPA had elevated serum gonadotrophine and low levels of PRL in control group. GH deficiency may be a hormonal marker when there is not hormonal hypersecretion.
P2.021

SCREENING TESTS IN PATIENTS WITH SHEEHAN'S SYNDROME

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Forty patients with typical obstetrical history of Sheehan's syndrome were reviewed retrospectively. Together with baseline laboratory values fifteen patients were evaluated by insulin hypoglycemia test, twenty seven patients by TRH and seven patients by LH-RH tests. Baseline hormone values suggested secondary hypothryroidism, hypogonadotropic hypogonadism and hypopituitarism. According to the result of the anterior pituitary stimulation tests, one patient (6.6%) showed normal growth hormone response to hypoglycemia. Nine patients (33.3%) who were clinically and biochemically hypothyroid demonstrated adequate TSH response to TRH. None of the patients showed normal prolactin response to TRH. Four out of seven amenorrhoeic patients (57.1%) had adequate FSH and/or LH responses to LH-RH.

It has been concluded that isolated anterior pituitary hormone deficiencies may occur in patients with Sheehan's syndrome. Prolactin response to TRH seems the most sensitive screening test for detecting Sheehan's syndrome in patients with typical obstetrical history.

P2.022

TSH PRODUCING PITUITARY ADENOMA LACKING SOMATOSTATIN RECEPTOR: CLINICAL AND BIOLOGICAL IMPROVEMENT BY SANDOSTATIN TREATMENT

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A TSH producing pituitary adenoma inducing hyperthyroidism was found in a 41-year-old patient. The serum T3 and T4 level was considerably elevated. TSH was 20 uIU/ml. Sella CT and MRI investigations revealed a microadenoma in the left part of the anterior pituitary. Octreoscan111 (Mallinckrodt) scintigraphy showed no accumulation in the region of the pituitary. The serum TSH level showed a further increase after TRH administration. FSH, LH, PRL, and ACTH reserve capacities of the pituitary have shown normal patterns. Oral administration of cyproheptadine, ranitidine, and dexamethasone caused a moderate increase of serum TSH level, while bromocriptine was able to suppress the TSH to 60% of the basal value. One week of Sandostatin (Sandoz Ltd.) treatment resulted in normalization of the T3, T4, and TSH levels. However, after one month of Sandostatin therapy, an escape phenomenon has been observed: by continuous treatment (600 µg Sandostatin sc) the TSH level was suppressed to 65% of the basal value accompanied by a moderate clinical improvement. The results suggest the beneficial effect of Sandostatin therapy on TSH producing pituitary tumours even in the absence of detectable somatostatin receptors.

P2.023

RESPONSES OF ALPHA-SUBUNIT TO TRH IN CLINICALLY NONFUNCTIONING PITUITARY TUMORS

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In 17 patients with clinically nonfunctioning pituitary tumors (NFP) alpha-subunit (alpha-SU) was measured by TRH. TRH test was performed in 10 patients. Three patients had elevated alpha-SU in the serum, while 11 secreted alpha-SU in cell culture. After administration of TRH, alpha-SU responses were ranged 65-111 µg/ml. No correlation was found between the level of alpha-SU in vivo or in vitro and the percentage of the elevation after TRH. Patients were divided into two groups according to the gonadotrophin secretion in vitro. The group which secreted gonadotrophins and alpha-SU had higher alpha-SU, LH and FSH responses after TRH administration, but only FSH responses were significantly different (P<0.05). We conclude that NFP and gonadotrophinomas cannot be distinguished by alpha-SU response following TRH administration.

P2.024

THYROID STIMULATING HORMONE LEVELS IN ACUTE TRAUMA

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After a severe injury the central nervous system undergoes various adaptations, responsible for the release endocrinological signals and symptoms. In order to evaluate their impact on the hypotalamus-pituitary-thyroid axis, we have performed a standard TRH-test in a group of 10 patients (9 males and 1 female, aged 17-57 yr), admitted to the Intensive Care Unit for a severe injury (Glasgow Coma Score <8). Basal TSH values ranged from 0.04 to 2.22 µU/ml (mean±SEM: 0.49±0.23 µU/ml). The peak response to TRH (200 µg i.v. as a bolus) ranged from 0.50 to 16.10 µU/ml (6.06±1.96 µU/ml). In the days following the acute event, TSH basal values showed a slight progressive increase (peak=0.96±0.49 µU/ml in the 4 day). When we considered retrospectively, we observed that the patients with a worse course of the illness (death or severe neurological outcome) had showed, in the acute phase, a higher peak response of TSH than the other subjects (11.55±3.06 vs 3.86±1.89 µU/ml).

The evaluation of the pituitary-thyroid axis in the acute phase of a severe injury could therefore cover a prognostic usefulness; in fact, the transient block of the hypothalamus-pituitary-thyroid axis, followed by a reactivation, could represent a mechanism of defense of the organism in the acute systemic stress. Further evaluations, in the days following the acute event, can clarify the meaning of this phenomenon.
P2.025

AN INCREASE IN THE BLOOD THYROID LEVEL AFTER METHYLENE BLUE IN RATS: THE INTERACTION WITH CARBIMAZOLE

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We found that estrogen-induced adenohypophysial growth is inhibited by an excess of thyroid hormones, but also by methylene blue (MB). For this reason anterior pituitary weight (AP), thyroid weight, T4, TSH, and cAMP levels in serum, thyroid and AP were measured in control rats, rats given methylene blue (MB) in their food, rats given the thyroid-blocking agent carbimazole (CARB) and rats fed both MB and CARB.

AP weight fell slightly after MB and strongly after CARB. MB partially inhibited the latter increase. The cAMP content of the thyroid rose after CARB, this increase was likewise blocked by MB, although MB alone slightly raised the thyroid cAMP concentration.

Previous observation showing that MB produced an increase in the blood T4 were confirmed. The T4 level fell, of course, after CARB and the decrease was partly inhibited by MB. The T4 content of the thyroid also fell after CARB, this decrease was completely reversed by MB. The blood TSH level fell slightly after MB and rose 7-fold after a CARB blockade of the thyroid, this increase was completely inhibited by MB. The TSH content of the AP fell after MB in both intact and CARB-fed animals. The cAMP content of the AP fell after MB, it was unaffected by CARB.

Thus, in some way, methylene blue raised the blood thyroid level in both intact and carbimazole-fed animals.

The it is question whether the marked inhibitory effect of MB on TSH secretion, with resultant reduction of hyperfunction of the thyroid, might be utilized potentially in the clinical treatment of hyperthyroidism, in the late phase of the administration of carbimazole, to suppress the latter undesirable effects.

P2.026

HORMONE RELEASE FROM EMBRYONIC PITUITARY CELL CULTURES

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It has been observed that cell differentiation processes of preterm infants are different from those of normal newborns. The present study aimed at the investigation of this phenomenon on an in vitro endocrine system. Monolayer cell cultures were prepared from 23-24 weeks human embryonic pituitaries after the interruption of pregnancy via misadministration of vital maturation-inducing substances. 14-day cultures were standardized by immunohistochemical procedures and hormone RIA-measurements. The kinetics of basal and specifically stimulated anterior pituitary hormones (prolactin, PRL; luteinizing, LH; folliculotropin, FSH; corticotropin, ACTH and thyrotropin, TSH) were followed. All the investigated hormones responded to their appropriate stimulants except for TSH that exhibited a markedly blunted reaction to thyrotropin-releasing hormone (TRH). These results support the presumption that the developmental delay of preterm infants may be in association with the hypofunction of their pituitary-thyroidal system. (Supported by ETT grant T-92/1990 and OKTA grant 1349).

P2.027

SOMATOSTATIN RECEPTOR SCINTIGRAPHY AND OCTREOTIDE TREATMENT IN PATIENTS WITH NON-FUNCTIONING PITUITARY ADENOMAS

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The so called "non functioning" or silent pituitary adenomas (NFPA or SL) represent about one quarter of all pituitary tumors and their common feature is the lack of hypersecretion of any biologically active pituitary hormone. Usually, the treatment of choice for these adenomas is surgical, the medical treatment is still far from satisfactory. The in vitro demonstration of somatostatin (5M) receptors on the cell membranes of these tumors, as well as in vivo by means of pituitary scintigraphy with octreoscan 61G-111, has suggested the possibility of clinical trials with 5M analogues in patients with NFPA. The aim of this study was to evaluate the existence of 5M receptors in NFPA patients and the treatment of receptors-positive patients with the 5M analogue, octreotide (OT). 11 patients (7F, 4M) mean age 39±5 years (27-54) were included in this study, all with NFPA. The diagnosis was based on clinical, radiological and endocrine findings. 5 normal individuals served as controls. Octreoscan 61G-111 scintigraphy was performed in all patients and controls. A semi-quantitative assessment was used in the evaluation of the images. 8 patients, 5F and 3M had positive scans and were put on OT 300μg daily for 3 months. None of the controls had a positive scan. In one out of 8 patients treated with octreotide the adenoma shrank, in one increased and in the rest patients octreotide did not induce any change in the volume of adenomas. We discuss our results on the basis of similar data from the literature.

P2.028

FUNCTIONAL AND MORPHOLOGICAL CHARACTERISTICS OF GH-SECRETING HUMAN PITUITARY ADENOMAS WITH HIGH AND LOW LEVELS OF THE ADENYLAZE CYCLASE ACTIVITY

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The rise of intracellular cAMP level in somatotrophs increases GH production and may play role in neoplastic cell growth. The aim is to investigate the relationship of the adenylate cyclase (AC) system state with functional activity and morphological features of different types of GH-secreting human pituitary adenomas. 23 acromegal patients tumors were divided into 2 groups (I and II) according to their AC activities. I included 17 low level basal AC activity macroadenomas with sparsely granulated cells, suprasellar extension or rapid invasive growth. II consisted of 6 tumors with high (by 7 fold) level basal (unstimulated by NaF) AC activity associated with invasive GAD. These tumors were densely granulated with tendency to be smaller in size and lower growth rates. GH serum basal levels have no significant difference in I and II patient groups. However, either in vitro GH-secretory activity and sensitivity to SRIF or in vitro and in vivo responses to TRH and BrCr were higher in II group. We conclude that II-tumors with elevated cAMP resting level are different from I-tumors either morphologically or functionally.


**P2.029**

**EFFECTS OF GALANIN ON GROWTH HORMONE RELEASE IN SOMATOTROPH ADENOMAS.**


There is increasing evidence that galanin modulates growth hormone (GH) secretion through effects on the hypothalamus. However, galanin has been also localized in anterior pituitary cells. The aim of this study was to investigate the effects of galanin on GH release in human somatotroph adenomas obtained by transphenoidal surgery from six acromegalic patients incubated in vitro. GH secretion by the adenoma was inhibited by 32.6 ± 5.31 %, 37.4 ± 4.6 %, 40.5 ± 2.31 %, 38.3 ± 3.6 %, and 29.6 ± 9.5 % of the basal release in five of the six adenomas during a 2 h exposure to galanin at a concentration of 5 x 10^-8 M. One of the six adenomas did not show a decrease in GH release after galanin. The findings support the fact that galanin, along with its hypothalamic effects, can modulate GH release directly at the pituitary level in some somatotroph adenomas.

**P2.030**

**TREATMENT OF ACROMEGALY WITH OCTREOTIDE: EFFECTS ON MARKERS OF BONE METABOLISM.**

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The effects of octreotide on bone metabolism in acromegaly are still debated. Methods: Fifteen patients affected by active acromegaly were studied before treatment and after 12 and 24 months of therapy with octreotide (50 to 100 µg subcutaneously tid).

Results: Treatment caused a significant fall of GH and IGF-I concentrations. Serum calcium and phosphate and urinary excretion of hydroxyproline after treatment disclosed no significant differences compared to pretreatment values. Urinary excretion of calcium significantly decreased after 24 months of treatment and urinary excretion of phosphate was significantly higher after 12 months of treatment. Osteocalcin levels were significantly lower after 12 and 24 months and intact parathyroid hormone (iPTH) concentrations were significantly higher after 12 and 24 months of therapy compared to pretreatment levels (iPTH: before treatment 31.9 ± 9.7, at 12 months 53.7 ± 26.2 and at 24 months 44.9 ± 21.2 mg/L). IGF-I levels during treatment were significantly higher than those in 20 healthy controls. Discussion: The reduction of osteocalcin during treatment with octreotide may be explained as a consequence of the fall of GH and IGF-I levels. The decrease of GH may reduce the renal synthesis of 1,25-dihydroxyvitamin-D, which causes an increase of iPTH. Octreotide may also produce osteoporosis and a decrease of the intestinal absorption of calcium so that iPTH secretion is furtherly stimulated.

**P2.031**

**DIFFERENTIAL EFFECTS OF OCTREOTIDE AND BROMOCRIPTINE ON IGFBP-1 IN ACROMEGALY.**

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Recently, it has been reported in literature that apart from its growth hormone inhibiting potency, octreotide has a stimulatory effect on the growth hormone independent IGF-binding protein, IGFBP-1. As the primary role of IGFBP-1 on IGF-1 action is thought to be inhibitory, the effects of IGFBP-1 might give octreotide an extra therapeutic value in acromegaly.

We studied the effects of octreotide and bromocriptine as compared to control on serum GH, insulin and IGFBP-1 in 25 untreated, non-fasted acromegalic subjects, aged between 32 and 77 years (mean age: 52 years). The effects of octreotide were studied for 24 h after a single dose (0.050 mg sc at 8:00 am). The effects of bromocriptine were studied for 12 h after a single dose (2.5 mg po at 8:15 am). The mean IGF-I value in these patients was 129 nmol/l (32 nmol/L). Octreotide significantly lowered the serum GH levels as compared to a control day (P < 0.001). The bromocriptine effect on serum GH was less pronounced, but still significant (P < 0.001). As compared to a control day, bromocriptine did not significantly alter serum insulin or IGFBP-1 levels. Octreotide induced a transient decrease in serum insulin levels. The overall 24 hours effects of octreotide on insulin levels as compared to control did not differ significantly. However, octreotide induced a significant increase in serum IGFBP-1 levels (P < 0.02). There was no significant correlation between the serum IGFBP-1 levels and the circulating insulin levels under octreotide therapy.

Conclusions: Octreotide and bromocriptine both significantly lower serum GH levels in acromegals. Additionally, octreotide (but not bromocriptine) induces an increase in serum IGFBP-1 levels.

**P2.032**

**LONG TERM TREATMENT OF ACROMEGALY WITH OCTREOTIDE: SIZE CHANGES OF PITUITARY TUMORS.**

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Objective: We wished to analyse the size changes of pituitary tumors in acromegalic patients under long term treatment with octreotide.

Patients and methods: We have treated 10 patients for up to 39 months (mean 28 months) with octreotide in varying doses (3 to 4 sc doses of 100ug, daily). All of them had already undergone previous therapy (surgery, bromocriptine and radiotherapy). Radiological evaluation of pituitary and parassellar lesions was performed before and after octreotide (CT scan or MRI). Growth hormone levels were measured each two hours, from 8 am to 8 pm, before and during treatment.

Results: In the 10 patients evaluated, one showed a slight expansion, two a slight reduction, and no significant changes in six. In the last one, a microadenoma was visualized during treatment. The growth hormone levels reduced in all but one patient, in whom the tumor remained stable. All the patients showed clinical improvement.

Conclusions: Of the 10 acromegalic patients under long term treatment with octreotide, only two showed a slight tumor reduction. All of them improved clinically with significant reduction of growth hormone levels. In our study, we did not find a correlation between the growth hormone changes and the tumor shrinkage.
P2.033
CARDIAC ABNORMALITIES IN ACROMEGALY.
A CLINICAL AND ECHOCARDIOGRAPHY STUDY.
Cardiac involvement occurs frequently in acromegaly and is an important cause of death. The aim of this study was to elucidate the existence of a specific acromegalic cardiomyopathy independent from arterial hypertension.
Thirty acromegalic patients (ACRO) (13M,17F, age 52±13), twelve hypertensive patients (HYP, 8M, 4F, age 48±14) and eighteen healthy volunteers (9M, 9F, age 47±10) were studied by ECHOCARDIOGRAPHY (ECHI).
Hypertension's diagnosis was made by history and blood pressure hotter recordings. In acromegalic patients current IGF-I and current GH (basal, during OGTG and after TRH and LHRH) were determined.
Left ventricular mass (LVM) was calculated and adjusted to the body surface area (LVM index). Stroke volume (SV) and cardiac output (CO) were determined by DOPPLER-ECHO. Isovolumetric relaxation time (IRT) and the left ventricular filling were obtained by PULSED-ECHO. IRT was corrected for the cycle length (CRT).
The active non hypertensive ACRO (group 1) had a higher LVM index, SV, CO, IRT (intraventricular septal thickness) and PWT (posterior wall thickness) than inactive non hypertensive ACRO (group 2) and volunteers (p=0.03). In group 1 a 75% had an elevated LVMindex, a 59% had an altered LV relaxation (CTRI>10ms), and a 25% had an abnormal LV filling. In group 2 a 20% had a elevated LVMindex, a 30% had a CTRI>10ms and 20% had an abnormal LV filling. All of the hypertensive active ACRO had an elevated LVMindex, a CTRI>10ms and an abnormal LV Filling.

GROUP (active non hypertensive ACRO) (n=18) acres (n=15) volunteers (n=15) LVM index (LVmi) (g/m²) (mean±SD) (LVmi) (g/m²) (mean±SD) (LVmi) (g/m²) (mean±SD) LVM index (LVmi) (g/m²) (mean±SD) LVM index (LVmi) (g/m²) (mean±SD)

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(p<0.05 group 1 vs 2).

Correlation between the years of activity in the active non hypertensive ACRO and the IRT, PWT and CTRI was observed (r=-0.851, r=0.877 and r=0.888). Correlation between current IGF-I and the current basal GH with cardiac abnormalities was not found. Hypertensive ACRO had a longer acromegalic activity in years than non hypertensive, in the active group (19 vs 11.6, p<0.05) and in the inactive group (15 vs 7.1, p<0.05). IGF-I level was higher in the hypertensive active ACRO than in the non hypertensive active ACRO.

These results suggest the existence of a myocardial disturbance in acromegaly independent from arterial hypertension.

P2.035
GONADOTROPIN SECRETION IN PATIENTS WITH ACROMEGALY.
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Hypogonadism is a distinct feature of acromegaly(A), even in the absence of hyperprolactinemia. We studied 24 h serum gonadotropins and α-subunit(SU) profiles (samples taken every hour) in 29 patients with A, mean age 44.7 yr. All had macroadenomas. Hypopituitarism(HP) was present in 5 and hyperprolactinemia(HG) in 10 patients. FSH and LH were determined by IRMA (MAIA clone, Serono) and free α-SU by RIA (Biomerca). Cross-reaction with intact FSH, LH, bFSH and bCG is less than 0.2%. AUCSU and AUCGH were calculated with repeated measures was used for data evaluation. During 24 h there was no significant changes in LH (F=0.6, p>0.05) and α-SU (F=0.5, p>0.09) levels. Significant changes in FSH concentration (F=2.5, p<0.001) were detected between 14 00 and 19 00 h. The pattern of secretion of these glycoproteins is preserved in patients with HG. These patients have higher levels of FSH (F=8,18, p<0.001), LH (F=75,4; p<0.001) and α-SU (F=81,0; p=0.032) in comparison with other patients. There are no differences in the pattern of secretion and FSH level between patients without and those with HP (F=2.99; p>0.07). In comparison with other acromegalic, HP is characterised by lower levels of LH (F=5,4; p=0.011) without changes in the pattern of secretion. Secretion of α-SU is unaffected by HP (F=2,1; p=0.1). AUCFSH/AUCGH in increased in patients with acromegaly (2.6±1.6; range from 0.8 to 17.5). LH secretion is more dependant on hypothalamic GnRH supplying than FSH. The consequence is increased FSH/LH ratio. This may explain hypogonadism in these patients. Whether the autonomous secretion of α-SU is in connection with increased FSH/LH ratio has to be clarified.

P2.034
TREATMENT OF ACROMEGALY WITH OCTREOTIDE NASAL POWDER.
Six acromegalic patients (3 with micro and 3 with macroadenoma of the pituitary) were treated with octreotide nasal powder (Sandostatin) for three months. All had active acromegaly with elevated serum GH (growth hormone) and IGF-I (insulin like growth factor I) levels. Serum GH levels (mean of 8 GH values measured at hourly intervals) fell below 5 ug/l in all patients: the effective dose of octreotide was 0.125 mg t.i.d. in three patients, 0.125 mg t.i.d. and 2 mg t.i.d. in other three respectively. IGF-I levels fell for more than 50% of initial values in all patients but normalised only in two with GH suppression below 1.0 ug/l. IGF BP3 levels fell in all patients but not into the normal range. Significant fall in fasting insulin levels without worsening of glucose tolerance was observed in patients who started with GH levels higher than 50 ug/l. After three months of treatment tumour shrinkage was observed on MRI in three macroadenomas of 46, 28 and 13% of initial tumour volume respectively. Two macroadenomas shrank also. No local side effects were noticed in the nose.
In conclusion, octreotide nasal powder is very efficient drug in treating acromegaly and was tolerated well by patients.

P2.036
ENDOCRINE PROFILE IN ACROMEGALIC PATIENTS WITH INAPPROPRIATE GH RESPONSE TO TRH/GnRH TEST.
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Inappropriate GH response to TRH/GnRH test may be a good prognostic feature in patients with acromegaly. Mean GH values in TRH/GnRH responders were lower than in non-responders. The aims of the study were to evaluate: 1) GH, PRL, LH, FSH and TSH response to TRH/GnRH test and 2) GH, glucose, insulin (IRI), glucagon (IRG) and B-endorphin (B-EP) serum concentrations during OGTG in 18 patients with acromegaly. Six healthy subjects served as controls (C). Seven patients (38,8%) with inappropriate GH response to TRH/GnRH (responders) had lower (p<0.04) GH, TSH and higher (p>0.04) basal serum levels as well as higher PRL, LH, FSH and TSH response to TRH/GnRH stimulation than non-responders. Basal IRI concentration and serum IRI secretion (AUC) during OGTG were lower in responders comparing with non-responders and not different than in C. Serum glucose, IRG, B-EP and their secretion during OGTG were not different in both groups. These results suggest that patients with GH response to TRH/GnRH test had near normal (excluding GH) hypothalamic-pituitary function and probably better insulin sensitivity.
P2.037

THERAPY OF ACROMEGALY WITH OCTREOTIDE: NASAL POWDER OVER 12 MONTHS

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The Somatostatin analogue octreotide nasal powder (Sandostatin, Switzerland) is a non-injectable drug with the same spectrum of pharmacological effects as the injectable octreotide. We treated 10 acromegalic patients (6 women and 4 men, mean age 42 years, ranging from 22 to 56) with octreotide nasal powder (ONP) for a period of 6 months and probed this therapy for another 6 months in case of a good response. Previously 9 of the patients had undergone transphenoidal and additionally 6 of them had been successfully treated with injectable octreotide (3-0.1 mg/sec.). This therapy was withdrawn for at least 2 days before the administration of ONP. The powder was applied 3 times a day in doses ranging from 0.25 to 2.0 mg. Regular rhinoscopic examinations of the nasal mucosa were performed in all patients throughout the study but revealed no relevant alterations. The effect of the therapy was evaluated by measurements of IGF-I levels and 8 hour profiles of hGH. According to the response two groups were determined: group A (6 patients) with hGH < 2 µg/l and group B (4 patients) with hGH > 2 µg/l. In group A mean IGF-I levels of the 8 hour profile before treatment was 5.0±0.9 µg/ml (mean ± SE) and IGF-I was 724±128 ng/ml. On day 14 of the ONP treatment the IGH levels were suppressed to 1.1±0.3 µg/l and IGF-I to 407±52 ng/ml. Similar results were obtained after 6 months (hGH 1.2±0.3 µg/l, IGF-I 427±84 ng/ml) and 12 months (hGH 1.1±0.3 µg/l, IGF-I 429±79 ng/ml). In group B hGH concentration at baseline was 14.3±5.8 µg/l, corresponding IGF-I levels 810±149 ng/ml. After 2 weeks of treatment IGF-I levels decreased to 6.7±2.0 µg/ml but IGF-I was nearly unchanged (770±41 ng/ml). Six months later these parameters were still abnormal in group B (hGH 10.3±4.9 µg/l and IGH-1 821±132 ng/ml), so therapy was discontinued. We presumed that the insufficient suppression of IGH in these patients was not due to poor resorption of ONP, because in the 8 hour profile there was no significant difference between the IGF-I response after application of the powder and after s.c. injection (hGH 8.1±4.1 µg/l). Obviously the higher initial GH concentrations in group B were the reason for the lack of normalization of IGH and IGF-I levels in this group, compared to group A. These results are comparable to the effects of the injective therapy, but the new administration form is more convenient and therefore the compliance of the patients was excellent.

P2.039

THE DESMOPRESSIN STIMULATION TEST IN PATIENTS WITH ACTH HYPERSECRETION


Recently a cortisol increase by desmopressin, an arginine vasopressin analogue, could be demonstrated in patients with Cushing's disease (CD) but not in patients with ectopic ACTH secretion. To further elucidate this new clinical test we have studied plasma ACTH and serum cortisol after 10 µg desmopressin intravenously in 7 patients with CD, 2 patients after bilateral adrenalectomy, 8 patients with Addison's disease (AD) and 10 normal individuals. In each patient with CD a desmopressin induced ACTH increase higher than 50 % above baseline could be demonstrated (baseline 100 % vs. 215 ± 56 % (15%), 193 ± 41 % (30%) and 152 ± 40 % (45%), respectively, X ± SEM). Serum cortisol showed a similar response from 19.5 ± 8.1 to 27.2 ± 15.0 µg/dl maximally. However, in two patients after bilateral adrenalectomy (macronodular hyperplasia in one case, suspected occult ectopic ACTH source in the other one), in patients with AD and in normal individuals desmopressin did not affect the ACTH secretion. Only 1. Desmopressin is able to stimulate the ACTH and cortisol secretion only in patients with CD but not in patients with ACTH-independent CS or ectopic ACTH syndrome. 2. In AD hypersecretion due to AD as well in normal individuals desmopressin is ineffective. 3. Desmopressin offers distinct clinical advantages in the diagnosis and the differential diagnosis of Cushing's syndrome.

P2.038

OBSTRUCTIVE SLEEP APNOEA IN TREATED ACROMEGALY - CORRELATION WITH AGE, IGH AND IGF-I LEVELS

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Irregular, heavy snoring and daytime sleepiness are common features of acromegaly (A). Only recently has the high incidence (30-60%) and clinical relevance of the sleep apnoea (SA) underlying these symptoms been recognized. Both diseases have a group of common symptoms and prognostic features: Increased cardiovascular and respiratory mortality, elevated incidence of hypertension and daytime sleepiness. These are very prominent in SA and often reversible under treatment. We studied the prevalence of SA in 42 treated A-patients and correlating factors. All patients underwent overnight MESA-4-polysonography (PS) measuring oxygen saturation, heart rate, snoring and body position. Blood samples for IGH and IGF-I were taken. A standardized questionnaire was answered by every patient. Wrist, index-finger and neck circumference were taken as indicators for hyperplasty of soft tissues. Treatment mode, duration of disease before first treatment and to PS were determined. Suspected SA was defined as more than 9 desaturations (of <94%) per hour of sleep time. Severity of SA was defined by the desaturation index (DI). Severity of SA was correlated with hormone levels, sex, age, body mass index and the other parameters mentioned above. Results: 38% of treated patients had suspected SA. The group without SA had an average DI of 3.65/µg. IGF-I averaged 351ng/ml and IGH 3.1ng/ml. In the group with suspected SA the DI averaged 24.5h, average IGF-I and IGH were 5386ng/ml and 7.04ng/ml respectively. Average age was 58 in the SA-group and 51 in the non-SA group. Simple regression analysis showed that age correlated significantly with DI (R=0.494, p=0.0009) whereas IGH and IGF-I levels did not. Sex distribution was equal in the suspected SA-group. Sixty two % of patients in the non-SA group were female. We conclude that SA is a common feature of A. In treated patients there is only a minor correlation between the hormonal form A and the DI of SA and that SA can persist after successful treatment of A.

P2.040

BASAL PLASMA CORTISOL ASSAYS VERSUS DYNAMIC TEST IN THE ASSESSMENT OF PITUITARY ADRENAL AXIS

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The insulin hipoglycaemia test (IHT) is time consuming and dangerous for patients. There is few information as to whether cortisol responses can be predicted from basal untimelimited cortisol levels. Our study was designed to evaluate the correspondence between resting cortisol levels and the response to IHT. Patients were performed on 113 patients (66 women, 47 men) aged 671 years with a variety of hypothalamic–pituitary disorders. Soluble insulin, 0.1–0.15 U/kg was given. Venous blood samples were taken at times 0,20,30,60,90,120 for measurement of glucose, cortisol and ACTH. Adequate hypoglycaemia was defined as a glucose level<2.2mmol/L and was achieved in 34 (79%) tests. The group I patients that had not been on steroid therapy (106 tests), and the group II patients that were on glucocorticoid replacement, which had been discontinued shortly before (28 tests). Normal cortisol responses were defined as a peak cortisol level >500µmol/L and normal ACTH response as an increment two fold in ACTH. There was a highly significant correlation between basal and peak cortisol levels (r=0.69, p<0.001) and between basal and stimulated ACTH levels (r=0.66, p<0.001). All cases with basal plasma cortisol concentration <165 nmol/l in both groups had subnormal ratios of ACTH response to hypoglycaemia (predictive value:1). In patients with basal cortisol between 165 and 415 nmol/l, 56% of normal cortisol responses were found. 57 of 59 cases with basal concentration >415 nmol/l showed a normal cortisol response (predictive value:0.96). We propose that morning plasma cortisol concentration <165 nmol/l confirms adrenocortical insufficiency irrespective of concurrent steroid replacement. Basal plasma cortisol>415 nmol/l is a strong indicator of normal pituitary adrenal reserve. If the only requirement is to exclude ACTH deficiency, a basal cortisol may give fully adequate information in over 60% of cases.
P2.041

GENETIC ASPECTS OF CUSHING’S DISEASE.

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140 patients with Cushing’s disease (CD) and partially their relatives from 19 families were examined by a series of genetic methods: immunogenetic, dermatoglyphic, isoenzyme, and biochemical. 135 lineages revealed 6 events of familial CD. Disease forms presenting filial segregation by Mendel law, corresponded to autosomal either recessive or dominant type of inheritance. Statistical analysis by an a priori method confirmed an autosomal-recessive type of CD inheritance. The hereditary burden of cardiovascular (27.2%), endocrine (24.4%), oncologic (27.2%), inflammatory (3%) and autoimmune (3%) diseases was discovered.

Certain genetic predetermination of CD development has been proved by genealogical, immunologic, dermatoglyphic, morphologic methods, clinical observations. Evolutionary, immunodeficient ideas of CD development has been suggested.

P2.042

THE NEUROHYPOPHYSIAL CONTROL OF WATER BALANCE: RECEPTOR-MEDIATED MOBILIZATION OF WATER CHANNEL AQUAPORINS


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Water balance is controlled in land vertebrates partly by an antidiuretic hormone, vasopressin (mammals) or vasotocin (birds, reptiles, amphibians). Water input and output occur at the level of specialized organs through epithelial cells. In addition to gut and kidney, amaranth Amphibia use the skin and the urinary bladder. Osmotically driven water transport involves a family of regulated non-regulated membrane water channels formed by specific proteins, the aquaporins. Vasopressin or vasotocin, acting on basal membrane receptors, trigger an increase in the number of apical hormone-regulated channels. Adaptive differential processing of prosaposcin led in amphibians to intermediates along with mature vasotocin: hydrol 1 (vasotocinyl-Gly-Lys-Arg) in the aquatic Xenopus and hydrol 2 (vasotocinyl-Gly), in semi-aquatic and terrestrial frogs and toads. Hydrol promotes water import through frog skin and bladder but not through the kidney. It is assumed that distinct receptors for vasotocin and hydrins, respectively, ensure specific reabsorption of internal (kidney) and external (skin) water. Reconstitution of processing has been performed in vitro using bovine secretory granule enzymes and synthetic pro-vasopressinyl peptides.

P2.043

EFFECTS OF VASOPRESSIN ANTAGONISTS ON ACTH AND CORTICOSTEROSE SECRETION IN STRESS CONDITION PRODUCED BY ETHANOL.

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A high dose of orally administered ethanol generates hemorrhagic lesions in the rat stomach, which may be prevented by simultaneous administration of plasma ACTH and vasopressin (VP) levels are elevated following oral ethanol administration, VP antagonists protect the rat stomach against ethanol-induced damage. Our aim was to investigate the effects of VP antagonists on ACTH and corticosterone blood levels following administration of a high dose of ethanol.

Female (n=5) rats were in a stress-free environment. After 24 h fasting, the rats received 1 ml of 350 ethanol orally. The VP receptor V1a receptor antagonist d(CH2)5-Tyr(Me)AVP (3.0 µg/kg i.p.) or the antidiuretic V1b antagonist d(CH2)5-Gly-Arg(8)-Tyr(Me)AVP (3.0 µg/kg i.p.) was injected 30 min before ethanol administration. Plasma VP, ACTH and corticosterone levels were determined by RIA at different times after treatment. At the end of the experiment, the stomach was removed and the lesions were analyzed planimetrically. After ethanol administration, higher plasma VP, ACTH and corticosterone levels were detected. V1a antagonist treatment significantly moderated the elevation of ACTH or corticosterone levels following ethanol administration. At the same time, the V1b antagonist protected the rat gastric mucosa against ethanol-induced damage. On the other hand, the V1a antagonist did not influence the elevation of the plasma ACTH or corticosterone concentration and the extent of gastric mucosal lesions.

These results indicate that VP receptors play an important role in the development of ethanol-induced gastrointestinal hemorrhagic lesions in the rat. We presume that the V1a antagonist can block the VP-mediated release of corticotropin, whereas the V1b antagonist protects the rat gastric mucosa against ethanol-induced damage.

P2.044

INTRACEREBROVENTRICULAR INJECTION OF ATRIAL NATRIURETIC PEPTIDE (ANP) REDUCES PLASMA VASOPRESSIN (ADH) LEVELS IN NORMAL AND DEHYDRATED CONSCIOUS RABBITS.

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Many studies have shown that although ANP consistently inhibits the ADH response to angiotensin II, KCl, hemorrhage and osmolarity changes, it has variable effects on basal ADH both in conscious and anesthetized animals (rats, dogs and sheep). In order to investigate the effects of centrally administered ANP on plasma ADH levels, 20 male albino rabbits were used. Measurements were made on restrained conscious animals one week after the implantation of an indwelling intracerebroventricular (ivc) cannula and an indwelling intravenous catheter. Animals were classified into 3 main groups, those with water available ad libitum (group A) and those who were deprived for 24 hours before blood sampling for hormonal determination (group B). Each group’s individuals were divided into 2 subgroups (1 and 2) of 5 animals each. The subgroup 1 animals were given, by ivc injection, 25 µl of artificial (a) CSF, whereas those of the subgroup 2 received human (h) ANP (1µg) in aCSF (25µl). Blood samples were collected at 0 min (control) and 30, 60, 90, 120 min following the ivc injection of either aCSF or hANP in aCSF. Plasma ADH concentrations were determined by RIA and the results were analysed by repeated measures ANOVA and the t-test (paired and unpaired) where appropriate. The analysis revealed that dehydration resulted in an increase of ADH (p<0.0001) and that ANP reduced ADH levels (p<0.05) in both A and B experimental groups. ADH levels were generally unchanged by time sampling, except for an increase (p<0.05) at 90 min in B1 group. In conclusion, the available evidence indicates that brain ANP may have a function in the control of ADH secretion even under basal conditions.
EVIDENCE FOR A DEFFECTIVE MATURATION AND FUNCTION OF DENDRITIC CELLS IN TYPE 1 DIABETICS. A FACTOR OF IMPORTANCE IN THE DISTURBED TOLERANCE TOWARDS β CELL ANTIGENS?

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Type 1 diabetes is an autoimmune disease in which β cells are destroyed by autoreactive T cells and macrophages. The disease is based on a dysbalance in immunoregulation that leads to the induction of humoral and cellular immune responses to an array of islet cell antigens. In type 1 diabetic patients we established a disturbed maturation of DCs from their precursors in the blood and a disturbed function of these generated dendritic cells (DCs), viz. a lowered capability of the cells to form clusters with autologous T cells. These DC defects in type 1 diabetics were independent from the duration of the disease. Similar and innate abnormalities in accessory cell function have been demonstrated in the animal models of type 1 diabetes (NOD mouse, BB rat). DCs are the T cell stimulating cells par excellence. Apart from playing a role in the initiation of immune responses, they are also involved in the shaping of the T cell repertoire in the thymus, and the generation of suppressor T cells in the periphery. Since DCs are of crucial importance in the triggering of naïve T cells, and since an optimal T cell stimulating action is earlier required for tolerance induction than for immunization, we hypothesize that the found DC defects form the basis for a disturbed T cell regulation in which induction of tolerance is stronger affected than immunization. This may easily lead to autoimmune disease.

FUNCTION OF ENDOCRINE PANCREATIC CELLS AND NEAR NORMOGLYCEMIA


To elucidate the impact of IDDM on basal and stimulated co-secretion of pancreatic hormones in patients with newly manifested IDDM (n=8, 2f, 6m, median age 26y, HbA1c at manifestation 10.7±1.4%) were infused with arginine (0.5g/kg BW) at t = 6 and 12 months after diagnosis of their disease, the patients were treated in functional insulin therapy, a multiple insulin injection schedule, immediately after diagnosis and aimed at optimized metabolic control. Basal and stimulated plasma concentrations were documented at timetervals for C-peptide, glucagon and somatostatin as was daily insulin demand (x±SD):

<table>
<thead>
<tr>
<th>Month after manifestation</th>
<th>1</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>7.8±0.8</td>
<td>5.65±0.44</td>
<td>6.38±0.76</td>
</tr>
<tr>
<td>Insulin demand (U)</td>
<td>24±9, 12.5</td>
<td>19.6±8.99</td>
<td>24.0±9.23</td>
</tr>
<tr>
<td>C-peptide (basal, pg/ml)</td>
<td>1.4±0.154</td>
<td>2.22±1.67</td>
<td>1.60±0.67</td>
</tr>
<tr>
<td>C-peptide (max, pg/ml)</td>
<td>3.9±0.68</td>
<td>3.64±1.27</td>
<td>2.00±1.20</td>
</tr>
<tr>
<td>Glucagon (basal, pg/ml)</td>
<td>154±3.2</td>
<td>191±4.3</td>
<td>171±9.6</td>
</tr>
<tr>
<td>Glucagon (max, pg/ml)</td>
<td>234±4.48</td>
<td>255±6.0</td>
<td>240±8.9</td>
</tr>
<tr>
<td>Somatostatin (basal, pg/ml)</td>
<td>13.2±1.6</td>
<td>13.0±1.3</td>
<td>15.0±6.3</td>
</tr>
<tr>
<td>Somatostatin (max, pg/ml)</td>
<td>16.1±4.1</td>
<td>15.0±4.3</td>
<td></td>
</tr>
</tbody>
</table>

Basal and stimulated plasma hormone concentration was retained and remained constant for glucagon and somatostatin throughout the observation period. An transient rise for mean C-peptide at 6 months reflects partial recovery of β-cell function, i.e. the honeymoon period.

The study demonstrates that IDDM does not co-impair endogenous glucagon and somatostatin release and confirms that normoglycemias is important for maintaining β-cell function.

EFFECT OF CLONIDINE ON THE ACTION OF GLUCOSE, TOBUTAMIDE, AND GLIBENCLAMIDE ON INSULIN RELEASE, CALCIUM- UPTAKE AND RB-EFFLUX OF MOUSE PANCREATIC ISLETS


Clonidine an agonist of alpha-receptors is known to inhibit insulin secretion in response to glucose and sulfonylureas. In the present study, the effect of clonidine (10 μM) on glucose (16.7 μM) tobutamide (100μg/ml) and glibenclamide (1 μM) induced insulin secretion, 45Ca++ uptake and their inhibitory action on βHKE efflux was studied. The 45Rb-efflux rate from isolated perfused islets was decreased significantly by tobutamide but not by clonidine in presence of non-stimulatory glucose concentration (3 μM). Clonidine did not affect tobutamide action. The Ca++-uptake into isolated islets was increased by glucose, tobutamide and by glibenclamide. It was not changed by clonidine in presence of non-stimulatory glucose concentration but was significantly reduced at a glucose concentration of 16.7 μM. Clonidine significantly reduced the effects of both tobutamide and glibenclamide on Ca++-uptake.

It can be concluded that clonidine exerts its inhibitory action on glucose – tobutamide – and glibenclamide – induced insulin secretion through decreasing Ca++ uptake. This action is not due to interference with potassium efflux.

METHYLGLXYLAL METABOLISM IN DIABETIC RATS

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Methylglyoxal (MG) is produced in the metabolism of lipids, carbohydrates and proteins by enzymatic synthesis, but it may be formed nonenzymatically from free autoxidation or by Maillard compounds fragmentation. If MG is produced in elevated concentration it is toxic and responsible for the formation of cross-links between proteins and/or nucleic acids. Glyoxalase (GlO1 and GlO2) are enzymes which metabolize MG, forming D-lactate. In the present study we induced diabetes in rats with streptozotocin (50mg/kg) diilated in citrate. A control group was injected with citrate. After 30 and 60 days, the rats were sacrificed by exsanguination after ether anesthesia. The following measurements were made: blood sugar by glucose oxidase method, plasma protein cross-links determination by fluorometric method. MG concentration of red cell, liver, kidney and heart by HPLC method and in the same organs we determined the glyceraldehyde activities by a spectrophotometric technique, measuring formation of 3,5-diacetylglutamine by GlO1 and for the GlO2 the decrease of this compound.

We found in diabetic rats an increase in plasma protein cross-links both after 30 or 60 days. In the there was increased activity of GlO2 after 60 days. In the liver there was an increase in MG concentration (p<0.001) at 60 days and GlO2 had decreased activity (p<0.001) both at 30 and 60 days. In the kidneys there was only an increase in MG (p<0.001) at 30 days. No changes were detected in the heart.

It is difficult to explain the results found, because they are not concordant in all organs. We think that perhaps the disposal of glutathione determines the pathway of MG metabolism. The liver has an alpha-eto-aldehyde dehydrogenase that transforms directly MG into pyruvate without GSH need. Also aldose reductase which has increased activity in diabetes metabolizes MG with high velocity.

So we think that our conclusions agree with other authors who think that when GSH concentration is diminished as happens in diabetes the MG is metabolized by other enzymes.
P2.049

DUCTAL HYPERPLASIA OF ADULT HUMAN ISLETS IN THREE DIMENSIONAL COLLAGEN GEL CULTURE: A POTENTIAL PATHWAY TO B-CELL PROLIFERATION

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The regenerating capacity of the adult human islet tissue is to date deemed limited, presenting difficulties in upscaling islet transplantation for Type I insulin dependent diabetes. Different culture techniques have been applied to facilitate B-cell proliferation without success. The cellular environment, in particular the extracellular matrix plays a critical role in the regulation of genetic expression and consequently in the function, proliferation and differentiation of cells exposed to growth factors rich media (Choongwongsa/ D et al 1978). Previously it has been shown that three dimensional (3D) culture of human islets or collagen (type I) can sustain the viability of human islets for up to 6 weeks.

Methods: In this study adult human islets isolated with the technique of Ricordi were embedded in a collagen gel system containing collagen type I (rat tail) and type IV (mouse), shown to play a role in the reformation of endocrine cells. Islets were cultured in RPMI 1640 (1% glucose) containing 10% or 20% fetal calf serum (FCS) and the morphological and functional changes were evaluated.

Results: 3D culture allowed islets maintain their spherical form. After 3-5 days in collagen type I-V formation of blob-like buds was observed in islets. The degree of budding was more pronounced in islets cultured in 20% FCS than 10% FCS. After 7 days the buds were liberated forming hollow (positive for insulin, chromogranin A and synaptophysin) and epithelial ductal nature of the cysts (positive for cytokeratin and CA 19-9), and negative for the fibroblast marker vimentin. Cysts were composed of a single layer of epithelial cells with an absence of collagen in the cyst lumen.

Discussion: The promotion of ductal hyperplasia in adult pancreatic tissue in vitro may prove to be promising as this is preceded by the subsequent differentiation of ductal tissue to endocrine cells as revealed in pancreatic embryogenesis and recently in adult rat tissue (Bonen-Weir S et al 1993). Investigation of the cellular source of the cysts (existing duct or transdifferentiation of contaminating exocrine tissue) and the subsequent differentiation of the ductal tissue is under investigation.

P2.050

"INSULIN DEPENDENT DIABETES MELITUS PREVALENCE IN GREECE"


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The prevalence of IDDM in Greece was established from clinical data sources and from the published data of National Statistical Service of Greece. The age and sex adjusted prevalence rate in 1990 was two times higher (95% confidence interval: CI 1.6 - 2.4) than the comparable rate in 1970.

The age adjusted prevalence for women was 1.9 times that for men (95% CI 0.8-3.1). The prevalence varied by age groups and was 1.6, 2.2, 2.2, 1.7 times higher in 1990 than in 1970 in age groups 0-4yrs, 5-9 yrs, 10-14 yrs and 15-19 yrs accordingly.

The annual prevalence rate per 100000 persons in 1990 was estimated to be 40.2 cases.

P2.051

ANALYSIS OF THE GROWTH HORMONE-INDUCED POTENTIATION OF INSULIN SECRETION CAPACITY FROM ISOLATED HUMAN FETAL ISLETS


In order to clarify the in vitro effect of growth hormone (GH) on isolated human fetal islets, this study was aimed to determine the requirements for optimal potentiating effect of GH (Genotropin, Kabi Pharmacia) on insulin secretion, concerning duration of incubation and dose dependency. The islets were isolated from pancreata of the 16-24 weeks gestational age fetuses by collagenase digestion, and cultured in the medium containing 10% fetal calf serum at 37°C, 5% CO2. The insulin secretion capacity was evaluated by determining insulin levels in the culture media after 1 hour incubation sequentially with 1.67 and 16.7nmol/l glucose and expressed as the percentage of the increase in insulin levels after the high-glucose stimulation. We found, first, that glucose-stimulated insulin response was maximal after 2 and 3 days of incubation with GH (GH+ vs. GH-): 1 day: 317.2+/-14.1 vs. 24.7+/-10.1; 2 days: 724.2+/-33.3 vs. 254.2+/-12.6; 3 days: 698.2+/-29.3 vs. 247.3+/-12.6. 4 days: 473.2+/-22.7 vs. 256.8+/-14.5; p<0.05(1000ug/l GH). In the second part of the study, we found that the insulin secretory response was maximal with 1000 and 1500ug/l GH (500ug/l: 404.4+/-27.1%, vs. 224.3+/-23.2 %; 1000ug/l: 673.0+/-28.7 vs. 259.4+/-101.0; 1500ug/l: 593.3+/-24.3 vs. 247.7+/-18.7%; 2000ug/l: 427.3+/-28.6 vs. 237.3+/-22.5 %) (3 days incubation). Our results signify that GH strongly increases the insulin secretion capacity in vitro, but this potentiation is a short-term and dose-dependent effect. The results imply that the effect might be used for potentiating of insulin secretion capacity in the pretreatment of isolated human fetal islets immediately before transplantation.

P2.052

CIRCULATING LEVELS OF INTERLEUKIN-1 IN NEWLY DIAGNOSED TYPE-1 DIABETIC PATIENTS

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Aim of the study was to investigate the serum levels of interleukin-1 (IL-1) in patients with insulin-dependent diabetes mellitus (IDDM) at the onset of the disease and after 3 and 6 months of insulin treatment. We studied 15 IDDM patients, aged 17-29, within 2 months after IDDM diagnosis and healthy controls (n=15). IL-1 serum levels were assayed by IL-1 mitogenic effect on murine thymocytes in the presence of the suboptimal concentration of the PHA. Levels of IL-1 were significantly higher at the onset of the disease compared to controls (p<0.001). The levels of TNF were reduced after insulin treatment compared with the onset of the disease (p>0.05), but were higher compared with control group (p<0.01).

Conclusion: high levels of cytokine IL-1 in newly diagnosed IDDM patients support the hypothesis that it may be involved in pathogenesis of islet B cell destruction.
**P2.053**

**IMPORTANCE OF C-PEPTIDE SECRETION FOR REMISSION DEVELOPMENT IN IDDM**


Institute of Endocrinology, Prague.

**AIM:** To evaluate the predictive value of C-peptide secretion (CPs) at the time of diagnosis of IDDM for development of spontaneous remission (R). **METHODS:** Basal and stimulated (arginine, glucagon, glucose) CPs were measured in 39 patients (17-37 years) within 5 weeks after diagnosis. The early and late phase of CPs were assessed (RIA) and analysed with respect to subsequent remission. **RESULTS:** We found: 1) higher basal and stimulated CPs (early and late phase) in R group after arginine, 2) min., R: 0.51, 0.13, non-R: 0.24, 0.13 nmol/l, p<0.05, 0.08 nmol/l, p<0.05, respectively. **CONCLUSION:** The early and late phase of CPs was found being of high importance for prediction of spontaneous remission of IDDM.

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**P2.054**

**ISLET AMYLLOID POLYPEPTIDE (IAPP) LEVELS IN ACROMEGALY**


The aim of the present study was to investigate whether plasma IAPP abnormalities are present to the disturbance of carbohydrate metabolism and insulin resistance which occasionally accompany acromegaly. 14 acromegalic patients were studied and compared with 18 controls matched for obesity. A standard 75g OGTT was performed and serum insulin, and plasma IAPP and glucose levels were determined. Basal IAPP levels, the area under the curve (AUC), the maximum increase from basal levels (Amax) and the IAPP to insulin ratio during the OGTT did not differ between patients and controls. These parameters did not differ either between the two subgroups of acromegalic patients, those with disturbed glucose tolerance (DTG, n=6) and those without (C, n=8). C vs DTG, x±SD, IAPP: 5.7±2.6 vs 5.2±3.4 pg/ml, AUCIAPP: 144±91 vs 93±68, AUCGlu: 651±207 vs 521±141, AUCIAPP/AUCInsulin: 0.225±0.08 vs 0.248±0.15, AmaxIAPP/MaxInsulin: 0.175±0.07 vs 0.161±0.09, p<0.05. We conclude that the new pancreas dopaminergic hypothesis may be involved in the disturbance of carbohydrate metabolism which occurs in a considerable number of acromegalic patients.

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**P2.055**

**"TRUE" INSULIN LEVELS IN TYPE 2 DIABETICS AND INSULINOMA PATIENTS AFTER EXTECTION OF PROINSULIN-LIKE COMPOUND**

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Proinsulin, 32-33 split proinsulin and 65-66 split proinsulin cross-react strongly in many insulin (IRI) radioimmunoassays. The aim of this work was to develop a simple procedure for the extraction of these proinsulin-like molecules from patients plasma before the insulin assays. We prepared monoclonal antibodies against C-peptide of human proinsulin with high affinity (constant of association 1.1 x 10^6/Mol) and specificity (amino acids residues 8-13 and 25-31 of C-peptide) which fully reacted with human proinsulin. These antibodies were immobilized on bromocyan-Sepharose. The extraction procedure was evaluated in three different radioimmunoassays in the presence of different concentrations of human proinsulin and C-peptide. Insulin concentrations in sera of control persons (n=50). Type 2 diabetes (n=45) and patients with insulinomas (n=46) were examined before and after extraction of C-peptide-like material using radioimmunoassay with cross-reaction for proinsulin 40%. In control group we found only small decrease of insulin immunoreactivity (RI) after proinsulin extraction (8.9±0.1 vs 7.5±0.2 MLI, mean±SEM, 10.9±0.7% of insulin immunoreactivity). Among Type 2 diabetics we found striking decrease of "true" IRI after extraction (21.4±2.9 vs 18.6±2.7 MLI, 16.9±1.5%, p<0.05 compared to controls). The highest proportion-like material we found in patients with insulinomas (25.3±1.1 vs 16.6±0.7 MLI, 32.8±1.3%, p<0.001 compared to controls). We conclude that extraction of proinsulin-like molecules from patients sera (using immobilized anti-C-peptide monoclonal antibodies) before insulin assay provides a simple procedure for the improvement of specificity of conventional insulin RIA. Supported by IGA 1434-3.

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**P2.056**

**EVALUATION OF SOME ENDOCRINE - METABOLIC PARAMETERS DURING ANIMAL PHALLODIUM POISONING IN RATS**

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Amanita phalloides poisoning is characterized by severe gastrointestinal symptoms followed by worsening of hepatic and renal function. Frequently, the clinical picture includes hypoglycaemia, related to hepatic damage. However, no correlation is shown between the hepatic failure degree and hypoglycaemia severity. The highest coincidence indicates that Am poisoning is followed by glycojenic hepatic depletion in humans and mice. Therefore, in mouse liver and muscles, Am toxin exhibit glycojenic synthesis by an in vivo mechanism. Studies evidenced, during experimental poisoning by alpha amanita, nuclear and cytoplasmic degenerative alterations in guinea pig and rat pancreatic alpha cells, whereas in mouse similar alterations occur in pancreatic beta cells. These observations suggest that alterations of glucoprotein hormones may be involved in the pathogenesis of hypoglycaemia after Am poisoning. No data are available on the levels of these hormones neither during experimental nor in human poisoning. Thus, we studied circulating glucose, insulin (IRI), C-peptide, glucagon (IGR), GH and cortisol in 10 patients admitted to intensive care units after acute poisoning by Am. Venous blood samples were drawn between 8:00 and 9:00 a.m. at least 1 hour after suspension of glucose infusion, for 24 consecutive days after admission. The control group consisted of 10 healthy subjects. Serum glucose did not differ significantly between these groups, whereas IRI, C-peptide and IGR were higher in poisoned subjects than in controls (p<0.01, p<0.01 and p<0.05, respectively). In poisoned subjects, a significant positive correlation was evident between IRI and IGR concentrations (r=0.89, p<0.001). C-peptide and IIR exhibited also a significant positive correlation (r=0.89, p<0.001). No difference was observed in cortisol, whereas a strong reduction of GH was evident in patients poisoned. Our data are consistent with the hypothesis that Am toxins stimulate IRI, C-peptide and IR release, likely by a cytokine mechanism. Increased levels of IRI in poisoned seem not related to stress, because in these patients GH and cortisol are not increased. It is difficult to explain the reduction of GH levels, even if toxins may affect GH secretion. Our study does not allow us to draw a pathophysiologic interpretation of hypoglycaemia during Am. poisoning. Moreover, we observed insulin-like antibodies, probably for therapeutic monitoring of poisoned patients. Nevertheless, our results show for the first time, that, in humans, Am. ph toxins alterate alpha and beta cellular function.
DESENSITIZATION OF PANCREATIC HORMONE SECRETION TO INCREASED CYTOSOLIC Ca2+: STUDIES IN PERMEABILIZED ISLETS

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Increased cytosolic Ca2+ is an important signal for the secretion of many polypeptide hormones, including the pancreatic hormones insulin (β) and glucagon (GL). We have used electrically-permeabilized rat islets to study directly the effects of cytosolic Ca2+ on the secretion of IN and GL. Permeabilized islets responded to increased intracellular Ca2+ with rapid increases in the secretion of IN and GL. However, Ca2+-induced secretory responses were transient with a rapid desensitization of the secretory process to elevated Ca2+ in both α-cells (50mM Ca2+: 4.6±0.2 pg GL/min/islet; 100mM Ca2+, 0.2min: 15.0±1.0; 100mM Ca2+, 31.35-31.35 min: 4.0±0.3) and β-cells (50mM Ca2+: 8.3±0.6 pg IN/min/islet; 10mM Ca2+, 0.2min: 31.1±4.0; 10mM Ca2+, 31.35-31.35 min: 10.3±2.7). The loss of secretory responsiveness to Ca2+ was paralleled by reduced Ca2+-dependent protein phosphorylation. Thus, in control (50mM Ca2+-treated) permeabilized islets activation of CAMK with 10mM Ca2+ resulted in the phosphorylation of endogenous protein of molecular weights 31-57kDa. Preincubation of permeabilized islets with 10mM Ca2+ greatly reduced (up to 90%) Ca2+-dependent IP3 of all substrates. Desensitization to Ca2+ was temperature-dependent implying an enzymatic component, but was not dependent upon the presence of ATP, suggesting that it was not caused by the autophosphorylation and resultant Ca2+-independence of CAMK. The Ca2+-dependent loss of CAMK activity was not due to the activation of Ca2+-dependent proteases since it was not prevented by the protease inhibitors E64 (10mM), TLCK (50mM), leupeptin (50mM) or leupeptin (50 mg/ml). The presence of the phosphoprotein phosphatase inhibitors okadaic acid, calyculin A and cantharidin (1mM each) enhanced Ca2+-induced phosphorylation in control islets (20±5% control, n=4), but did not prevent the Ca2+-induced loss of CAMK activity in islets pretreated with 10mM Ca2+. The cellular mechanisms which underly the loss of responsiveness of pancreatic endocrine cells to increases in cytosolic Ca2+ are therefore still unclear, but may play an important role in regulating the secretory responsiveness of polypeptide endocrine cells to prolonged physiological/pathophysiological stimulation.

NPY release from perfused rat islets of normal and dexahemethone treated rats is differentially modulated by glucose

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Neuropeptide Y (NPY) has been demonstrated to be synthesized within pancreatic islets. The measured release of NPY from rat islets, isolated from normal rats and rats treated with dexahemethone (DEX) for 10 day periods, perfused with low (2.8 mM) and high (20 mM) glucose concentrations. NPY release was detected from islets of normal rat perfused in the presence of low glucose. During the initial 40 min period the geometric mean NPY release was 2.10±0.95% (confidence interval 1.6-2.6) amol/10 min. NPY secretion was 71% decreased to 0.6 (0.3-1.1) amol/10 min when the glucose concentration was increased to 20 mM during 40-80 min, it then rose to 1.8 (1.4-2.4) amol/10 min with low glucose, and subsequently fell to 0.9 (0.5-1.4) amol/10 min during high glucose. NPY release from islets of DEX-treated rats was 2.7 (1.0-7.4) amol/10 min during perfusion with 2.8 mM glucose from 0.40 min and was not significantly different from the rate of NPY release by normal rat islets. NPY secretion was elevated 6-fold (P<0.001) on perfusion with high glucose between 40-80 min. The decay of NPY from DEX-treated rats compared to low glucose. In this period NPY release was 50-fold higher (P<0.001) from islets of DEX-treated rats compared to islets of normal rat. NPY release was therefore decreased by high concentration glucose in the normal rat islets whereas it was enhanced by high concentration glucose in islets of DEX treated controls. NPY release from DEX-treated islets when chromatographed on Fast Protein Liquid Chromatography (FPLC) and Sephadex G-50 eluted similarly to that of synthetic NPY standard indicating secretion of authentic NPY. The effect of glucose on NPY secretion indicates that release of NPY was from endocrine cells as no nerve terminals remaining in the islets would not be expected to respond to glucose. Furthermore, these results show that islet NPY release is responsive to change in endocrine status.

PCO A MODEL OF PRIMARY DEFECT OF INSULIN RESISTANCE

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Hyperinsulinemia in PCO has been attributed to insulin resistance. Hyperandrogenism has been implicated to contribute to this abnormality. However, it has not been established whether the beta cell insulin secretory capacity is normal in this syndrome. Therefore to determine which is the primary defect, i.e., evaluated insulin secretion and insulin sensitivity by performing hyperglycemic clamps (180 min to 180 mg/dl in ten lean and ten obese BMI > 26) women with PCO (FH=93+6 and 88+5 mU/L respectively N.S.) Second phase insulin release (SII) was no different in the lean groups (SII: 45±5 and 47±3 mU normal and PCO respectively N.S.) and in the obese groups (SII: 88±10 and 78±8 mU normal and PCO respectively N.S.). Sensitivity index (SI) as assessed by the hyperglycemic clamp was significantly reduced inrkn women with PCO (SI normal=21±2.5 SI PCO=11.4±1.9 mU/min/mU). SI in the obese PCO although reduced, did not reach statistically significant difference, compared with the obese normal women (SI normal=20±4 SI PCO=7.2±1.9 mU/min/mU). N.S. SI assessed by hyperglycemic clamps correlated well with the one assessed by hyperglycemic clamps in the above mentioned groups (r=0.78). These data suggest that: 1) There is no insulin secretion defect in PCO 2) Insulin resistance seems to be the primary defect in this syndrome 3) Androgen contribution to insulin action defect seems to be of not significant importance. We conclude that insulin resistance is the primary defect in carbohydrate metabolism in women with PCO. Thus PCO could be a useful model for studying genetic defects of insulin action.

A NOVEL CHEMIUMINESCENCE IMMUNOMICTRIC ASSAY TO MEASURE ERYTHROPOIETIN IN SERUM

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This new chemiluminescent assay, for the assessment of erythropoietin (EPO) levels in serum, can be used in patients with hematoletic disorders and in follow-up studies in patients in which EPO is used as a therapeutic agent (renal failure).

This is a chemoluminescent enzyme immunometric assay utilizing a polyclonal antibody, raised in sheep against human recombinant EPO, which is biotinylated and a mouse monoclonal antibody against EPO which is labeled with an acridinium ester. We measured release of EPO from different cell lines of the EPO molecule, present in the sample, to form a sandwich. The samples/standard containing EPO is incubated simultaneously with both antibodies, which bind without competition. The formation of a soluble sandwich complex occurs only in the presence of EPO molecules, which bridge the two antibodies. Addition of an avidin coated plastic bead (6 mm) allows binding of the sandwich to a solid phase by means of the high affinity interaction between avidin and biotin. After a four hours incubation on a rotator (20°C), the beads are washed and the amount of bound/labelled antibody is measured in a luminometer. The amount of Relative Light Units is directly proportional to the concentration of EPO in the sample. The dynamic range of the assay is from 5 to 1500 mEU/ml. The recovery in 3 different sera, spiked with hr-EPO was 105% and also dilution of 3 sera containing high levels of EPO, with zero standard, run parallel to the standard curve. The intra assay variation ranges from 3.2%-10.5% (at 6 levels: 3.1-1099 mU/ml), while the inter assay variation ranges from 4.1%-10.5% (at 7 levels: 6.8-1065 mU/ml). The sensitivity of the assay is approximately 1 mU/ml. The chemi assay was compared to a commercial EIA ELA and RIA and it showed that there is a correlation of 0.98 and 0.97 respectively.
P2.062

POSTPRANDIAL GALLBLADDER EMPTYING IN DIABETICS RELATED TO GASTRIC EMPTYING RATE AND PANCREATIC POLYPEPTIDE


Functional disturbances of the alimentary tract is common in diabetics but it is unclear whether this is due to autonomic neuropathy, elevated blood glucose or secondary hormonal changes. Our primary aim was to investigate the postprandial gallbladder emptying (GBE). A normal pattern is considered to be composed of 1) a lag-phase of less than 22.5 min, 2) a maximal emptying in less than 3 h and 3) the emptying being continuous: pauses or refilling events may appear, but not for more than 13.5 min. Our material comprised sixteen males: Non-diabetics (n=4, age 23-27), non-insulin dependent diabetics (NIDDm) (n=4, age 50-65) and insulin dependent diabetics (IDDM) (n=4, age 16-20). The duration of diabetes was < 7 year, all were well controlled (HbA1c < 10%) and no one had signs of late complications. Methods: Gamma camera scintigraphy: GBE; continuous infusion of 85Tc-Methotrexate (40Bq/h), Gastric emptying rate (GER); an oneder (1400 KJ, 60% fat tagged with 40 MBq 85Tc-sulfur colloid and 150 ml water mixed with 8 MBq 111In-DTPA. Blood glucose (BG); Refluxol R. Pancreatic Polypeptide (PP); RID. Results: All NIDDm had normal GER. In IDDM had abnormal GER pattern, all together delayed GER. The abnormalities were unrelated to BG and PP level. But all had an early postprandial peak of PP (15-45 min versus 60-150 min in IDDM with normal GER, 75-135 min in NIDDm and 30-105 min in non-diabetics). In the two hours following the PP-peak the mean PP values were higher in NIDDm (114 pmol/l) versus 54 in IDDM and 47 in non-diabetics. Conclusion: Disturbed postprandial emptying of the gallbladder in insulin dependent diabetics is related to disturbed gastric emptying and to an early release of Pancreatic Polypeptide (fast vagal response following ingestion). In non-insulin dependent diabetics the release of Pancreatic Polypeptide is prolonged but without influence on gastric and gallbladder emptying.

P2.063

CCK 33 CAN INDUCE CL - SECRETION IN HT-29CL19A CELLS.

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It has been shown that CCK can influence the ion-transport activity of isolated guinea pig intestine via a TTX sensitive mechanism (1). This indicates that CCK has an effect on the enteric nervous system. But it cannot be excluded that CCK has also a direct effect on the epithelial. In fact, it has been found that in cultured human colon carcinoma cells cells (Lo Vo) CCK can influence the transport on these cells. To study the presence of receptors and the possible short term effect of receptor stimulation we used the human colon carcinoma cell line HT-29CL19A. We have shown earlier that these cells can be used as a model for investigation of intestinal secretion (2).

We have studied the electrophysiological effects of CCK on these cells grown as a confluent monolayer on permeable, transluent Falcon filters and mounted in an Ussing-type experimental chamber. Conventional microelectrode technique was applied to monitor the intracellular potential with respect to the apical side and transepithelial potential was measured as usual. Transepithelial resistance and the fractional resistance of the apical membrane were calculated from voltage deflections induced by transepithelial current pulses. Application of CCK-33 to the serosal side induced a dose dependent and transient depolarization and concomitantly a transient serosa positive transepithelial potential change and increase of the short circuit current. The transepithelial resistance decreased transiently as well, whereas transepithelial resistance did not change. These electrophysiological phenomena suggest that CCK 33 can induce the transient opening of apical Cl' channels. In the presence of a supramaximal concentration of forskolin (activator of adenylhycyclase) a further increase of the short circuit current could be induced by CCK. This suggests that another Cl' channel than the CAMP dependent channel can be activated by CCK or, alternatively that more CAMP-dependent channels can be activated. The CCK-A and B receptor blockers L364.718 and L365.980 were both effective. These results indicate that in this model of intestinal epithelial cells serial CCK has a direct effect on ion-transport activity.

P2.065

SOMATOSTATIN-RECEPTOR IMAGING OF GASTROENTEROPANCREATIC NEUROENDOCRINE TUMORS

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Since gastroenteropancreatic neuroendocrine tumors express in a high percentage somatostatin receptors in vitro, visualization of these receptors in vivo by somatostatin-receptor scintigraphy appears to be feasible for the search of gastroenteropancreatic neuroendocrine tumors. Applying the recently developed, indium-labeled somatostatin anologue 111In-pentetreotide to 60 patients with gastroenteropancreatic neuroendocrine tumors, we investigated the diagnostic value of pentetreotide-receptor scintigraphy in comparison to conventional imaging techniques and endoscopic ultrasound. Expression of somatostatin receptors was observed in the majority of patients (16/25 in foregut carcinoids, 23/24 in midgut carcinoids and 10/11 in metastatic carcinoids with unknown primary). Comparative imaging by computer tomography, fluorine-18 PET and somatostatin-receptor scintigraphy in 15 out of 60 patients; in 20 patients, however, tumor tissue was detected by somatostatin-receptor scintigraphy that had escaped conventional imaging techniques. Moreover, in patients with midgut carcinoids somatostatin-receptor positivity correlated with serum chromogranin A levels and the functional tumor state. Thus, 111In-pentetreotide scintigraphy is a practical, safe and sensitive procedure for in vivo imaging of gastroenteropancreatic neuroendocrine tumors.

P2.066

ASSOCIATION OF G-PROTEIN HETEROTRIMERS WITH REGULATED VESICLES


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The association of heterotrimeric G-proteins with large dense core vesicles from bovine adrenal medulla (chromaffin granules) and small synaptic vesicles from rodent and bovine brain was analysed by immunofluorescence microscopy and Western blotting. Both vesicle types contain G-protein α-, β- and γ-subunits. In addition, two β-subunits, β3 and β2, as well as γ-subunits were found. Interestingly, the two vesicle types differ in the pattern of γ-subunits. On purified chromaffin granules one α2γ-subunit was detected by Western blotting but no α2-subunits. By contrast, small synaptic vesicles contain two α2γ-subunits (α2γ1 and α2γ2) and two αq-subunits (αq1 and αq2). Thus chromaffin granules and small synaptic vesicles possess complete sets of heterotrimeric G-proteins consisting of α2-, β3- and γ-subunits. Functional properties like transmitter storage and/or exocytotic membrane fusion may be modulated by the various G-protein subunits. Work was supported by the Deutsche Forschungsgemeinschaft.

P2.067

INCREASE IN BLOOD GASTRIN AND INSULIN CONCENTRATION OF DIABETES MELLITUS PATIENTS WITH GASTRODUODENO-PANCREATIC (G-D-P) SYSTEM DAMAGE

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Background. The upper intestinal system is the primary mechanism for glucose absorption, early insulin response in cooperation with enteroinhibitory axis, and carbohydrate digestion. The goal of present work is to investigate the influence of G-D-P system on hypergastrinemia and hyperinsulinemia production mechanism in diabetes patients.

Methods of investigation. Functional. pH of intragastic and duodenal content was measured continuously in situ without exhausting the gastric content. Biochemical: the blood sugar content, radioimmunological: determination of insulin, C-peptide, gastrin and trypsin in blood by RIA kit (FRance) Totaly 720 patients with diabetes were observed. Data were statistically treated on a computer by means of program BMDP produced by California Univer. USA.

Results. In our experiments gastroduodenitis had some connection with diabetes, especially for patients with NIDDM (p=0,033); usually these patients had not only reduced intragastic pH values, but also increased gastrin (p=0,05) and insulin (p=0,05) concentration. In IDDM patients high gastrin (p=0,05) and insulin level (p=0,047±0,0042) combined with reduced C-peptide level distinguished chronic pancreatitis in diabetes patients from chronic pancreatitis without diabetes. Increase gastrin concentration correlates with increase of insulin concentration (p=0,05). The increase of gastrin concentration correlates well with: duration of disease (p=0,01), the increase of trypsin (p=0,005), insulin (p=0,05) and C-peptide (p=0,05). Cluster analysis indicates that gastrinopathy closely correlates with g-d-p system disturbance symptoms.

Conclusion. The increase of gastrin concentration in blood could be one of the mechanisms to achieve the activation of the whole g-d-p system and, in this way, to facilitate the homestasis of carbohydrates. Hypergastrinemia and hyperinsulinemia are among the earliest symptoms of malignations of the g-d-p system in diabetic patients.

P2.068

FEATURES OF HORMONAL AND AUTOCRINE REGULATION OF HUMAN MALIGNANT MELANOMA CELL GROWTH

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Fast malignant growth for melanoma tumours seems to be connected with features of selective hormone action mechanisms and expression of autocrine growth modulating factors (AGMF)-genes. We showed that melanocyte stimulating hormone, α-MSH, and its analogue, NLe4, D-Phe4-α-MSH, exert inhibitory or stimulatory influence on the growth of human melanoma cells of different phenotypes. These differences are determined by features of hormonal signal transduction for various cell phenotypes. Considerable distinctions were observed for Adenylate Cyclase complex functioning and for another G-protein-bound systems (levels of myo-inositol phosphates IP2, IP3, phosphatidylinositol (Phosphatidylinositol Kinases). More over, the cells of one phenotype have expressed their own GF-s, which do not effect on the growth of another cell phenotype. So, we carried out the isolation of AGMF-s, which are produced by M6-line of pigmented human malignant melanoma cells. Selective and universal GMR-s for another cell phenotypes, including anealcanotic human malignant cell line, BZ0, were among them. These AGMF-s do not have any effect on the growth of human lung fibroblasts.
**P2.069**

INSULIN-LIKE GROWTH FACTOR-II (IGF-II), TYPE 2 IGF RECEPTOR AND IGF-BINDING PROTEIN-2 GENE EXPRESSION IN RAT LUNG ALVEOLAR EPITHELIAL CELLS: RELATION TO PROLIFERATION

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Pulmonary alveolar type 2 cells act as a reservoir of stem cells which can be induced to proliferate during periods of lung growth and repair following lung injury. Despite the importance of this process, the mechanisms that regulate type 2 cell proliferation have not been well characterized. We show in this study that IGFBP-2 accumulates to high levels in culture medium of growth-arrested type 2 cells, by either serum-deprivation or oxidant exposure. This is associated with an increased expression of IGFBP-2 mRNA. Study of the other components of the IGF system also reveals induction of IGF-II and type 2 IGF receptor mRNA during the process of type 2 cell block of proliferation. When growth-arrested cells are allowed to resume proliferation, the level of expression of IGFBP-2, type 2 IGF receptor and IGF-II rapidly decreased. Despite the similarities in the timing of induction, it is likely that these components are not necessarily linked to mediate effects through a single pathway. Indeed, we show that addition of conditioned media from growth-arrested cells on proliferative cells results in a down-regulation of IGFBP-2 and in an increased expression of IGF-II and type 2 IGF receptor mRNA. Treatment of the cells with various concentrations of IGF-II affects only the level of expression of type 2 IGF receptor, whereas IGF-I and insulin appear to influence only the expression of IGF-2. From the results presented in this study, it can be suggested that IGFBP-2, IGF-II and type 2 IGF receptor may play an important role in the transition of lung alveolar epithelial cells in and out the cell cycle.

**P2.070**

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2 IS ELEVATED IN MALIGNANT PLEURAL EFFUSION.


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IGF-I and its binding proteins (IGFBPs) are altered in sera of patients with malignancy, infection and other diseases causing pleural effusion. We investigated IGF-I/IGFBP-2 expression in pleural effusions formed in these diseases. IGFBP-2 as determined by Western ligand and immunoblotting was markedly increased in effusions of malignant solid tumors (1.82±0.42 arbitrary units, n=6) compared with exudates of lymphoma (0.27±0.13, n=3) or infection (0.63±0.23, n=3) as well as with transudates (0.23±0.1, n=4, p<0.05). Moreover, in effusion of solid tumors IGFBP-2 levels were higher than in corresponding sera suggesting local production of this binding protein. The demonstration of IGFBP-2 in solid tumor cells by immunohistochemistry further supports this possibility. IGFBP-3, the major binding protein in sera of these patients was faintly seen in some effusions and absent in others. In contrast, all effusions contained a 31 kDa protein which is presumably the proteolytic product of IGFBP-3. IGF-I levels were significantly different only between transudates and exudates. Our work demonstrates the existence of the IGF-1/IGFBP system in pleural fluids from different etiologies and implies the use of IGFBP-2 as a potential marker of malignant effusions.

**P2.071**

USE OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-1 (IGFBP-1), IGFBP-3 AND IGF-1 AS BIOCHEMICAL MARKERS IN PREPUBERTAL CHILDREN


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[IGFBP-1] and [IGFBP-3] are members of a family of proteins, present in extracellular fluids, that binds IGFs and modulates their biological activity. IGFBP-1 and IGFBP-3 are GH dependent whereas IGFBP-3 is insulin regulated. Variations in nutritional status are also important in modifying serum levels of these proteins.

AIMS: Comparing serum levels of IGF-I, IGFBP-1 and IGFBP-3 between BMI above 90% and BMI below 100% healthy prepubertal children.

**MATERIAL AND METHODS:** From a representative sample of 215 children collected from General Pediatrics in School-aged Population from the Autonomous Community of Madrid [24]. Serum levels of IGF-I, IGFBP-1 and IGFBP-3 were measured by radioimmunoassay (Nichols) in these prepubertal children (aged 7 to 10 years) with a BMI higher than 90% and weight above 95th percentile. Statistical analysis was performed applying the Student's t and Mann-Whitney tests for mean comparison.

**RESULTS:** Our results are shown in the following table:

<table>
<thead>
<tr>
<th>BMI Percentile</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (ng/mL)</td>
<td>24</td>
<td>315.95</td>
<td>161.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IGFBP-1 (ng/mL)</td>
<td>24</td>
<td>4.39</td>
<td>2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IGFBP-3 (ng/mL)</td>
<td>24</td>
<td>2.38</td>
<td>1.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**CONCLUSIONS:** - Serum IGF-I and IGFBP-3 levels are higher in fat than in low-weight children, whereas they could be associated with the new biological markers of total body fat in prepubertal healthy population.

- Serum IGFBP-1 levels are low in fat children and elevated in thin children, probably related to the variations of the insulin-like associated to the amount of fat.

- These three biochemical parameters clearly separate fat and lean prepubertal healthy children.

**P2.072**

ISOLATION AND CHARACTERISATION OF A BOVINE INSULIN-LIKE GROWTH FACTOR-1 GENE PROMOTER

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Bovine insulin-like growth factor-1 (IGF-I) is a 70-kDa acid mitogenic peptide with important functions in the regulation of somatic growth, development and differentiation. The liver is the main site of synthesis of IGF-I, although it is also synthesised in numerous other tissues.

In order to study transcriptional regulation of the bovine IGF-I gene we have isolated a bovine genomic clone which contains exon 1. Sequence analysis of this clone shows that the sequence of exons 1 and the 5' flanking region, is well conserved with other species which have been studied including human, rat and sheep. We mapped the major transcription start site of exon 1 by primer extension analysis. This indicates a 5' UTR of 280 - 295 nucleotides. Promoter activity was measured by cloning regions of the 5' flanking region into pBlCa 6' and E and transferring these constructs into Hep G2 and Hela cell lines. Activity was seen in both cell lines. Sequence analysis of the promoter regions showed the absence of typical proximal promoter regulatory regions such as TATA or CCAAT boxes. DNAse 1 footprinting of a 200 bp region 5' to the start of transcription shows several protected regions.

In conclusion we have isolated a functional promoter for bovine IGF-1 and identified potential transcription factor binding sites.
P2.073

STIMULATION OF ENDOMETRIAL CANCER CELL GROWTH BY TAMOXIFEN IS ASSOCIATED WITH INCREASED PHOSPHORILATION OF THE IGF-I RECEPTOR.

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Endometrial carcinoma is the most common malignancy of the female genital tract in Western countries. The major growth stimulators of this tumor are estrogens, but paradoxically, tamoxifen, a known antiestrogen, also stimulates its growth. The estrogen mode of action can be partially explained by the modulation of the IGFs autocrine or paracrine mechanisms. The purpose of the present study is to examine the involvement of the IGF system in the estrogen and tamoxifen stimulated growth of these cells by quantitating the IGF-I receptors, and their phosphorylation as well as the secretion of IGFBP. IGF-I was found to be a potent mitogen for the stimulation of Ishikawa cell proliferation but at low concentration of the peptide this stimulation was largely dependent on the presence of estradiol. Binding experiments using labeled IGF-I showed that estradiol caused a 2.5-fold increase in the number of high affinity receptors. This effect was not observed in tamoxifen treated cells. In addition, the phosphorylation of the IGF-I receptor was increased by estradiol but not by tamoxifen. These results suggest that the IGF-I receptor may be involved in the regulation of estrogen induced cell proliferation.

P2.074

EGF RECEPTORS IN HUMAN PROLACTINOMAS.

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Epidermal Growth Factor receptors (EGF-R) have been observed in the normal rat and human pituitaries. EGF is not only a mitogenic factor. It is able to stimulate PRL secretion, and has some differentiating effects on GHy cells (stimulation of PRL secretion, inhibition of both GH- and PRL-secretion and cell proliferation, induction of dopaminergic receptors). EGF-R were studied in 18 surgical biopsies of human PRL-secreting pituitary tumors and compared to 22 non-PRL-secreting tumors. Tissue specimens from human benign postheral hypertension (BPH) were used as positive controls. Membrane were incubated with a single concentration of [125I]EGF (1 nM), with or without a 100-fold excess of cold EGF. In some cases increasing concentrations of [125I]EGF were used to perform Scatchard analysis. Scatchard plots obtained on pituitary tumor samples displayed the presence of two classes of binding sites with high and low affinities (Kd of about 0.8 nM and 40 nM, respectively), such results being similar to those obtained with BPH specimens, though the corresponding Kd were slightly different (C. Labranca et al. J. Steroid Biochem. 1989, 34, 499-504). Values obtained with a single point saturation assay were shown to correlate with the binding capacity for the high affinity binding site, and a greater variability in EGF-binding was observed. EGF-R expression was significantly higher in prolactinomas than in the other pituitary tumors (91 ± 39.1% vs. 35.4 ± 21.5 fmol/mg protein, P = 0.038), and tended to be higher in female than in male prolactinomas (147 ± 8 vs. 73.9 ± 34.3 ± 15.3 fmol/mg protein, P = 0.070). Interestingly, the highest values of EGF-R were observed in microprolactinomas, though the mean EGF-R expression was not significantly higher in micro- than in macro-prolactinomas (232.6 ± 123.2 vs. 36.6 ± 12.9 fmol/mg protein, P NS). The presence of EGF-R in human prolactinomas supports a direct effect of its endogenous ligand, EGF, or TGF-a, on tumoral lactotrophs. The relatively higher EGF-R expression in female tumors suggests a possible regulation by sex steroids, as reported in breast and prostate tumors.

P2.075

THE OLIC ACID MODIFIES EGF-INDUCED MITOTIC CYCLE IN EGF-R-T17 CELLS.

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Proliferation control is cross-shaped for the study of the actions that make on cells growth factors and hormones. They make up a basic approach to solve important problems such as embryogenesis, tissue repair, cancer and so on. In a lot of cases, the technique of thymidine incorporation is used as an index of cellular proliferation. When we made a time course on thymidine incorporation during 36 hours in EGF-T17 cells we noted that the cells which have been treated with oleic acid (30 μM) and stimulated with EGF (10 nM) presented twice of counts incorporated of thymidine that its controls (EGF-stimulated cells) (EGF= 114±28±5±71 A, OA+EGF= 225±7±14±285 (cpm), stimulated cells 24 hours). This results suggested that oleic acid favored the mitogenic effect of EGF. So as to obtain to what we evaluated cell proliferation by cold cell direct counting using a Coulter counter, and the result was that oleic acid did not modify EGF-induced cell proliferation (Control; 100±5, EGF, 143±21±54, OA, 94±5, OA+EGF 137±6±12%. over control). This result was not confirmed neither by means of a 72 hours cell counting. We determined the thymidine incorporation directly in DNA, and we are observed that twice of thymidine incorporated in the monolayers that pretreated with oleic acid and lately stimulated with EGF, this result was confirmed when we was determined the presence of [3H]-thymidine in the nucleus by autoradiography. After excluding an experimental devise, we studied by flow cyometry, and we are observed that cells treated with oleic acid and then stimulated with EGF, accelerates the entrance in the mitotic cycle, but this change in the cellular cycle, did not involved an increase in cell proliferation. Oleic acid modifies the effect made by EGF on the cellular cycle. It advances in but cells have regulating mechanisms which induced a late synchronisation to normal cycle. As a minor conclusion we can say that the method which consists of the incorporation of thymidine is not commendable to describe cellular proliferation.

P2.076

EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETAO MODULATE TUMOR CELL MOTILITY AND ADHESION IN FOLLICULAR THYROID CANCER.


We investigated the role of the extracellular matrix (ECM) components collagen IV [C IV], fibronectin [FN], laminin and matrigel for tumor cell motility and adhesion in 3 follicular thyroid cancer (FTC) cell lines from 1 patient (FTC133-thyroid, FTC236-lymph node, FTC238-lung metastasis). Also, the effects of epidermal growth factor (EGF) and transforming growth factor beta 1 (TGFβ1) on adhesion were analysed. Tumor cells were grown in serum-free media (HS). Adhesion (attachment to the ECM components) and chemotactic migration (penetration of 8 μm pore polycarbonate membranes versus ECM) were tested using the MTT assay. Unstimulated, cell motility was highest in both metastases. After 8 hrs, locomotion of all FTC was stimulated by C IV (FTC133: 236%, FTC236: 229%, FTC238: 138%, p<0.05) and by FN (FTC133: 229%, FTC236: 19%, FTC238: 15%, p<0.01). Also, all FTC attached best to C IV and FN. EGF (10 ng/ml) inhibited tumor cell adhesion significantly, whereas TGFβ1 (10 ng/ml) was stimulating (table). Compared to FTC133, both metastases had a higher basal migration, a smaller basal adhesion and a loss of sensitivity to both growth factors.

<table>
<thead>
<tr>
<th>Tab.</th>
<th>(%) of adhesion</th>
<th>H5</th>
<th>EGF (10ng/ml)</th>
<th>TGFβ1 (10ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>FTC133</td>
<td>HS</td>
<td>21</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Collagen IV</td>
<td>14</td>
<td>25</td>
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<tr>
<td></td>
<td>Fibronectin</td>
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<td>34</td>
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<tr>
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<td></td>
<td>Collagen IV</td>
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<td></td>
<td>Fibronectin</td>
<td>21</td>
<td>17</td>
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</tbody>
</table>
P2.077

G PROTEIN MUTATIONS IN ENDOCRINE NEOPLASIA.
EA Williamson, 'S Johnson, P Kendall-Taylor, J.L Jameson, FE Harris. Departments of Medicine and Pathology, University of Newcastle upon Tyne NE2 4HH, UK.

Mutations of Gsa (gap) resulting in the constitutive activation of adenylyl cyclase have been described in a number of endocrine conditions. We have examined the tissues of patients with more than one endocrine disorder, or with metastatic disease for gap and gip (G12α) mutations, using PCR, oligonucleotide-specific hybridisation and direct DNA sequencing. Only one specific mutation was identified, encoding a change of arginine to cysteine at codon 201 of Gsa. Patient1 carotid body tumour gap, hyperplastic adrenal wild-type (wt); Patient2 phaeochromocytoma gap, hyperplastic adrenal wild-type (wt); Patient3 Nelson's syndrome, hyperplastic adrenal gap, corticotroph adenoma wt; Patient4 parathyroid hyperplasia and adenoma gsp; Patient5 malignant phaeochromocytoma and metastatic tissue gap; Patients6-8 adrenocortical and normal thyroid and parathyroid tissue gsp. Leucocyte DNA only showed wt sequence. We conclude that gsp mutations may be of oncogenic significance in a number of endocrinopathies and that some patients with more than one endocrine disorder may harbour the same G protein mutation.

P2.079

A METHOD FOR THE EVALUATION OF p53 ABERRATIONS IN HUMAN MALIGNANT TISSUE USING SINGLE STRAND CONFORMATION POLYMORPHISM ANALYSIS (SSCP).

Introduction. Human malignant tumours may occur in human malignomas and, if so, if these are associated with the presence of p53. Methods. Malignant tissue was obtained from the operating theatre and immediately stored at -80°C. Tissue was homogenized and genomic DNA was extracted. Radioactive PCR was performed with [3P]-dCTP using primer sets specific for each of the exons 5 through 9. Thirty-five cycles with 30° annealing at 37°C and 45° extension were carried out at 26M MgCl2. When the length of the PCR product exceeded 250 base pairs, as with exons 5 (271 bp) and 8 (258 bp), the product was split with a restriction enzyme (Pvu II and Hinfl respectively). The product(s) obtained were electrophoresed on 8% polyacrylamide gel containing 5% glycerol, and autoradiographed. Cell lines with known mutations were used as controls: i.e. SKBR3 cells for Exons 5; T47D and EVSA-T cells for Exons 6; EVSA-T cells for Exon 7; and HT-29 cells for Exon 8. For Exons 9, cells with a known mutation were not available.

Results. Thus far six malignomas could be tested by this extensive method for p53 analysis. The control cells clearly showed the presence of mutations. None of the malignomas specimens, however, showed a deviation from the normal pattern.

Preliminary conclusion. If p53 aberrations occur in human malignomas, their incidence must be low. Consequently, any such aberrations are not related to the presence of progesterin receptors.

P2.078

G PROTEIN MUTATIONS IN THYROID NEOPLASIA.
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G proteins have a central role in the transduction of extracellular signals via the TSH receptor to intracellular adenylyl cyclase. Abnormalities in G protein function can be expected to result in major changes in thyroid cell function and growth. We have studied the prevalence of activating mutations of Gsa (gap) and G12α (gip) in thyroid neoplasias from Boston, USA (n=41) and Newcastle, UK (n=66). Tumour DNA was amplified by PCR and examined for gap and gip mutations using oligonucleotide-specific hybridisation. An invasive papillary carcinoma (USA), demonstrated a gip mutation at codon 205 (glutamine to arginine). A follicular adenoma (UK) demonstrated a gap mutation at codon 179 (arginine to cysteine) and a Hürthle cell adenoma (USA) demonstrated a gap mutation at codon 201 (arginine to cysteine). These data suggest that although activating mutations of Gsa and G12α are uncommon in thyroid neoplasia, they may have oncogenic roles in some tumours.

P2.080

NON-RADIOACTIVE SCREENING FOR MUTATIONS IN MEN 2A FAMILIES.
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*Department of Surgery,** Institute for Hormone and Fertility Research, University Hamburg, Germany.

Recently, non-conservative amino acid mutations of the ret proto-oncogene have been reported in more than 90% of MEN 2A families but not in control individuals. The mutations convert cystein residues of the extracellular cystein-rich domain of the receptor tyrosine kinase. We implemented a non-radioactive procedure to screen the affected exons of the ret gene for mutations. DNA-fragments (156bp) obtained by PCR were subjected to single stranded conformational polymorphism analysis. A shift in the migration of the ssDNA indicated a mutation. To assess whether the mutation leads to an amino acid exchange, the amplified DNA-fragments were subjected to dideoxy sequencing utilizing a digoxigenin as a non-radioactive label. The products of the sequencing reaction were separated in a direct blotting electrophoresis system and detected on the nylon membrane by chemiluminescence after incubation with phosphatase labeled digoxigenin antibody. The complete analysis can be performed within 24 hours. 30 members of 4 MEN 2A families have been tested by this method. In all 4 families mutations in the same codon (codon 380, cyto) were detected causing different specific amino acid substitutions in every family. 12 individuals who were already clinically identified as MEN 2A patients had the mutation in codon 380. A 5 year old clinically unaffected boy was identified as gene carrier. This non-radioactive method for the detection of mutations in the ret proto-oncogene is a rapid and safe procedure to identify individuals being at risk for MEN 2A.
P2.081
GROWTH FACTOR RECEPTORS CD30 AND NGFR ARE EXPRESSED IN NORMAL AND NEOPLASTIC ENDOTHELIA
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Based on the casual observation of a coactivity for Ki-1 of capillary endothelia in lymphomatoid papulosis and non-specific adenitis, this study is concerned with the expression of the CD30 antigen in reactive and neoplastic lesions of endothelial origin and a possible correlation with the expression of nerve growth factor receptor, another member of the same growth receptor superfamily.
A total number of 120 benign and malignant vascular tumors as well as reactive capillary proliferations was examined with the monoclonal antibodies BerH2 and ME20.4. Additionally, a panel of endothelium-specific markers was used in order to verify the endothelial origin of the lesions. Immunohistochemistry was performed on paraffin sections using the APAAP technique.
A reactivity with both antibodies was observed in at least 50% of benign and malignant vascular neoplasms, and to a lesser extent in reactive lesions and native endothelium. As a rule, only a subset of endothelial cells, ranging from 10 to 70%, was labeled. The distribution pattern was not specific, and the expression of the antigens was not correlated. Although a positive reaction was more frequently noted in malignancy, the difference was not significant. Interestingly, the cells of Kaposi's sarcoma and endothelium of telangiectatic granulomas never reacted with either antibody.
We conclude that endothelium can express either CD30 or NGFR, and that the presence of these antigens is not linked to a particular differentiation. They possibly reflect states of cellular activation, and may act as ligands for a number of yet unidentified growth factors, which might play a role in cellular growth and maturation.

P2.082
ISOLATION AND CHARACTERIZATION OF A LYMPHOCYTA PROLIFERATION ACTIVATING PEPTIDE (LPAP) FROM HUMAN BLOOD FILTRATE
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1 = Lower Saxony Institute of Peptide Research, Foxdor Lynxen Str. 31, D-30625 Hannover (FRG)
2 = Centro de Microscopía Electrónica, Universidad Nacional de Córdoba, RA-5000 Córdoba (Argentina).
Nh2-Node lymphoma cells react on stimulation by lactogenic substances like prolactin by an increased proliferation rate. Our attempt was to isolate lactogenic compounds from human blood filtrate with strong stimulatory activity on this cell line.
Peptide-extraction was carried out using 1000 ml of human hematofite (HF). Peptides were adsorbed to aminic acid, the peptide concentrate was then chromatographed on reversed-phase C8-HPLC. Proliferative activity in the fractions obtained was assayed with Nh2 lymphoma cells and cell proliferation was quantified with a cell counter. Active fractions were processed over a series of 7 successive RP-HPLC steps resulting in a pure fraction as judged by capillary zone electrophoresis. Mass-spectrometry, amino acid analysis, and amino acid sequencing revealed the structure of a blood-derived peptide of 13.8 kD called lymphocytoma proliferation activating peptide (LPAP) highly related to interleukin and cima cell protein. The proliferation induced by LPAP by far exceeds the stimulation caused by prolactin and fetal calf serum.
The proliferative activity of this peptide on Nh2 cells represents an unknown biological activity until date. With a further improved isolation technique we are able to isolate sufficient amounts of LPAP in a two-step procedure from HF as a major source for future studies regarding its biological relevance.

P2.083
BLOOD LEVELS OF CATECHOLAMINES AND CORTICOSTEROIDE IN RATS AFTER PARTIAL HEPATECTOMY
J. Knopf, R. Krivan, D. Jureckova, M. Ruznak, I. Rajeckova
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Adrenal hormones are probably not essential for the process of liver regeneration but the onset of regenerative process is facilitated in their presence.
In conscious rats following partial hepatectomy (PH) two waves of increase of plasma levels of norepinephrine (NE), while only one peak of epinephrine (EPI) or corticosterone was recorded. The first peak of NE increase was significantly higher in both PH and sham operated rats. The second rise seems to be specific for partial hepatectomy. During the regenerative phase (period of 24 h) the increased levels of NE were found. In the rats exposed to immobilization stress for 20 min 24 h after partial hepatectomy or sham operation no increase of plasma levels of NE was recorded. Plasma levels of EPI and corticosterone were increased after sham operation, but their rise was more pronounced after PH and still elevated 24 h after PH in comparison with sham operated rats. In both, the PH and sham operated rats immobilization caused a similar increase of plasma levels of EPI and corticosterone.
The results show that in conscious rats following partial hepatectomy the increased plasma level of NE might be accounted for its role of comitogenic factor in rat liver regeneration. The results indicate that the liver containing both the parench and the daughter hepatocytes in rat after immobilization possessed a similar sensitivity regarding to plasma levels of catecholamines.

P2.084
POSTSECRETORY DOWNREGULATION OF ACTIVE TRANSFORMING GROWTH-FACTOR-ß (TGFß) IN ISCHEMIC/DIABETIC RETINAL ANGIOGENESIS IN MAN
A. Pfeiffer, J. Spranger, R. Meyer-Schwickerath, M. Klein, H. Schatz, Med. Klinik Bergmannsheil, Ruhr University, Bochum, Germany.
Chronic ischemia triggers new vessel outgrowth mediated by release of angiogenic factors or a reduction of inhibitory factors. The eye allows precise grading of angiogenesis. We studied the role of TGFß which is well measurable in the vitreous.
METHODS: TGFß1 + 2 was measured by TGFß-antibody reversible inhibition of CCL64-cell growth. TGFß was measured by elisa, in human vitreous obtained after vitrectomy or enucleation. "Labile" macromolecular bound biologically inactive TGFß was activated by acidification prior to assays.
RESULTS: Biologically active TGFß1 + 2 was decreased to 47 ± 8 pg/ml (n = 8) in massive angiogenesis due to ischemia compared 382 ± 73 pg/ml in controls (n = 9, p < 0.01) or proliferative diabetic retinopathy (269 ± 67 pg/ml, n = 11). Total TGFß1 + 2 was 4.8 ± 0.65 ± 0.3 ng/ml in all groups. Total TGFß1 + 2 was decreased in ischemia to 1.0 ± 0.4 ng/ml (n = 7, p < 0.025) compared to 3.3 ± 0.3 ng/ml in controls (n = 11) or 4.2 ± 0.4 ng/ml (n = 17) in diabetes. CONCLUSION: In controls TGFß1 + 2 was the dominant type of TGFß in the vitreous. Ischemia appears to trigger a balanced increase in TGFß and a decrease in TGFß-production which are released in the labile or inactive form. The massive downregulation of biologically active TGFß to 12 % of control levels suggests a postsecretory mechanism of regulation and fits with an inhibitory function of TGFß on angiogenesis.
P2.085

GERM-_LINE MUTATION IN EXON 11 OF THE RET PROTO-ONCOGENE IN MEN IIA

*Ambrosch A., **Hainzl A., *Fliedner T., **Gehler T., **Bayer J., **Maulik L.M., **Farkas B.A., *Lehbett H., **E. Medizinische Klinik, Endokrinologie, Universität Mainz, FRG; **Institut für Humangenetik, Universität Mainz, FRG; **Cancer Research Center, Department of Endocrinology, University of Cambridge, UK.

Multiple endocrine neoplasia IIa is a dominant inherited endocrine cancer syndrome, that is characterized by medullary thyroid carcinoma (MTC), phaeochromocytoma and hyperparathyroidism. Recently L.M. Maulik et al. (1) found germ-line missense mutations in the RET proto-oncogene, a receptor tyrosine kinase gene expressed in medullary thyroid cancer and phaeochromocytoma. Missense mutations in distinct MEN IIA families but not in sporadic MTC, phaeochromocytoma or normal controls were described.

Material: EDTA-blood samples from distinct MEN IIA families and from normal controls were collected. Method: First, patient’s DNA from 5 ml lytic of peripheral blood cells was isolated by standard techniques. We performed a PCR with the primers CRT19A (5′AAGCTTGAAGGACCCTCCGCTGCTTG 3′) and CRT19B (5′GATACGGAGGCTCGTATCC 3′) that amplified a 310 base-pair region of exon 11 encoding a transmembrane domain of the tyrosine kinase in chromosome 10q11.2. After gel- and column-purification of the amplified fragment cycle sequencing to detect RET missense mutations was used. Results: In two distinct families with MEN IIA a heterozygous missense mutation in codon 380 (base pair 1829, TGC→TTC, Cys→Phe and TGC→TAC, Cys→ Tyr) and in normal controls was found. Conclusion: These data confirm the suggestion that replacement of codon Cys 634 residue may disrupt normal RET protein conformation, possibly altering RET signal transduction and leading to MEN phenotype. We now have a genetic tool for family screening and early discrimination of MEN IIA genotypes. 1. Maulik et al., Nature 363:458-460 (1993).

P2.086

RISK OF COLONIC CARCINOMA IN ACREMAGELIC PATIENTS: EVALUATION BY ENDOSCOPIC SCREENING AND IMMUNOHISTOCHEMISTRY

A. Rozencz, M. Terszad, E. Leonardi, G. Tapper, S. Cappia, A. Pia, G. Rainoldi, P. Pasotti, G. Baretta, A. Augeri, Department of Clinical and Biological Sciences, Chiusa of Internal Medicine, G.Secretary of Pathology, S. Luigi Hospital, University of Turin and Temoendocrinology, 3 Cross Hospital, Chiusa, Italy.

Full colonoscopy was performed employing a flexible colonoscopy in 31 acromegalic patients (pts), 11 M and 20 F aged 27-45 years (mean: 52.2), and in 236 controls, 127 M and 109 F aged 23-84 years (mean: 50.1), referred for hormonal abnormalities, who were considered as controls. The prevalence of colorectal adenomas or hyperplastic polyyps was higher in acromegalics than in controls (38% vs. 26% 10% p<0.01), respectively) but the prevalence of carcinoma was superporable (5% vs. 29%). No significant difference was found between acromegalics with or without colorectal adenomas as for clinical and hormonal data, albeit pts with adenoma were younger (median: 50.5 years, range: 27.5-59 years, 39-66 p<0.05). As opposed age pattern was observed in the control group, thus the cumulative prevalence of adenomas yielded significant differences only in the <30 year age group (60% in acromegalics vs 7% in controls, p<0.01).

Expression of the nodular antigen Ki-67, a proliferative index, and of p53 protein, a cell cycle regulatory protein implicated in colorectal tumorigenesis, was studied immunohistochemically using specific monoclonal antibodies and the avidin-biotin-peroxidase complex technique. 15 colorectal adenomas and 19 hyperplastic polyyps from 11 acromegalics were analyzed and compared with a corresponding set of polyyps from 20 age-matched non-acromegalic subjects. The percentage of Ki-67 stained cells was significantly lower in colorectal adenomas of acromegalics than in controls (14%, 8.30 vs. 25%, 13.5, p<0.05). The p53 immunostaining was observed in 51/5 (33%) adenomas of acromegalics as compared to 14/15 (95%) of controls (p<0.05). Also the number of p53 positive nuclei in any section was significantly lower in the acromegalic group (p<0.01). In conclusion, acromegaly carries an increased risk of adenomas polyyps, especially in younger pts, who usually display a more aggressive disease. Immunohistochemical patterns, on the other hand, suggest that colorectal adenomas of acromegalics have a low potential for malignant progression. These findings may help to explain the discrepancy between the prevalence of adenoma and that of carcinomas in the face of early occurrence of adenomas and relatively long life expectancy of young acromegalics.

P2.087

BOMBESIN PLASMA AND TISSUE LEVELS IN PATIENTS WITH BENIGN AND MALIGNANT BREAST DISEASE

A. Miletic, J. Drozdzewski, D. Jędzzejuk, W. Biedenik, Department of Endocrinology, Medical Academy, Wroclaw, Poland.

Bombesin (BN) is a peptide involved in development of some malignant lesions. We have shown previously, that BN plasma levels in breast cancer patients is higher in samples taken from vicinity of the tumor (thoracodorus vein-TDV) than in peripheral circulation.

42 patients undergoing surgery for breast cancer and 63 women with BBD were included into the study. 22 healthy women formed a control group.

Levels of BN, E2, progesterone, prolactin and PRL were measured in peripheral blood, and in the cancer group also in TDV by means of RIA. Tissue samples for BN estimation were weighed, sliced, dehydrated in acetic acid and incubated in 10°C. Than samples were homogenized and ultracentrifuged. BN concentration were measured in supernatants using RIA. BN levels in cancer tissue samples did not differ from those in BBD samples (2.85+1.6 ng/ml and 5.07+2.14 ng/ml respectively). BN level in peripheral blood was 9.241+29.09 pg/ml and was significantly lower than in plasma from TDV (136.4+41.45 pg/ml, p<0.001). It was also lower than in BBD subjects (104.77+57 pg/ml, p<0.001).

Basing on above results the initial hypothesis that BN may be a marker for breast cancer may be excluded.

P2.088

STRESS HORMONES IN ANOREXIA NERVOSA: Correlation with BMI and percent weight loss.

Rob D., Jorge Z., Cerno I., Galvíos-Teles A.

Endocrine Unit. Santa Maria University Hospital. Lisbon. Portugal.

Starvation alone cannot explain all of the alterations of the hormonal profile in Anorexia Nervosa (AN). There are other factors, including the severity of the illness and the stress, that contribute to the hormonal disturbances. The aim of this study was to investigate the effect of weight reduction on the stress hormones ( cortisol (PRL), growth hormone (GH) and cortisol (C) in AN correlation with BMI and the percent weight loss (%WL) with these hormones.

Thirty-six individuals with AN diagnosed under the DSM-IV criteria 3.5 (9.7% 2) girls and 2.8% of boys aged 17-23 years (range 27-23 years) were included. Serum PRL, cortisol, GH, and testosterone were determined by RIA methods. A standard TRH test was performed (200 μg TRH IV) for PRL determination at 0, 30, 60 and 90 min in 18 (50.9%) subjects. An insulin hypoglycemia test (IHT, 0.15 U/kg short-acting insulin IV) for GH and C determinations at times 0, 30, 60 and 90 min was performed in 15 (56.6%) and 20 (41.7) subjects, respectively. The total and the incremental areas under the curves (IAUC and IAUC for PRL. GH and C responses to stimulations tests were approximated with the trapazoidal rule. Linear correlations were calculated according to the method of Pearson.

The results are shown in the table:

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>PRL (ng/ml)</th>
<th>GH (ng/ml)</th>
<th>C (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (mean ± SEM)</td>
<td>12.4 ± 2.29</td>
<td>3.77 ± 0.46</td>
<td>19.07 ± 1.05</td>
</tr>
<tr>
<td>Shy, with high basal level</td>
<td>5.15 ± 2.89</td>
<td>2.87 ± 0.45</td>
<td>5.10 ± 2.99</td>
</tr>
<tr>
<td>AUIC (mean ± SEM)</td>
<td>15.4 ± 3.08</td>
<td>7.29 ± 4.16</td>
<td>5.53 ± 3.27</td>
</tr>
<tr>
<td>IAUC (mean ± SEM)</td>
<td>9.40 ± 1.12</td>
<td>16.35 ± 3.10</td>
<td>5.64 ± 3.03</td>
</tr>
</tbody>
</table>

The PRL response to TRH test was normal in 14 and delayed in 4 subjects, with a peak response occurring after 60 min. The GH response to IHT was normal in 10, delayed in 2 with a peak response occurring after 60 min and absent in 3 subjects. The C response to IHT was normal in 13 and absent in 7 subjects. There was no significant correlation between the IAUC for PRL, GH or C and BMI or WL.

Conclusions: 1. The majority of the patients studied with AN had normal basal levels of stress hormones. 2. An important percentage of the group studied had high basal concentrations of GH, more frequently than another stress hormones. 3. When stimulations tests were performed we found an abnormal response of PRL in 22.9% of of GH in 33.3% and of C in 33.3% of patients.
THE EFFECT OF CYPROTERONE ACETATE, ETHINYL ESTRADIOL THERAPY ON PITUITARY RESPONSE TO EXOCYTIC CRH

Nicola R. Coopits, M.D.; Gabriella Soutier; Ph.D., Gigante Clinic-Endocrine Unit, Taranto, Italy.

Cyproterone acetate (CPA) in combination with ethinyl estradiol is a widely used therapy for hirsutism in females. CPA has glucocorticoid-like properties, tested mostly in animals. To determine the possibility of pituitary-adrenal function suppression in patients treated with CPA-ethinyl estradiol therapy, we tested the pituitary response to the administration of exogenous corticotropin-releasing hormone (CRH).

We studied 18 hirsute female patients (age range: 8-25). Ferriman and Gallway score range: 20-32) receiving daily therapy with 0.03 mg of ethinyl estradiol from day 3 to day 23 of the menstrual cycle and 50 mg (patients 1-10) or 100 mg (patients 11-18) of CPA from day 3 to day 25 of the menstrual cycle. Simulation tests (using 0.1 mg of CRH (Corinichia Bischof) for a bolus injection, were performed in the morning (between 8:00 and 9:00 am). All patients were tested twice before starting therapy in mid follicular phase, and six months later during therapy on day 13 of the menstrual cycle, 10 hours after the last dose of CPA.

Before therapy all patients had a normal corticotropin response (Alpegro immunoradiometric assay, Nichols Institute) to exogenous CRH (The mean plasma corticotropin concentration increased from 5.8 ± 1.4 pmol/L to a maximum of 11.9 ± 6.6 pmol/L after CRH).

During therapy 4 patients had a normal corticotropin response to CRH and 12 patients had a blunted response. In the blunted response group of patients the mean plasma corticotropin concentration increased from 3.9 ± 1.5 pmol/L to a maximum of 6.5 ± 5.5 pmol/L after CRH.

All patients receiving the higher dose of CPA (100 mg per day) were in the blunted response group. The difference between basal and peak corticotropin concentration was statistically significant (P < 0.001) in all patients. These results support the existence of a glucocorticoid-like activity of cyproterone acetate in humans.

The degree of glucocorticoid-like activity seems to be dose-dependent. Further work will be necessary to determine the clinical relevance of our findings.

THE IMPORTANCE OF EARLY DIAGNOSIS IN MEDULLARY THYROID CARCINOMA

A. Libroso, G. Di Sacco, A. Grassi, U. Verga and F. Muratori, Department of Endocrinology, Niguarda Hospital, Milan, Italy.

Early diagnosis and surgical treatment of familial medullary thyroid carcinoma (MTC) is essential to decrease the likelihood of metastatic spread. In such situations, the presence of a family history of MTC is a valuable diagnostic guide.

In this study, we considered five subjects (aged 10 to 45) from three MEN 2A families treated for 1 to 4 years. Their CT scans (for 10 to 20 days) were reviewed.

The results demonstrated that the presence of hyperplasia in the parathyroid gland was not a reliable indicator of MEN 2A. A more comprehensive approach to the diagnosis of MEN 2A is required.

A NEW SCINTIGRAPHY IN THE DIAGNOSIS OF MEDULLARY THYROID CARCINOMA RECURRENCE: OCTREOSCAN

A. Libroso, U. Verga, F. Muratori, G. Di Sacco, A. Grassi, G. Vecchietti, M. Pessa*, F. Barba* Dept of Endocrinology, I Dep of Radiology, Dept of Nuclear Medicine, Niguarda Hospital, Milan, Italy.

Twelve patients with recurrent medullary thyroid carcinoma (MTC) (previous total thyroidectomy) were studied. All patients underwent a pentagastrin scan (octreoscan) and we compared our results with conventional imaging techniques (ultrasonography (US), computed axial tomography (CAT), magnetic resonance imaging (MRI), 131I MIBG and 99mTc (V) DMSA scintigraphy).

The results showed that the presence of C cell hyperplasia was in all cases associated with a positive octreoscan scan. The presence of C cell hyperplasia was not detected in the 3 patients with a negative octreoscan scan.

These data suggest that the octreoscan scan is a valuable tool in the detection of MTC recurrences.
P2.093

**EFFECT OPERATIVE WEIGHT REDUCTION ON HYPOTHALAMO-PITUITARY-ADRENAL, GONADAL AND THYROID AXES FUNCTION IN OBESE WOMEN WITH THE DISTURBANCES OF MENSTRUAL CYCLES**

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There are many conflicting reports in literature concerning hormone secretion in morbid obesity and after operative weight reduction. The aim of the study was to evaluate of GH, LH, PRL, TSH, FSH, insulin, sex steroid hormones, thyroid hormones SHBG, IGF-I, as well as the areas under the curve for GH, PRL and cortisol in 21 obese women, and in 18 women with mild obesity who had undergone jejunoileostomy. Disturbances in GH, PRL and cortisol release appear in both non-operated women. Upper body is associated with disturbances in gonadotropin, thyrotropic, sex steroid and thyroid hormones secretion and synthesis of IGF-I and SHBG. Sex hormones excess, higher levels of IGF-I, and lower levels of SHBG may result from hyperinsulinaemia. The consequence of the reduction in body mass after jejunoileostomy is the normalisation of the gonadotropin, thyrotropic, triiodothyronine, sex hormones, SHBG and IGF-I concentrations.

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P2.094

**GENOMIC AND NON-GENOMIC ESTRADIOL-17 BETA EFFECTS IN THE NERVOUS TISSUE**


Intraperitoneal injection of estradiol-17 beta into rat increases the activity of brain regulatory enzymes hexokinase, phosphofructokinase and pyruvate kinase while it has no effect on non-regulatory enzymes (phosphoglycerate dehydrogenase, aldolase and glyceraldehyde-3P-dehydrogenase). The activation was maximal (160-185%) at the 4 h of hormone action and prevented if protein biosynthesis was blocked by actinomycin D, which indicates an action related with de novo biosynthesis mostly in the neurons. This conclusion is confirmed in with neuroblastoma C1300 cell culture where the induction of the activity is more expressed. From the other hand estradiol application to the rat brain synaptosomes suspension brings redistribution of soluble, weakly and firmly membrane-bound fractions of pyruvate kinase (but not enolase). Since this in vitro effect develops within 5-10 min of hormone action we speculate about the existence of short-term non genomic way of estradiol action. Possibly, genomic effects of estradiol is connected with the long term activation through de novo biosynthesis while short-term effects mainly involved enzymes activity redistribution as a possible way of the glycolysis regulation.

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P2.095

**AN EXPERIMENTAL PARADIGM FOR QUANTITATION OF ESTROGEN DEVELOPMENTAL TOXICITY IN THE RAT UTERUS**

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Estrogens are known human and animal developmental toxicants, inducing abnormalities in male and female reproductive tracts and other tissues. There is growing concern regarding the effects of estrogens (including pharmaceuticals, phyoestrogens, pesticides, mycotoxins and industrial chemicals) on the health of humans and wildlife. For several estrogens, we have quantitated dose-response relationships at different developmental stages in the rat uterus using 5 day treatment regimes. Diethylstilbestrol (DES) and ethynylestradiol (EE) but not 17β-estradiol (E2) induce fetal uterine growth in late pregnancy. This is a translational bioassay for estrogens. DES, EE, present, coumestrol (CM), zearalenone (Z-O), zearalanol (Z-OL), ciomione citrate (CC) and tamoxifen (TAM) induce uterine growth. In the neonate, DES and EE are about 100-fold more potent, while Z-ONE, Z-OL and CM are about 10-fold less potent than E2. All have parallel dose-response curves, unlike CC and TAM which are partial in this system. However, CC and TAM inhibit the differentiation of uterine glands as does high dose, longer duration DES treatment, while E2, EE, and lower doses of DES delay gland appearance. Following developmental treatment with DES, adult animals display hypotrophic uteri and lowered estrogen receptor (ER) levels. These studies demonstrate that dose-response relationships among the various estrogens can be endpoint-dependent and are difficult to predict from immature or adult studies. Differences in placental transport, binding to serum proteins, metabolism, developmental processes, ER levels and inherent tissue sensitivity can explain unpredictability. Our system can be used across developmental stages to quantitate the potency and developmental toxicity of the wide variety of estrogens to which humans and wildlife are exposed.
SEX HORMONES AND SERUM WHOLE BLOOD MAGNESIUM AND CALCIUM LEVELS IN POSTMENOPAUSAL WOMEN.

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The influence of sex hormones on magnesium metabolism is still controversial. The serum and full blood levels of magnesium and calcium were estimated by means of atomic spectrophotometry in 21 women aged (40-49) with surgically induced menopause before and after 3-month therapy with conjugated estrogens (Oestro-feminal manufactured by Mck)

The serum calcium levels in menopausal women before institution of the treatment were statistically significantly higher (p=0.05) - 3.58 mmol/l than those during the luteal phase - 3.19 mmol/l, while no differences were found in relation to the follicular phases. The levels of magnesium in menopausal women did not differ from those in women during the luteal and follicular phases

The replacement therapy resulted in reduction of magnesium levels and increase of calcium levels in both serum and whole blood (statistically significantly)

The above observations justify the hormone replacement therapy as a method of prevention against osteoporosis in women with surgically induced menopause.

ESTRONE INTERACTION WITH HUMAN UTERUS

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The present study was undertaken to understand the molecular interaction and mechanism of action of estrone (E1), a less potent estrogen, in human uterus. The uterine myometrial and endometrial tissues were incubated separately with [3H]-estrone, homogenized and centrifuged for separation of nuclear; mitochondrial, microsomal and cytosol fractions. The binding studies were carried out by gel filtration and results confirmed by sucrose density gradient. The metabolism of E1 was seen by TLC. The subcellular distribution showed that major site of localization of E1 was myometrial cytoplasmic fraction, while the maximum uptake in endometrium was seen in mitochondrial + microsomal fraction. E1 gets bound to the endometrial and myometrial cytosol estrogen binding proteins. The capacity of metabolising E1 to E2 of endometrium was found to be 4.6 times higher than that of the myometrium. The local availability of E1 in the human uterus, its binding to receptor proteins and its higher metabolism in endometrium regulate the availability of the more potent estrogen viz. estradiol and thus modulate the action of estrogen in the human uterus.

EFFECTS OF ANTI-ProGESTAGENS DURING THE PERI-OVULATORY PERIOD IN FEMALE RATS

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Progesterone plays an important role in the events which occur during the peri-ovulatory period. Progesterone is involved in initiation and maintenance of the gonadotropin surge, rupture of the follicle wall and transport of ova in the fallopian tube. Antiprogestagens which inhibit the physiological effects of progesterone are excellent tools to investigate the role of progesterone in these processes. We studied the antiprogestagens ORG 31710, ORG 33628 and RU 486 which differ in potency and selectivity. The antiprogestagens were administered orally to female rats at proestrus either before or after the gonadotropin surge. We examined the ovulation and the uterus for the presence of ova. Ovulation was determined by the presence of fresh corpora lutea in the ovariens. We found that all three antiprogestagens, which have been shown to significantly attenuate the gonadotropin surge, inhibit ovulation when administered before the gonadotropin surge. Supplementation with progesterone partially restores normal ovulation. Restoration of normal ovulation was most evident when progesterone was combined with ORG 33628. Administration between the gonadotropin surge and ovulation does not inhibit ovulation but accelerates the transport of ova from oviduct to uterus. This accelerated transport can not be reversed when ORG 31710 and RU 486 treated animals are supplemented with progesterone. The accelerated transport observed after ORG 33628 treatment however, can be significantly reversed with additional progesterone. In a pregnancy test we found that fertilised ova do not implantate in antiprogestagen treated animals. These data suggest that antiprogestagens inhibit ovulation predominantly by means of a reduction of the gonadotropin surge. Furthermore, antiprogestagens accelerate the transport of ova from the oviduct to the uterus. Reversal of these antiprogestagenic effects after progesterone supplementation is most evident after treatment with ORG 33628. This indicates that the mode of action of ORG 33628 is not completely comparable to that of ORG 31710 and RU 486.
**P2.101**

**DISSOCIATION OF LH AND FSH RESPONSES IN CASES WITH HYPERPROLACTINEMIA AND POLYCYSTIC OVARY SYNDROME**

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We conclude that the hypothalamo-hypophyseal dopaminergic tone has been indicated as one of the cause of polycystic ovary syndrome (PCOS). To evaluate this hypothesis 38 women divided in 4 groups a) control group n=10; b) PCO and normal PRL n=12; c) PCO with hyperprolactinemia n=6; d) hyperprolactinemia without PCO n=10. All patients were intravenously administered TSH 400 µg and 10 mg of methostranol, in different days, with extractions being carried out at ±30, 0, 30, 60, 90 and 120 minutes for PRL, FSH, LH, TSH. The results obtained were evaluated from a TADPOLE III informatic program. Basal PRL is greater (p<0.01) in group c) than in a) and b). Basal PRL is greater in group d) than in group a) and b). Basal LH is greater (p<0.05) in group a) and b) (p<0.05). Basal LH is greater in group b) than in group a) and group c) than in group a) (p<0.05). No significant differences were obtained for any of the parameters among the different groups although the different behavior of the maximum absolute increase and maximum percentage increase of LH and PRL in the case of hyperprolactinemia (dopaminergic antagonism).

We conclude that there is no decrease of the hypothalamo-hypophyseal dopaminergic tone in any of the PCO subgroups.

**P2.102**

**VARIOUS ADRENAL ENZYMIC DEFICIENCIES IN POLYCYSTIC OVARY SYNDROME (PCO)**


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Considering the high prevalence in our population of PCO, we investigate the presence of mild errors in steroid biosynthesis in 150 untreated hyperandrogenic Caucasian women (aged 17-28 yr) from South Italy, compared to 50 normal women age-matched. Morning blood samples were obtained in precocious follicular phase, in basal condition and after ACTH (1-17 alactacto) stimulation test. F, S, 17OHP, T, DHEA, DHEAS and D4 plasma levels were assayed by RIA and the adrenal response evaluated as Stimulus Under Curve Areas (AUC) calculation. Enzymatic defects in steroidogenesis were diagnosed by S, DHEA and 17OHP AUC > of mean + 2 SD. A non-classic 21-hydroxylase deficiency (NC210HDH) was found in 22 patients (170HP AUC: 5244 ± 1322 ng/dl*6 h), mild 11-hydroxylase deficiency (11OHD) in 6 (S AUC: 4236 ± 870 ng/dl*6 h), mild 3o-ol-dehydrogenase deficiency (3BDDH) in 2 (DHEA AUC: 157 ± 21 ng/ml*6h), while 122 hyperandrogenic women comprise the non-adrenal hyperandrogenic population (NONADRENAL).

**P2.103**

**EXERCISE-INDUCED HORMONE RESPONSES IN GIRLS IN THE COURSE OF SEXUAL MATURATION: A THREE-YEAR FOLLOW-UP STUDY**


The aim of the study was to test the dependence of exercise-induced hormone responses on sexual maturation in girls. 34 girls (initial age 11-14) were studied during a three year period. Venous blood samples were taken for RIA determination of cortisol, insulin, somatotropin, 8-estradiol, progesterone and testosterone before and after 20-min cycling exercise. Sexual maturation was evaluated by bone age, Tanner's maturation stages and menarche. The exercise caused a pronounced increase in cortisol and a decrease in insulin blood levels. Somatotropin level was highest in maturation stage 1-2 when the growth spurt took place, low, maximum level of estradiol (maturation stage 0-1) associated with the increase, and high estradiol level (stage 2-3, bone age 12-14) with decrease of the hormone level in exercise. In advanced maturation significant changes were not found. The basal progesterone level increased and testosterone became detectable with maturation. Exercise responses of both hormones were insignificant.

**P2.104**

**GONADOTROPHIC FUNCTION AND STARVATION IN ANOREXIA NERVOSA**

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Anorexia is one of the symptoms required for the diagnosis of Anorexia Nervosa (AN) in young females, according to the DSM III criteria. Low levels of gonadotropins are well documented in females and males with AN. However, weight loss alone does not explain low levels of gonadotropins and amenorrhea, once this one occasionally precedes weight loss and goes on after weight gain.

Our aim was to study correlation between basal LH or FSH levels and Body Mass Index (BMI) or percent weight loss (% W.L.). Correlation of BMI and % W.L with LH and FSH responses to LH-RH stimulation were also analyzed.

**Population:** Fourty five patients with AN, according to DSM III R criteria (43 girls [95.6%] and 2 boys [4.4%]) aged 17.33 ± 2.33, BMI 15.20 ± 2.43, % W.L. 32.93 ± 12.27.

**Methods:** A standard LH-RH test was performed (100 µg LH-RH i.v.) for LH and FSH determination at 0, 30, 60 and 90 min in 27 (60%) subjects. The total and the incremental area under the curves (AUC) for the serum LH and FSH responses to LH-RH was approximated with the trapezoid rule. Analysis was performed with linear correlation of Pearson method.

**Results:** 1. The LH and FSH responses to LH-RH stimulation were statistically significant (LH: p < 0.04. FSH: p < 0.001). 2. A positive significant correlation was found between BMI and basal LH values (r = 0.58, p < 0.007). 3. A negative significant correlation was found between % W.L and basal LH values (r = 0.37, p < 0.02). 4. A positive significant correlation was found between BMI and basal serum FSH values (r = 0.38, p < 0.05). 5. No correlations were found between % W.L and basal FSH values (r = 0.24; pNS). 6. BMI, % W.L and IACU of LH. FSH.

**Conclusion:** Starvation interferes with basal levels of gonadotropins. However, pulsatory responses to stimulation is not correlated with starvation. It seems that there is a hypothalamic dysfunction rather than starvation factors.
P2.105

RELATIONSHIP BETWEEN SEX HORMONE BINDING GLOBULIN AND BODY WEIGHT IN WOMEN WITH ANOREXIA NERVOSA

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The data reported concerning the serum levels of sex hormone binding globulin (SHBG) in women with anorexia nervosa are insufficient and controversial. It is the purpose of the present study to gain better insight into this problem.

We have evaluated serum levels of SHBG, oestradiol, testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in 19 women with anorexia nervosa. The diagnosis was established according to the criteria of Feingold et al. (1972).

Clinically and biochemically the patients were euthyroid. Seven practically healthy age-matched women with normal weight and regular menstrual cycles served as controls. All hormonal levels were measured by time-resolved fluoroimmunoassay.

The serum SHBG concentrations in patients with anorexia nervosa (165.27 ± 63.57 nmol/L, X ± SD) were significantly higher (p<0.05) than those in the control group (96.21 ± 14.38 nmol/L, X ± SD), but the levels of oestradiol were significantly lower in the patients (0.089 ± 0.06 vs. 0.186 ± 0.17 nmol/L, p<0.05). In the anorectic women the ratio of testosterone to SHBG was also significantly lower (p<0.02). Correlation between SHBG and body weight (r = 0.761) was found out in the patients with anorexia nervosa. The alterations in SHBG concentrations were not associated with the changes in testosterone, LH, FSH, free triiodothyronine and free thyroxine levels. The findings from this study suggest that the body weight might influence the SHBG level, which could be used as a reliable diagnostic index of nutritional status in the disease discussed.

P2.106

THE INFLUENCE OF THE DIABETES ON ESTROGEN METABOLISM IN FEMALE RATS

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The estrogen biotransformation in the interconnection with the estrogen status in female diabetic rats was studied. The animals with the alloxan diabetes, and control ones were injected with 3H-estradiol. The analysis of the glucuronides, sulphates and unconjugated estradiol metabolites in urine fractions was carried out by means of the enzyme hydrolysis. The uterus weights were considered as an indicator of the estrogen status. Our results revealed an almost 2-fold increase of the total estradiol metabolites excretion in diabetic animals. The data indicated a perfect concordance between the volume of excretion of the glucuronide (4.0-fold increase) and total estradiol metabolites. Additionally, the uterus weights correlate significantly with changes in estrogen metabolism and glucose plasma levels. In conclusion, these findings suggest that the diabetic hyperglycemia may be one of the most important factor of this phenomenon.

P2.107

DEFORMABILITY OF RED BLOOD CELLS IN POSTMENOPAUSAL WOMEN

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It is well established that decline in the estrogen production after menopause is associated with the development of the ischemic heart disease (IHD). One of the potential risk factors in IHD is deterioration of the blood rheology, especially the reduction of the deformability of the red blood cells (RBC). Deformability means the ability of the RBC to change their shape without changes in the cytoplasm volume. Some studies indicate that RBC deformability may depend upon cytosolic calcium content. On the other hand, it has been reported that RBC cytosolic calcium concentration rapidly declines after castration and may be restored by the estrogen treatment.

The aim of the present study was to evaluate the deformability of the RBC in women either after spontaneous or surgical menopause and to determine its relation to the hormonal findings. Deformability of the RBC was investigated according to time required to complete filtration of the whole blood through Nucleopore filters of 5 μm, taking into account the hematocrit value. Serum estradiol, LH and FSH levels were measured with ELA method (BioMerieux).

The results are as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mean)</th>
<th>n</th>
<th>RBC deformability index (mean ± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Spontaneous menopause</td>
<td>47.60 (53.3)</td>
<td>24</td>
<td>0.042 ± 0.016</td>
</tr>
<tr>
<td>B</td>
<td>Surgical menopause (0 - 6 months after operation)</td>
<td>45.58 (50.9)</td>
<td>17</td>
<td>0.014 ± 0.009</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>19.42 (38.4)</td>
<td>68</td>
<td>0.054 ± 0.011</td>
</tr>
</tbody>
</table>

Conclusions: 1. Deformability of the RBC in both groups of menopausal women was significantly decreased in comparison with the control group.

2. The lowest deformability index was observed in women after surgical castration.

P2.108

INVESTIGATION OF THE DISCREPANCY BETWEEN DIRECT AND EXTRACTION OESTRADIOL ASSAY

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Discrepancies have occasionally been reported between oestradiol measured in the same serum by direct and extraction immunoassays. We have investigated the basis for the four fold greater value obtained by direct assay of sera when compared with assay of the same sample after extraction with ether and reconstitution in zero standard, using a commercially available kit (OPL, double antibody).

Multiple sera were obtained from subject (A) taking oestradiol valerate for the relief of menopausal symptoms and subject (B) attending a clinic for assisted conception. Oestradiol valerate did not cross react in this direct assay. Recovery, after extraction and reconstitution, was better than 80% in all cases. Sera after incubation with [3H] labelled tracer containing displacing agent were analysed by G25 sephadex chromatography. More than 94% of the radioactivity in the case of (A) was recovered in a low molecular weight fraction whereas in the case of (B) 44%-63% of the radioactivity was eluted in the void volume corresponding to the serum protein fraction. Sera from (B) was assayed for oestradiol using the direct method and the unbound fraction after separation was also analysed by G25 chromatography. Of the total radioactivity added to the assay 48%-52% was eluted in the void volume.

These results suggest that the discrepancy in situation (A) is due to cross reacting metabolites whereas in (B) the discrepancy can be accounted for by binding of the tracer to serum protein.
The relationship of maternal postpartum glycemia and infant birth weight in patients with gestational diabetes mellitus

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We have studied the relationship of fasting blood glucose and postpartal/one hour after meal/blood glucose levels with infant birth weight in 16 patients with gestational diabetes mellitus/GDM/. The diagnostic criteria for GDM were those of O'Sullivan-Mah. The characteristics of study patients are: mean ± SD: age 32±0.6 yr; maternal weight 82±15.3 kg; fasting blood glucose 5.2±0.78 mmol/l; postprandial glucose 7.5±1.06 mmol/l. Delivery details /mean ± SD/ gestational age 39.7±1.5 wks; infant birth weight - 3970±630 grms. The caesarean section was performed on 31.25% of patients and vaginal delivery in 68.75%, respectively. The mean postprandial blood glucose levels correlated significantly with infant birth weight /p<0.001/. The mean fasting blood glucose levels did not correlate with infant birth weight /p = 0.206/. In conclusion, the postprandial glycemia significantly influences the fetal growth and neonatal size in patients with GDM.
COORDINATE GENETIC CONTROL OF
MICROSOMAL STEROIDOGENIC ENZYME
ACTIVITIES IN MOUSE LEYDIG CELLS

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In previous experiments with inbred mice, we found
the genetic variations in plasma testosterone to be of
very high adaptive value, and positively correlated with
reproductive success and social dominance. This
interstrain diversity can serve as a good model for
studying the genetic control of testicular steroidogenesis
and clarifying its key determinants. Factors for this
purpose, interstrain differences in CAMP-dependent
signaling pathway of tesntosterone production in
micr0somal steroidogenic enzyme activities in Perchlor-
purified Leydig cells between six inbred mouse strains
(All, A$c, C57B1/6J, DD, YT and PT) were studied.
Testosterone content in incubation medium was measured
by RIA. The enzyme activities were assayed using
micropreparative HPLC.

Significant interstrain differences were found in the
testosterone productions in response to increasing
concentrations of hCG and d, b-CAMP. There were
significant positive interstrain correlations between
maximal testosterone productions in response to the
stimulants. The results suggest that the inherited
differences in responsiveness of Leydig cells may be due
to biochemical events distal with respect to stimulation of
CAMP production. Significant positive interstrain
differences in Δ5-3β-hydroxysteroid dehydrogenase-isomerase,
17α-hydroxylase, C17-20-Iyase and 17-ketosteroid
reductase activities were demonstrated. Significant
positive interstrain correlations between the activities
of micr0somal steroidogenic enzymes were established.
It was concluded that the activities of micr0somal
steroidogenic enzymes were under coordinate genetic
control and correlated with maximal testosterone
production.

P2.116
NORMAL HUMAN TESTIS AS A SOURCE OF HUMAN CHORIONIC GONADOTROPIN β (HCGB) AND HCGβ-LIKE MOLECULES

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The α/β-heterodimeric HCG and its free subunits are
synthesized in the developing placenta. Moreover, they
serve as well established tumor markers for testicular
carcinoma. Recently, however, low levels of HCGα, HCGβ
and hko-HCG were also found in the sera of non-pregnant,
healthy individuals and were expected to be of pituitary origin.
Analyses of serum, urine and hydrocele fluids of human
testes by ultrasensitive highly specific time-resolved
fluoroimmunoassays for hCGβ revealed that this molecule is
found at higher concentrations in hydrocele fluid than in
serum (<5 pg vs. 400 pg/ml). This prompted us to
investigate a putative testicular origin of hCGβ at both
the protein and mRNA level. HCGβ could be immunologically
detected in minute amounts (approx. 100 pg/mg tissue wet
weight) in cytosolic extracts of normal human testes obtained
from men undergoing orchidectomy for previously untreated
prostatic cancer. Final proof for the testicular production of
hCGβ has been achieved by reverse transcriptase polymerase
chain reaction (PCR) with primers specific for products of
hCGβ genes1&2 (yielding three alternative splicing
products) and hCGβ genes 3, 5, 7, & 8, PCR-product specificity
was verified by Southern-hybridization. We conclude that,
in addition to its role, hormonal action during pregnancy,
hCG-like molecules are eutypically produced in the human
testis pointing to possible autocrine or paracrine functions.
Supported by the Tuba-Foundation, Innsbruck, Austria.
THE EFFECT OF A PURE ANTIANDROGEN RECEPTOR BLOCKER ON LIPID METABOLISM IN POLYCYSTIC OVARY SYNDROME

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Androgens influence adipose tissue metabolism in women with polycystic ovary syndrome (PCO). It is not known whether the effect of androgens on lipid profile in PCO is mediated through direct effects on specific androgen receptor or indirect through other mechanisms, i.e., enhancing catecholamine lipolysis or altering insulin sensitivity. The main aim of this study is to assess the long-term effect of a pure androgen receptor blocker, Flutamide, on lipid profile in PCO. Twelve women with PCO aged 18-28 years, BMI: 29.6±1.2, WHIR: 0.83±0.02, received Flutamide 500 mg daily, for 12 weeks. The following were determined before and after treatment: 1) Androgen levels, 2) Insulin sensitivity index (SI), as assessed by the euglycemic hyperinsulinemic (70µU/ml) clamp technique, 3) Catecholamine plasma levels, before and after three hours of euglycemic clamp, 4) Baseline lipid profile: cholesterol (CH), Triglycerides (TG), HDL, and LDL. Results: BMI and WHIR did not change after the treatment. 1) Testosterone levels were above normal range and showed no change. There was a significant decrease in Androstenedione and 3α-Androstendiol 5.2±0.5 vs. at baseline, p=0.02; 8.76±0.25 vs. 7.09±1.03 ng/ml, p=0.05 respectively. 2) SI (glucose infusion rate/insulin resistant) showed no change. 3) Catecholamine levels remained unaltered before and after treatment. 4) Lipid profile improved, showing a significant reduction in CH and TG levels: 178.25±48.51 vs. 148.67±37.59 mg/dl (p=0.006) and 75.59±48.25 vs. 57.92±39.44 mg/dl (p=0.004), respectively. HDL and LDL showed no statistical change.

Conclusion: Androgens may have a beneficial effect on lipid profile in women with PCO. These results suggest that the androgen effect on lipid metabolism is receptor-mediated.

46XX KLINEFELTER’S VARIANT: PHENOTYPE, TESTICULAR, HISTOLOGICAL FEATURES AND HORMONAL PROFILE

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We present the case of a phenotypic male patient, 26 years of age, who was complaining of lack of, or insufficient ejaculation in spite of normal libido and sexual performance. Our patient had deficient growth of axillary, facial and body hair. The development of his pubic hair was deficient and horizontal. His penis was of normal length and diameter. His testes were hypoplastic and soft (volume 3.4 ml according to Prader orchidometer) and were located in the scrotum. His skin was thin and reddish and his body fat was increased. He was of normal height and weight, with normal body proportions and no evidence of gynecomastia.

The diagnostic procedures included: measurement of serum T, T4, FSH, LH, testosterone, growth hormone after GHRR test and clomidin test, TRH test, LHRR test, HCG test, clomiphene test, karyotype, testicular biopsy, and semen analysis. A high FSH level (29 µg/ml, normal range 0.9 - 9.8 µg/ml) and low testosterone (5 ng/ml, normal range 3 - 14 ng/ml) was found in addition to azoosperma, hypoplasia of seminiferous tubules, and a 46XX karyotype with undetected Y chromosome.

Our patient has a male phenotype, male psychosocial and sexual identity with very few female features. He is suffering from hypergonadotrophic hypogonadism with testicular hypoplasia, aplasia of seminiferous tubules, azoosperma, and 46XX karyotype. Cytogenic and molecular genetic analysis will help to elucidate the role of translocated (SRH: sex determining region Y) or mutated chromosome genes (TDF: testing determining factor) which are responsible for male sex determination. Psychological support and hormone replacement therapy will be necessary in order to overcome his problems.

OVARIAN STEROIDGENESIS IN HIRSUTE PATIENTS

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Ovarian steroidogenesis defects are a suspected cause of hyperandrogenism and polycystic ovary disease. A specific and direct test is needed to define these defects. We studied ovarian steroidogenesis by simultaneously blocking the adrenal and stimulating the ovaries.

25 successive hirsute patients were studied. No patient was under contraceptive pills or other steroids containing drugs. None had evidence of thyroid or hepatic disease.

Patients were evaluated between the 7th-8th day of spontaneously occurring menstrual cycle. Blood was collected for basal measurements at 1:00 pm of test day I, and 1500 h was then administered i.v. at 11:00 am. 1 mg danazol was given p.o. in the next test day 2 at 7:00 am and was again collected.

Both cortisol and DHEA were significantly decreased on day 2, suggesting adequate adrenal suppression (190 µg/dl). 3 µg/dl for cortisol and 255 µg/dl versus 187 µg/dl for DHEA, p<0.01). Estradiol significantly increased on day 2 suggesting adequate ovarian steroidogenesis stimulation (152 µg/dl versus 95 µg/dl, p<0.01). Patients with polycystic ovaries by sonographic criteria had no different test response at the 0.5 significance level. Also patients with a later proved anovulatory cycle had no different test response at the same significance level.

However, considering the basal androstenedione level on day 1, patients with higher androstenedione levels had a much higher Estradiol response (127 vs. 77 µg/dl, p<0.01), as well as a much higher 17β-hydroxyprogesterone and progesterone increase (1.9 versus 1.0 and 2.1 vs. 0.3 µg/dl, p<0.01).

If we consider androstenedione to be mainly or ovarian origin, that the data suggest ovarian hyperandrogenism to result of both an increased ovarian steroidogenesis with simultaneous increased ovarian aromatization, this is how increased pathway flow without specific blocking estrogenic defects. This agrees well with other recent data.
P2.121

CHANGES IN INSULIN-LIKE GROWTH FACTOR-11 RECEPTOR/MANNOSIDE-6-PHOSPHATE RECEPTOR DURING GROWTH AND ATRESIA OF OVINE FOLLICLES.

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To assess a potential role of insulin-like growth factor-II/Mannose-6-phosphate (IGF-II/M-6-P) receptor in the ovary, [125I]-IGF-II binding was studied on whole oocyte ovarian membranes and on whole ovarian sections. Competition studies with IGF-II, IGF-1 and insulin were performed to estimate the specificity of [125I]-IGF-II binding. On the ovarian sections, labeling was quantified after micro-autoradiography by image analysis. Furthermore immunohistochemistry experiments using a specific polyclonal antibody raised against the IGF-II/M-6-P receptor were performed on ovarian sections.

Specific and affinity binding sites for IGF-II were present on whole ovarian membranes. On membranes, the Kd value was close to 0.2 nM, IGF-I was approximately 1000 fold less potent than IGF-II to displace binding of [125I]-IGF-II from whole membranes as well as from granulosa and theca cells on ovarian sections. Insulin did not displace [125I]-IGF-II binding. Cross-linking experiments followed by SDS-PAGE electrophoresis under reducing conditions revealed that [125I]-IGF-II binding predominantly involved a 260 KDa protein, the size of which is compatible with that of the IGF-II/M-6-P receptor.

Furthermore, both autoradiography and immunohistochemistry experiments demonstrated that IGF-II/M-6-P receptor is present at high levels in granulosa follicles and in theca of healthy follicles. Conversely, very low levels were found in granulosa of healthy follicles and in the theca of atretic follicles.

The involvement of the IGF-II/M-6-P receptor in ovarian tissue remodeling and in mediating IGF-II action during the processes of folliculogenesis and atresia remains to be determined.

P2.122

TRANSFORMING GROWTH FACTOR Ø REGULATES PRORENNIN PRODUCTION BY BOVINE THECA CELLS IN VITRO

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Bovine ovarian theca cells have been reported to synthesize and secrete prorenin and renin activities. (1). Although the expression of this prorenin-angiotensin-system seems primarily to be under gonadotropin control, increasing evidence suggests that other intracellular factors are involved in the regulation of this enzyme system. (2). We have investigated whether transforming growth factor Ø (TGF-Ø), itself a product of theca cells (3), can alter the production and secretion of prorenin and renin activities in an autocrine fashion.

Addition of recombinant TGF-Ø (0.01 - 10 ng/ml) to percoll-purified bovine theca cells in a serum-free cell culture system resulted in a significant, dose-dependent stimulation of LH-induced prorenin synthesis and release. Prorenin production by unstimulated cells however was not effected. In comparison, neither basal nor LH-stimulated progesterone production was influenced by addition of TGF-Ø, demonstrating that the peptide had no influence on cell number or viability. The stimulatory effect of TGF-Ø appeared to be exerted at a site distal to the LH-stimulated cAMP production since the 8βR-cAMP as well as the Forskolin-stimulated prorenin production was also markedly enhanced by TGF-Ø, whereas the gonadotropin-induced cAMP formation was unaltered. Thus, we provide evidence that the regulation of the ovarian prorenin-angiotensin-system is not only subject to gonadotropin control, but is finely tuned by a number of locally acting factors including TGF-Ø.


P2.123

THE PATTERN OF STEROID PRODUCTION BY PERIFUSED PREOVULATORY RAT FOLLICLES

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In order to determine the detailed pattern of follicular steroid release before ovulation, the ovarian follicles of female Wistar rats displaying a regular 4-day oestrous cycle were isolated every 2 or 3 hours on the day of pro-oestrus and subsequently perifused with Eagle's medium for 3 hours. In the collected perifusates oestradiol, androgens and progesterone were measured by specific RIA's.

The follicles removed in the morning and afternoon secreted predominantly oestradiol and androgens, while those isolated in the evening and night released mainly progesterone. FSH- or LH-supplemented media stimulated oestradiol and androgen release only when the follicles were removed before the endogenous LH surge. Although the follicular content of androgens was high and the pattern of their release followed that of oestradiol, androgen output was very low. These results indicate that (1) there is a shift in steroid release after the precoccubative LH surge: the follicles stop secreting oestradiol and androgens and start producing progesterone; (2) androgens as substrates for aromatization are selectively stored inside the follicles.

P2.124

INTERLEUKIN 6 IN HUMAN PREOVULATORY FOLLICLE

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Numerous studies have shown that cytokines including interleukin-6 (IL-6) are capable of exerting profound effects over ovary at the periovulatory period. Here, we attempted to demonstrate that IL-6 is locally produced by ovarian granulosa cells (GCs) at ovulation time. Using bioassay, IL-6 activity was detected in follicular fluids and in supernatants of cultured oocyte-cumulus complexes (OCC) from 22 patients undergoing in vitro fertilization therapy. Total RNA was isolated from follicular aspirates, reverse transcribed and submitted to Polymerase Chain Reaction using oligonucleotide primers corresponding to known cDNA sequences for IL-6. To localize IL-6 mRNA in situ hybridization analysis was performed using a [35S] IL-6 riboprobe. The expression of IL-6 protein was assessed by immunohistochemical staining. Progesterone (P) production and aromatase activity were assayed in GC cultures added or not with recombinant human (h) IL-6.

Biological IL-6 activity in OCC cultures was not related to fertilization rates. A 126 basepair band characterized the amplification product of IL-6 transcripts on gel electrophoresis. The distribution of transcripts was more dense 15% versus 3% stained cells in GC enriched preparations than in original follicular preparations; it was not significantly modify by the presence of macrophage contaminants. Immunohistochemical staining were positive on GC enriched preparations. Biological IL-6 activity and in situ hybridization signals dropped after a 72 h culture period. Adding rh IL-6 had no effect on steroidogenic activities of GCs in cultures free of macrophages whereas it increased P production and aromatase activity in cultures including macrophage contaminants. The present study provides strong support for the view that IL-6 is transiently produced by GCs as the preovulatory follicle at ovulation time and suggests that IL-6 might be an intronovarian regulatory factor concerned with steroidogenesis.

P2.125

**EFFECT OF VARIOUS DOSES OF CORTISOL ON THE CONCENTRATION OF STEROIDS IN CULTURE GRANULOSA CELLS FROM BUFFALO.**

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Ovarian cysts are a serious cause of reproductive failure in buffalo and cattle because they occur frequently and prolong the calving interval. Present study was designed to examine the effect of cortisol on the amount of steroid hormones secreted in cultured granulosa cells. Ovaries of non-pregnant buffalo were collected, granulosa cells were isolated and viable cells were plated in plastic dishes with culture medium. The amount of estradiol 17β and progesterone secreted by the granulosa cells increased to over the 10 day culture period. Cell viability was not significantly affected by cortisol treatment. DNA content was not significantly affected by the addition of cortisol. Cortisol in high concentration (1μM) inhibited the secretion of estradiol 17β. As progesterone production was increased, these data are suggestive of that cortisol selectively suppresses specialized functions in bovine granulosa cells. Therefore, conclusion that cortisol stress may directly inhibit the functional maturation of the follicle and thus induce ovarian cyst.

P2.126

**STEROIDIOGENESIS IN OVARIUS OF FASTING RATS.**

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The effect of fasting on the ovarian steroidogenesis in adult rats was investigated. Adult female Wistar rats were fasted (received water ad libitum, but no food) for 7 days. The fasting caused a significant reduction of body weight, ovarian weight and plasma concentrations of FSH, LH and Progesterone (P), with a slight non-significant reduction of estradiol (E2). Ovarian steroid content and steroid production by incubated (4h) ovaries were measured by RIA. In both conditions fasting did not affect the values for P, but reduced significantly those of 17α-hydroxyprogesterone (17α-OH-P), androstenedione (54A), testosterone (T) and E2. Daily ip administration of 10 IU LH during the 7 days fasting, enhanced strongly the amounts of all steroids in the controls. In the fasted animals, only values were obtained comparable to the unstimulated control values (except for P).

These results indicated that fasting causes a block between P and E2, which can not be reversed by in vivo LH administration.

Incubations with labeled 17α-OH-P and T — in which metabolites formed were measured by β-counting after chromatography — showed a reduced conversion of 17α-OH-P to 54A, but a strong increase of the conversion of T to E2. It can be concluded that a period of 7 days fasting has, without excluding the effect of a reduced gonadotrophic stimulus, a direct inhibiting effect on the activity of the 17α-hydroxylase — C17,20 lyase enzyme complex which converts P to 54A via 17α-OH-P.

The activity of the enzymes leading to P and of the aromatase were not modified or rather enhanced.

P2.127

**NALTREXONE IS UNABLE TO MAINTAIN THE GONADOTROPIN ACTIVITY INDUCED BY PULSATILE GnRH ADMINISTRATION IN WOMEN WITH HYPOGALAMIC AMENORRHEA.**


It has been clearly shown that endogenous opioids play a regulating role in the control of gonadotropin secretion only in the presence of gonadal steroids. However, in functional hypothalamic amenorrhea (HA), chronic administration of an opioid receptor antagonist (Naltrexone) has been shown to restore gonadotropin secretion and ovarian function. To further study these conflicting results, a group of 5 women with secondary functional HA (absence of withdrawal bleeding after gestagen administration, low frequency and amplitude of LH pulses and normal body weight) were studied.

GnRH was administered IV at a dose of 10 μg/pulse every 90 min to induce ovulation and was maintained until menorrhea occurred. The opiate antagonist, Naltrexone (Nal), was administered at a dose of 100 mg/day (50 μg, BID), starting on day 10 of the luteal phase induced by pulsatile GnRH administration. Ovulation was monitored with repeated plasma estradiol (E2) levels, ultrasonographies and progesterone levels on day 20-24 of each treatment cycle.

On day 12 of pulsatile GnRH administration, plasma levels of E2 increased from 2.1±0.3 to 71±5.5 pg/dl and LH pulses followed each GnRH pulse during the frequent sampling study. Ovulation occurred in the 5 women during pulsatile GnRH treatment and the luteal phase lasted 14.3±2 days with normal 2 and 6 levels. During Nal treatment, plasma E2 levels fell to preovulatory levels and remained low. No follicular growth occurred and the pulsatile GnRH study showed dramatically reduced frequency, amplitude and mean plasma levels of LH. When pulsatile GnRH was reinitiated, ovulation resumed in all patients.

In conclusion, Naltrexone, started during priming with pulsatile GnRH administration, was unable when continued alone to maintain gonadotropin secretion and to stimulate ovarian function in women with HA. Thus, the role of endogenous opioids in HA and the effect of Naltrexone even in the presence of ovarian steroids is really doubtful.

P2.128

**PULSATILE LH RELEASE DURING FOLLICULAR PHASE IN GILTS: EFFECTS OF RESTRAINMENT AND ENDODGENOUS OPIOIDS.**


Endogenous opioid peptides (EOP's) play a role in the negative feedback of progesterone on the Luteinizing Hormone (LH) release during the luteal phase of the estrous cycle. In addition, EOP's are activated during stress and have been implicated in the inhibitory effect of stress on LH secretion. However, whether stress and/or EOP's modulate the release of LH during the follicular phase is still a matter of dispute.

Experimental setup: The estrous cycles of 5 chronically restrained (tethered) and 8 loose housed gilts equipped with permanent jugular vein catheters were synchronised by treatment with the progesterone agonist altrongest for 21 days. Subsequently, 3 of the restrained and 4 of the loose housed gilts were treated with the EOP-antagonist naltrexone during 6 days. The other animals served as controls. On days 2, 4, 5 and 6 of the period of naltrexone administration, bloodsamples were taken every 12 minutes during 12 hours for LH analyzed for LH.

Neither chronic restraint nor naltrexone treatment affected the release of LH. In all animals, a time effect was found on the pulsatile LH release; on day 2 the puls frequency was higher (p<0.05) than on days 4, 5 and 6. On day 5 the puls were wider and the interval between the pulses was larger as compared to day 2 (p<0.05). On day 6 the amplitude of the pulses was higher (p<0.05) than on days 2, 4 and 5. The preovulatory LH surge occurred on average on day 6 (range day 4-9). There was a positive correlation between the day of the preovulatory LH surge and the height (r=0.62; P=0.03) as well as the peak-valley ratio of the pulses on day 2 (r=0.69; P<0.01).

Stress and/or EOP's do not seem to modulate the release of LH during the follicular phase of the estrous cycle. After an initial high frequency and low amplitude LH release on day 2, the pulsatility decreases until day 6. On this day the amplitude is higher compared to day 2. In addition, a relatively high amplitude on the second day of the follicular phase predicts a delayed LH surge.
PULSATILE LH RELEASE DURING THE LACTATION PERIOD IN RELATION TO THE METABOLIC STATUS AND THE TIME OF FIRST OVULATION IN SOWS.

P.129

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The pulsatile LH release during lactation and the relation of these pulses with metabolic hormones, metabolic status and the interval between weaning and ovulation (IWO) was investigated in 17 sows. After parturition the number of piglets was standardized at 9 and the sows were weaned at day 22 after parturition. Bloodsamples were taken every 12 minutes during 12h on day 109 of pregnancy and on days 7, 14, 21 and 22 after parturition. Ovulation was detected by scanning the ovaries ultrasonographically every 4 hours from 18h after onset of oestrus onwards. The number of LH pulses during 12h (mean ± s sem) on days 109, 7, 14, 21 and 22 were 2.3 ± 0.4, 1.2 ± 0.4, 1.2 ± 0.4, 1.7 ± 0.4 and 10.8 ± 0.4, respectively. The mean LH peak concentrations on those days were 29.9 ± 3.4, 33.5 ± 4.1, 30.7 ± 4.5, 38.9 ± 3.3 and 6.5 ± 3.1 respectively. The number of LH pulses on day 22 tended to be positively correlated with the average insulin and glucol levels on day 21 (r=0.47; p<0.06 and r=0.48; p=0.02 resp.). Ovulation occurred between 126 and 214h after weaning. Sows that lost less than 12.5% of body weight showed a shorter IWO than sows that lost more than 12.5% (IWO 145 and 168h resp. p<0.06). The number of LH pulses on day 22 correlated negatively with the average LH pulses during the IWO (r=-0.45; p=0.08 and r=-0.71; p=0.002 resp.). The number of LH pulses during 12h on day 22 is 1.1 ± 0.9 for sows with a short IWO (<144h) and 8.6 ± 0.3 for sows with a long IWO (>144h).

In conclusion: The number of LH pulses is supressed during lactation and has increased dramatically on the day of weaning (day 22). The number of LH pulses on day 22 depends on the amount of weight loss during the last week of lactation and on the insulin and glucol levels on day 21 of lactation. The higher the number of LH pulses on day 22, the sooner ovulation occurs.

ROLE OF THE PROGESTERONE RISE AND THE INHIBIN DROP IN PROESTRUS ON THE SECONDARY FSH SURGE IN ESTRUS

P.130

M. Thibau, C. Bellido, A. Ruiz, J.E. Sanchez-Crando. Dept. of Physiology. Faculty of Medicine, University of Cordoba, Cordoba, Spain. During the estrous cycle of the rat, the secretion of LH and FSH is low except in proestrus when the primary surge of gonadotrophins takes place. LH secretion falls by midnight of proestrus while that of FSH continues elevated throughout estrus and falls by noon of estrus. LH and FSH primary surge is coupled to the secretion of LHRH but the secondary surge of FSH is not. And its control is not completely known. It is well established that the secretion of FSH in estrus depends on both the LH surge and the permissive absence of circulating inhibin. The antiinhibotropins RU486 blunts the primary LH and FSH surge and abolishes the secondary surge of FSH. The present study was carried out to study whether, in addition to the LH primary surge-dependent fall in ovarian inhibin secretion in proestrus, the rise of progesterone in proestrus afternoon and evening is responsible for the secondary surge of FSH in estrus. We studied the effects of a LHRH antagonist (LHRH-A) (1 mg sc at 0900 h on proestrus) and the antiprogesterone RU486 (0.4 mg sc at 0900 h on proestrus) on both LH primary surge and FSH secondary surge.

Afterwears, we tried to reverse the effect of the LHRH-A on FSH secondary surge with an ovulatory injection of KC (10 IU sc at 1700 h on proestrus) and with progesterone (3 mg sc at 1500 h proestrus) and/or anti-inhibin serum (0.3 ml iv at 1900 h on proestrus). Thirdly, the role of the ovary in the control of the secretion of FSH on early estrus was analyzed in ovariectomized rats at 1500 h on proestrus. Our results indicate that the LHRH-A blocked both the LH and FSH primary surge and the secondary surge of FSH and that RU486 and ZK299 blunted the LH and FSH primary surges and abolished the FSH secondary surge. In conclusion, MCT totally reversed the effect of the LHRH-A, the treatments with progesterone or anti-inhibin serum restored only in part the secretion of FSH on early estrus, and the combined treatment completely reversed this secretion. Finally, ovariectomy did not modify the concentration of FSH on estrus either in oil or RU486-treated rats. These data indicate that the LH primary surge dependent drop in inhibin secretion, together with the actions of extra ovarian oestradiols that are blocked by RU486 and ZK299 (probably adrenal progesterone), might be responsible for the release of FSH on estrus.

SECRETION OF FSH AND LH: KALLMANN'S SYNDROME VERSUS ACQUIRED HYPOGONADOTROPISM

P.131


In a previous paper (1993) we affirmed a different behaviour of FSH and LH in idioopathic hypogonadotropism: FSH pulsatility can be relatively preserved while LH pulses are reduced or abolished. We wondered if patients affected by hypogonadotropism with peripheral onset of LHRH-A, in whom all levels were very low. The ultradian behaviour of LH and FSH was evaluated taking blood samples every 15 minutes during 9 hours and using a sensitive and specific immunoradiometric assay. Pulses were analyzed by Santos and Bartolin (1985) and burst (30 min) were calculated by the linear trapezoidal rule.

In our study, 17 patients, aged 22-55 years: 4 men and 2 women affected by Kallmann's syndrome (KS), 2 men and 1 woman affected by congenital hypopituitarism, 8 men by pituitary adenomas with peripheral onset of LHRH-A and 1 woman by idiopathic hypogonadotropism. LH and FSH were measured every 30 min in 20 normal males, 14 healthy postpubertal boys and 7 healthy postpubertal girls.

These data show that: 1) 7 levels rise in a similar manner in THAL and IHH; 2) a more dramatic increase in I-LH, b-LH and FSH is observed in THAL; 3) only b-LH significantly rises in THAL, while i-LH and i-FSH levels are unchanged.

In conclusion, a gonadal impairment is not evident in our THAL. Indeed, a early damage of the hypothalamic-pituitary unit could be present. The pulsatile LH administration seems to partially improve their secretion of bioactive LH.

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**P2.134**

**LEUPROLIDE TESTING IN PCOS: PATHOPHYSIOLOGICAL IMPLICATIONS**

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A case of 17a-OG-progesterone (170P; 5.39 mg/ml, 7.8 mmol/l) in response to GnRH analogs (M gavefis, Buclerim) is negative in women with nonagenetic (bilateral hyperthermia and hypogonadism) and negative in the presence of a negative ACTH test (excluding Cushing syndrome). Our study evaluated this test in 40 premenopausal PCOS patients (mean: 27 ± 5 yrs). The LH/FSH ratio in the presence of a negative ACTH test was significantly lower (p < 0.05).

**P2.135**

**DISPARATE EFFECTS OF HA966 AND FSH VIA THE GONADOTROPIN SURGE-INHIBITING FACTOR (GnSIF) ON GnRH-STIMULATED LH RELEASE IN ADULT AND PREPUBERTAL FEMALE RATS**

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Excitatory amino acids (EAA’s) play an important role in the regulation of the preovulatory gonadotropin surge. Effects of the partial glycine-site antagonist of the N-methyl-D-aspartate (NMDA) receptor complex 1-hydroxy-3-amino-pyrollidone-2 (HA966) on the gonadotropin releasing hormone (GnRH)-stimulated luteinizing hormone (LH) release in female rats were studied. Adult and 19 days old rats were injected with respect to 100 and 50 mg/kg body weight HA966 or saline during 10 days or 2 hr before sacrifice. Pituitary glands were incubated with 1000 mg/ml GnRH. In order to investigate whether HA966 affects the gonadotropin-releasing hormone (GnSIF) action which is known to antagonize the action of GnRH, animals were also injected with Metrelin (10 IU follicle-stimulating hormone; FSH) on days 1 and 2 of diestrus.

In adult animals, both HA966 injection protocols caused a decreased LH release. In combination with FSH no additional effect was observed. FSH alone more strongly reduced LH release than chronic administration of HA966.

In prepubertal rats, both HA966 injection protocols caused an increased LH release. In combination with FSH, LH release was not distinguishable from control animals.

In conclusion, in adult female rats HA966 suppresses LH release, which supports a stimulatory role of the hypothalamo-pituitary-gonadal axis. FSH (GnSIF) does not further enhance the antagonistic action of HA966.

In prepubertal rats due to low concentrations of ovarian steroids, a stimulatory effect of HA966 is found. In this case, FSH (GnSIF) is able to restore HA966-induced increased LH levels to normal control values.

These observations support the idea that NMDA effects on LH release depend upon the steroid milieu at the time of administration.

**P2.136**

**LHRH AND TRH INCREASE INTERLEUKIN-1ALPHA PRODUCTION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS**

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The neuroendocrine and immune systems are totally integrated and share a common set of hormones, neurotransmitters and polypeptides, and their receptors. Lymphocytes have been shown to produce IL-1 and TNF in response to LHRH and TRH, respectively. We evaluated the influence of LHRH and TRH on IL-1 and TNF production by normal human peripheral blood mononuclear cells (PBMC) in vitro. IL-1 and TNF were stimulated following a suboptimal concentration of staphylococcal enterotoxin A (SEA) (100 pg/ml). Blood was obtained from five women with regular menstrual cycles. PBMC, isolated by Ficoll-Hypaque, were cultivated in EAA’s media plates at 37°C in a humidified atmosphere of air 2% CO2 at a concentration of 10^5 cells/0.5 ml in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. Cell responsiveness was checked for 24, 48, and 72 hr. Each plate contained unstimulated and stimulated cells with or without LHRH (2.5 x 10^6), TSH (1 M U/ml) or TRH plus LHRH. In the supernatants were used whole for titration of IL-1 activity, using MUS cells as indicator cells and VNS (human serum) as challenge virus. Each assay included the international standard for human IFN- which was tested on 2 different IFN- specific analysis. Samples were tested at least twice in triplicate to test activity. IL-1 activity was expressed in IL-1 units and of the IFN- specific activity was calculated using specific anti-IFN monoclonal antibodies. The minimum detection limit of IFN-1 was 10 units/10^6 PBMC. The microplate reduction assay was used whole for titration of IFN- activity using MUS cells as indicator cells and VNS (human serum) as challenge virus: Each assay included the international standard for human IFN-2. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC. The sensitivity of this assay was determined by titration of known IFN-2 activity as shown in IL-1 units and units/10^6 PBMC.
P2.137

SECRETION OF FREE hCG IN THE ABSENCE OF HOLO-hCG BY NON-TROPHOBLASTIC CELLS IN VITRO

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Low levels of hCG-immunoreactivity can occasionally be detected in patients with various non-trophoblastic tumors, such as cancers of the cervix, pancreas, stomach, colon, bladder as well as malignant lymphomas. In contrast to trophoblastic tumors, these malignancies seem to produce the free ß-subunit of hCG in excess of the hCG-hCG molecule.

To further elucidate the in vitro secretion of hCG-immunoreactivity from non-trophoblastic tumors, we measured free hCG, free ß-subunit and holo-hCG in supernatants from various established cell lines derived from epithelial cancers or from malignant lymphomas by highly specific and sensitive immunoradiometric assays. Significantly elevated levels of free hCG could be detected in cell lines from gastric carcinomas (CRL 1739: 0.30 ng/ml, KATO 0.11 ng/ml), from a ductal breast carcinoma (T47D: 0.33 ng/ml) and from a carcinoma of the uterine cervix (CaSk: 1.1 ng/ml). No hCG-immunoreactivity could be detected in cell lines from colorectal carcinoma, adenocarcinoma of the breast and from various B-cell and T-cell lymphomas. For comparison, however, free hCG could be well detected in various non-neoplastic epithelial cell lines, which had been transformed spontaneously (HiCa: 0.31 ng/ml) or after infection with SV40 (SV40: 0.17 ng/ml) or HPV16-virus (HPK 1a: 1.54 ng/ml, HPK 2a: 3.31 ng/ml). No hCG immunoreactivity could be detected in the supernatant of normal human fibroblasts.

Neither the free ß-subunit nor the holo-hCG molecule could be detected in any of the supernatants investigated.

When subjected to gel chromatography (Superdex 200, Pharmacia), the elution position of the hCG immunoreactivity in cell supernatants was virtually identical with the purified hCG-subunit (NTI CR125B).

In conclusion, secretion of the free ß-subunit of hCG is not restricted to trophoblastic cells, but can also be found in various cell lines from non-trophoblastic malignant tumors and also in some non-neoplastic epithelial cells. These cell lines may represent useful tools for the investigation of the regulation of free hCG secretion in non-trophoblastic cells.

P2.138

EFFECT OF CHRONIC ANDROGEN RECEPTOR BLOCKADE ON GONADOTROPIN SECRETION AND POLYCYCLIC DEVELOPMENT IN HYPERTROPHIC WOMEN

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The effects of androgens on gonadotropins (GT) secretion in women are not completely understood. It has been proposed that in hypertrophic state, androgens either directly or by aromatization to estrogens drive the abnormal GT dynamics which characterize this syndrome. This abnormal GT secretion could be responsible for the alteration in follicular development and ovarian steroidogenesis in polycystic ovary syndrome (PCO). The aim of this study was to examine the effect of long term androgen receptor blockade on pulsatile GT secretion and follicular growth in 8 PCO patients treated for 30 days with daily oral doses of 750 mg Flutamide (F). Blood samples were collected over 10 min intervals for 6 h, before treatment, on day one and 30 of F administration. To assess the effects of F on the pituitary, a GNRH test (100 mg iv) was performed after pulsatile studies. LH and FSH were determined in all samples; while PRL, testosterone (T), DHEAS, E2, and 17-OHP were in selected samples. In addition, follicular maturation was assessed by transvaginal ultrasound, and LH and E2 determination between the 8th to 30th day of treatment. For pulse analysis, an algorithm based on a threshold method was used. Long term F treatment was followed by a decrease in LH pulse amplitude (day 0: 6.94±1.2 and day 30: 4.64±0.6 IU/L, P < 0.01) associated with a decline in LH response to GNRH (0.05), LH pulse frequency, FSH, PRL, E2, T, and 17-OHP levels were not modified. In contrast, DHEAS decreased respect to day 0 (15.6±1.2 to 7.2±0.6 umol/L, P<0.01). Ovulation and follicular maturation did not take place in any patient. In conclusion, F administration to PCO patients was followed by a significant decrease in LH amplitude and LH release in response to GNRH administration. These findings suggest that androgens facilitate the LH secretion at the pituitary level in PCO women. However, the changes on LH secretion by F in these patients were unable to induce follicular maturation or ovarian cyclicity. (Supported by Fondecyt #193/92)

P2.139

HUMAN PLACENTAL PROGESTERONE (P) AND OESTROGEN (E) PRODUCTION IN VITRO ARE UNAFFECTED BY ACTH, CRH AND IGF-1.

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Several reports have suggested a stimulatory role for ACTH and a modulatory role for IGF-I on placental P and E production. As placental P synthesis has long been considered to be under autonomic regulation, such findings might have a significant impact on the understanding of placental steroid production during pregnancy and in relation to the onset of labour. The aim of this study was to assess the effect of ACTH, CRH and IGF-I on the production of P, oestradiol (E2) and oestrone (E3) in human placental tissue.

To date, cotyledons from placentas of 5 women who had undergone normal labour were minced and incubated at 37°C in Dulbecco's Modified Eagles Medium with/without ACTH (100nmol/L), CRH (100nmol/L) and IGF-I (2.6 and 52nmol/L) in the presence/absence of NADPH for a period of 24 hours (6 dishes with approx. 300 mg of tissue per dish in 3 ml of medium, were used for each experiment). Small aliquots of medium were removed at fixed time intervals throughout the incubation period and levels of the respective steroid assayed by RIA. Mean 24 hr control values (pg/mg protein) ranged from 11.7-25.6 mg/ml, 306-835 ng/ml and 236-708 ng/ml for P, E2 and E3 respectively. No stimulatory effect of ACTH, CRH or IGF-I was seen on P, E2 or E3 production. The addition of NADPH (1mmol), a cofactor requirement in cytochrome P450 SCC and P450 aromatase activity, did however significantly raise P and E2 production from control levels in 4 out of 5 placentas at 1,2,3 and 24 hrs (P<0.001, at 24 hrs). The absence of ACTH, CRH and IGF-I in the presence of NADPH had no effect on this. NADPH enhanced P and E2 production. Interestingly E3 production was unaffected by the presence of NADPH.

This study suggests, in contrast to previously reported findings, that ACTH and IGF-I do not have a stimulatory effect on the short term production of P, E2 and E3 in placental tissue minces. NADPH, however, significantly increases P and E2 but not E3 synthesis.

P2.140

GROWTH HORMONE HYPERSECRETION IN RECENT ONSET TYPE I DIABETES MELLITUS

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To investigate the effect of residual beta cell function on GH secretion in type I diabetes mellitus, two groups of diabetic subjects were studied: group A - with recent onset diabetes (less than 12 months, mean 7 months) and type I diabetes (mean 11 years) and group B - with long standing diabetes (mean 14.2 years).

Ten healthy subjects served as controls. Spontaneous GH secretion was measured hourly during 24 hours and analyzed with Pulsar program. Group B (TP negative) had higher number of secretory peaks than group A: 6.7 ± 5.0 (p<0.05) and slightly higher mean 24-hr GH level: 7.07 ± 4.90 ng/ml (p<0.05) Other parameters of peak analysis (amplitude, length, frequency and interval) were similar in both groups. In CP negative patients no difference between mean daytime and nighttime GH levels was observed: 5.34 ± 8.74 ng/ml, respectively, while in healthy subjects and in CP positive patients nighttime GH levels were higher than daytime GH levels. The whole diabetic group (n=18) had higher mean peak GH levels than healthy controls: 5.98 ± 2.38 ng/ml (p<0.01) and higher mean peak amplitude: 14.20 ± 5.49 ng/ml (p<0.05) while number of peaks were not different: 5.8 and 5.7 respectively. The results of this study indicate that residual beta cell function plays no major role in GH hypersecretion of diabetes. However, it may contribute to minor qualitative changes in the pattern of GH secretion by increasing the number of secretory peaks and stabilizing the typical night/day difference in GH secretion.
P2.141

GH RESPONSES TO GHRP-6 IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS.

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Insulin-dependent diabetes mellitus (IDDM) is often associated with elevated growth hormone levels. The mechanism responsible for these alterations remain unclear. GHRP-6 has been shown to be a potent and selective secretagogue for GH in normal subjects. In order to gain further insight into altered GH secretion in IDDM, GHRH (100 μg), GHRP-6 (90 μg) either alone or in combination were administered i.v. to 6 IDDM patients and 6 normal volunteers. Venous blood samples were collected from 30 to 150 minutes at different intervals. Plasma GH levels were measured by IRMA. Non-parametric statistics were used to evaluate the results (Mann-Whitney’s test or Friedman’s two-way analysis of variance when appropriate).

GH responses to GHRP-6 were significantly greater in IDDM patients in comparison to normal volunteers. GHRP-6 appeared to be a potent stimulus for GH release in IDDM patients, showing similar responses to those elicited by GHRH. The simultaneous administration of GHRH and GHRP-6 induced a massive release of GH in IDDM patients (AUC) alone: 5311±169 mg/ml, GHRP-6: 5221±1019 mg/ml, GHRH+GHRP-6: 8728±1023 mg/ml, Friedman’s test, p<0.02).

In conclusion, GHRP-6 is a potent releasing factor in IDDM patients as it is in normal subjects. However, the effects of combined administration of GHRP-6 and GHRH were only additive. Thus, although basal GH secretion is elevated in the IDDM and the ability to respond to both GHRH or GHRP-6 is enhanced, there is still considerable reserve of GH in the somatotroph cells ready to be secreted on demand.

P2.142

EFFECT OF GROWTH HORMONE RELEASING HORMONE (GHRH) ON GH, PROSTAGLANDIN E2 (PGE2) AND INSULIN IN DIABETIC PATIENTS.

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In a previous paper we have demonstrated a link between GH and PGE2 in their action on GH in normal subjects. On the other hand in diabetes mellitus various disturbances of GH and PGE2 secretion have been reported.

In the present study the response of GH, PGE2 and insulin to GHRH (1 μg/kg, i.v.) was investigated in 12 non-insulin dependent poorly controlled diabetics (8 males and 4 females) and 11 normal volunteers (6 males and 6 females).

After GHRH injection GH increased in diabetics from 2.28±0.52 μg/ml to 14.76±1.29 μg/ml at 30 min (p<0.001). This response was not statistically different compared to the control subjects. The basal values of plasma and urinary PGE2 were significantly lower in diabetics than in control subjects.

In 22 patients we have observed an increase of PGE2 in normal subjects (9.8±0.44 pg/ml at 0 min; 14.5±1.3 pg/ml at 90 min, p<0.05 and did not have any effect on PGE2 in diabetics. Plasma insulin decreased significantly after GHRH in both normal subjects and diabetics at 60 min.

We conclude that GH response to GHRH is not significantly impaired in poorly controlled non-insulin dependent diabetes. The lack of an effect of GHRH on PGE2 in diabetic subjects provides further evidence of preserved PGE2 synthesis or metabolism in this disorder. The physiological significance of the GH suppressive effect on serum insulin remains to be explained.

P2.143

CIRCULATING GH MOLECULAR FORMS IN PRADER-WILLI SYNDROME.

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Short stature is one of the clinical features of Prader-Willi syndrome (PW). It has been previously demonstrated in some patients both normal and reduced GH responses to standard provocative tests, while analyses of spontaneous GH nocturnal secretion suggest the presence of GH neurosecretory dysfunction. Given the biological activity of GH directly correlates with its different secreted and circulating molecular forms, this study evaluated the relative proportions of the main circulating forms of GH in 4 PW patients (2 males and 2 females). Mean age 26.3±1.5 years; height percentile according to Tanner; BMI 18±6; IGF-I 7±0.1, SGA 73±0.1, with the aim to better define the origin of their growth delay. The patients (1 male and 1 female with normal karyotype; the other with chromosome 15q11-q13 deletion) underwent provocative test with GHRH (1 mg/kg iv), i.v.-propranolol (60 mg p.o. 60 min before the test); blood samples were taken at 30, 0, 15, 30, 45, and 60 min. The obtained sera were analysed by means of gel filtration on Superose 75 column (Pharmacia), equilibrated and eluted with PBS 0.14 M, pH 7.6, fast a flow rate of 6 ml/minute collecting 1.5 ml fractions. GH levels were measured by immunoradiometric assay (Nichols). As controls, we studied 5 healthy subjects, well-matched for sex and age. The data were analysed by means of Student’s t test for unpaired data.

Significant differences were observed in the percentages of GH molecular forms between PW and control group; high-basic peptides (6.4% vs 15.6±1.6; big27±12.8 vs 22.1±3.5; little=69±8.6) vs 58±9.4. Nevertheless in the PW male patient with a normal karyotype, a marked increase in the contents of high molecular weight and reduced biological activity was observed; big3; big3; big27; little=23.5; little=38.5. On the basis of these preliminary data, it is possible to suggest that structural and/or functional anomalies in secreted GH may represent, in some subjects, one of the pathogenic mechanisms responsible for short stature in PW.

P2.144

IMPAIRED GROWTH HORMONE STIMULATION IN OBSESE PATIENTS - IS IT RELATED TO DECREASED PARASYMPATHETIC DRIVE?

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In order to examine whether impaired growth hormone (bGH) stimulation in human obesity is related to decreased parasympathetic drive, 20 otherwise healthy obese patients (body mass index (BMI) > 30 kg/m²) and 10 healthy control subjects (BMI < 25 kg/m²) were studied. IV bolus injections of dexamethasone (DMX) or placebo were given in random order according to a double-blind trial design. In controls, DMX increased bGH levels by maximally 1.9±0.6 ng/ml (p<0.01). In 8 patients, bGH was not influenced by DMX (non-responders). In 12 patients (responders), bGH was stimulated by maximally 1.0±0.2 ng/ml (p<0.05 vs. controls). In all subjects, DMX increased the coefficient of cardiac beat-to-beat variation (CV) as index of parasympathetic activity (p<0.05). In patients, both baseline and stimulated CV were decreased in comparison to controls (p<0.05), but responders did not differ from non-responders. Patients had higher blood pressure, higher HbA1c and lower somatomedin C levels than controls (p<0.05), but again responders did not differ from non-responders. Moreover, age was similar in responders and non-responders. In contrast, non-responders differed from both responders and controls in higher free thyroxine concentrations (p<0.05). Conversely, cortisol levels after DMX were decreased in responders in comparison to non-responders and controls (p<0.05). Thus, the present study confirmes decreased parasympathetic drive in obese patients which, however, does not explain impaired bGH stimulation. It will have to be studied which relation may exist between bGH release and carbohydrate consumption.
ROLE OF FREE FATTY ACIDS IN THE PATHOGENESIS OF SOMATOTROPE HYPOACTIVITY IN OBESITY
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In human obesity there is a reduction of basal and stimulated GH secretion, whose pathogenesis is still unclear. The increased free fatty acid (FFA) levels could play an important role, since the increase and the reduction in FFA levels can inhibit and stimulate, respectively, GH secretion. To clarify these mechanisms in 13 obese female patients (19-57 yrs; BMI: 37.5 ± 1.7 kg/m²) we studied the effect of arginine (ACX), an anti-lipolytic drug, on the GH response to GHRH (10 µg/kg iv) alone or combined with arginine (ARG), 0.5 g/kg iv, which likely inhibits the hypothalamic somatostatin release.

In 6 patients ACX was administered acutely (500 mg po), while in 7 a prolonged treatment was performed (256 mg 11.12 for 4 days). FFA levels in obese patients were higher than in normal subjects (0.05 ± 0.05 vs 0.38 ± 0.03 mmol/l, p<0.01) and were significantly reduced either after acute (0.12 ± 0.03 mmol/l) and prolonged ACX administration (0.06 ± 0.02 mmol/l). In obese patients the GH response to GHRH was lower than in controls (AUC: 207.3 ± 93.3 vs 1424.5 ± 184.5 µg/L, p<0.01). ARG potentiated this response in both groups, but in obese patients it was clearly lower (p<0.01) than in controls (605.9 ± 217.5 vs 389.6 ± 386.0 µg/L) in obese patients. Acute ACX administration enhanced the GH response to GHRH (531.8 ± 184.9 vs 235.6 ± 91.1 µg/L, p<0.01), but it did not affect that to GHRH+ARG (573.7 ± 168.7 vs 547.4 ± 147.6 µg/L). Prolonged ACX treatment enhanced the somatostatin release to GHRH (1156.3 ± 380.8 vs 690.0 ± 228.8 µg/L, p<0.05) but did not normalized it. These results show that the reduction in FFA levels clearly increases the reduced somatostatin response to GHRH in obesity. After prolonged treatment the effect of ACX is more marked and additive with that of arginine. Therefore, the increased FFA levels in obesity seem to play an important role in the pathogenesis of somatotrope hyposecretion. This study was partially supported by a grant of M.U.R.S.T.
THE EFFECT OF LONG-TERM PREDNISONE TREATMENT ON THE GROWTH HORMONE/INSULIN-LIKE GROWTH FACTOR 1 (GH/IGF1) AXIS.

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Due to a lack of longitudinal data, the effect of long-term prednisone treatment on the GH/IGF1 axis is controversial. We therefore performed a prospective controlled study in which we followed fasting morning levels of GH, IGF1, and IGF1 binding protein 3 (IGFBP3).

Methods: Ten euthyroid Graves' ophthalmopathy patients were treated with a 3 month oral prednisone course (60 mg for 2 wks, 40 mg for 2 wks, 30 mg for 4 wks, 20 mg for 4 wks, after which the dose was tapered to 0). 8 patients treated with orbital irradiation served as controls.

Results: Pretreatment serum GH, IGF1, and IGFBP3 were similar in both groups. In contrast to controls, prednisone induced a rapid (maximum at 2 weeks) increase in IGF1 levels, which was sustained during the entire treatment period. 18 ± 1.6 (mean±SE) at baseline to 24 ± 3.1 (mean±SE) at 3 months (P<0.001). IGFBP3 levels remained unchanged during the first month, and tended to rise only after 2 months. GH levels did not change during prednisone as compared to controls. 12 ± 1.6 day 0. (P<0.01). All patients returned to normal at 6 months after start of treatment.

In conclusion, long-term prednisone treatment suppresses GH levels whereas it induces a rise in circulating IGF1 (in contrast to what is generally thought). This increase in IGF1 is not due to a rise in IGFBP3 levels. These findings suggest that GH treatment is more likely to prevent prednisone-catabolism than IGF1 administration.

GROWTH HORMONE SECRETION AFTER DEXAMETHASONE STIMULATION IN HYPOTHYROID PATIENTS: EFFECT OF LONG TERM THERAPY

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In addition to direct effects on growth hormone (GH) synthesis in the pituitary, thyroid hormones may alter neuroregulation of GH secretion. Recently, it has been shown that acute administered corticosteroids are a potent stimulus of GH secretion. The aim of study was to investigate whether changes in thyroid hormones levels influence Dexamethasone (DEX) induced GH secretion in hypothyroid patients before and after long term treatment (i-thyroxine 100-150 mcg/day for 12+0.9 months). Six patients (1 F, 5 M, aged 55.1+4.5 yrs; BMI: 27.96+6.2 kg/m2) with primary hypothyroidism (T4:21.78+5.89 amol/l, TSH: 39.32+4.05 mlU/l) were studied and after long term treatment (T4: 112.41+2.45; TSH: 4.11+0.01) and their results were compared to age and sex matched control group. GH levels were measured (RIA Delphi, mlU/l) in response to DEX (4 mg) and GHRH (1 mcg/kg) injected iv, in a separate study, before and after relevant therapy. No significant difference was found between controls and hypothyroid patients in response to DEX (basal value: 1.48+0.45 vs 1.34+0.55; peak value: 11.64+2.72 vs 8.76+2.24; AUC: 199.83+256.63 vs 120.67+315.65 mlU/min) and GHRH (basal value: 2.01+0.73 vs 1.97+0.69; peak value: 22.53+7.67 vs 14.91+3.69; AUC: 187.96+525.85 vs 109.83+312.41 mlU/min) stimulation (p>0.05). GH response before and after long term therapy to DEX (basal: 1.13+0.41; peak: 5.86+1.05; AUC: 110.6+312.42) and GHRH (basal: 1.72+1.21; peak: 13.53+7.82; AUC: 1114+225.12) was not significantly different (p>0.05). In conclusion, growth hormone response to acute dexamethasone stimulation was not impaired in hypothyroid patient. Long term replacement therapy didn't change growth hormone response to dexamethasone stimulation.

INFLUENCE OF ANDROGEN SUPPRESSION BY LH-RH AGONIST TREATMENT ON GH REGULATION IN THE ADULT RAT


Pubertal levels of sex steroid hormones have dramatic effects on growth hormone (GH) regulation, increasing its secretion without altering its clearance. The mechanism(s) by which the gonadal steroid hormones influence GH secretion is uncertain. Moreover, it is well known that males with precocious puberty have higher levels of circulating IGF-I than normal children of similar chronological age: after gonadal suppression with a gonadotropin-releasing hormone agonist (LH-RH agonist), IGF-I levels and mean spontaneous nocturnal GH concentration decrease. Presently, LH-RH agonists are used to obtain gonadal suppression in multiple situations besides precocious puberty. Thus, the aim of this work was to analyze the effect of gonadal suppression with a LH-RH agonist on pituitary GH in the adult rats. 90-day-old male Wistar rats (i.e. adult or post-pubertal rats) were treated subcutaneously with high doses (65µg/day) of an LH-RH agonist (Leuprolrelin) for one month; controls received vehicle alone. After sacrifice, blood samples were collected from cervical vessels and pituitaries processed for peptide and RNA extraction. Peptides were quantified by RIA and mRNA by Northern-blot. Following LH-RH agonist treatment, testosterone levels were undetectable, serum GH levels dramatically decreased (p<0.01 vs control group), pituitary IR-GH increased (p<0.01 vs control group) without modification of GH mRNA signal level and pituitary IGF-I content increased (p<0.05 vs control group)

This study shows that treatment with LH-RH agonist interferes with GH secretion, without affecting pituitary GH gene expression, leading to a GH deficiency situation. Consequently this indicates that androgens may influence the somatotropic axis in the adulthood, with a stimulatory effect.

THE RELATIONSHIP BETWEEN GROWTH HORMONE EXERCISE RESPONSE, BRANCHED-CHAIN AMINO ACID CHRONIC INTAKE AND LEAN MASS CHANGE IN TRIATHLON ATHLETES


Many studies have been made to investigate the anabolic effects of Growth Hormone (GH), and of some of these studies showed that GH-induced protein synthesis increases after training program in athletes, others haven't the same results. Our previous works showed how oral intake of branched chain amino acids (BCAA), main amino acids oxidized by muscle during physical exercise, can affect athletic performance. Others demonstrated that acute BCAA oral intake impaired GH response during the stimulation tests. Aim of this study was to investigate the GH response during muscular exercise after oral BCAA chronic intake and their anabolic effect through lean mass changes in athletes. Eleven well trained triathlon athletes after BCAA or placebo chronic oral intake (0.2g/Kg/day) for one month, underwent a cycle ergometer exercise test at 75% of their VO2 max for one hour and the last 15 min of exhaustion. Blood samples were drawn at 30' (before BCAA intake), 0' (beginning exercise), 60' (end of exercise) and 120' (recovery) minutes to plasma GH assay. The athletes were studied after 4 h of fasting and following a standardized diet with well-known protein contents for the whole experimental period. Lean mass was assayed by bioelectrical impedanceadnometry analysis (BIA) and by anthropometric method. Results: plasma GH increments were highest at the end of exercise in treated athletes than before oral BCAA chronic treatment (60 min: 33.8 ± 13.6 vs 12.2 ± 2.0 ng/ml, p<0.05). This phenomenon is not present in the placebo group. Lean mass measurement in both BIA as well as in anthropometric method shows increase after BCAA chronic treatment (p<0.03 and p<0.01 respectively). Conclusions: the relationship between the lean mass changes and the increase in plasma GH level at the end of exercise and after BCAA chronic treatment, seems to suggest that an anabolic effect of endogenous GH to protein synthesis might be linked to BCAA chronic intake which could be oxidized saving as well as giving endogenous amino acids to protein synthesis.
**P2.153**

**FRACTURED RADIATION PROTOCOL AND GH SECRETION IN BONE MARROW TRANSPLANTATION (BMT).**
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Long term effects on growth and GH secretion in children under gone single dose total body irradiation (TBI), as conditioning regimen (GR) for BMT, have been reported. Aim of the study was to evaluate the early and late effects of the low total dose fractionated TBI on GH secretion after BMT in adult. We studied 12 pts (7 M, 5 F; 16 to 73 years, mean 30) with refractory chronic myelogenous leukaemia in the chronic phase. GR consisted of cyclophosphamide (60 mg/kg iv at 6th and 7th day before BMT) plus TBI (170 mrad three time at day on 2nd and 1st day before BMT, total dose 1000 mrad, dose/rate 25 mrad/min).

Before GR, GH levels were similar in all pts (1.5±0.2 ng/ml) but one (a 36 year old woman) showed a markedly higher value (14 ng/ml) even though within the normal range, in the first month after BMT an increase (+p=0.02) of GH serum levels (5±4±1.2 ng/ml) has been observed, 30-60 days after BMT, GH levels were 2.4±0.7 ng/ml, 90-180 days after BMT, GH values were similar to those detected before GR (1.4±0.2 ng/ml) even in the patient with the initial higher value (11.2±9.2 ng/ml). 180-360 days after BMT, an unexpected increase of GH (7.0±3.5 ng/ml) has been observed in 5/7 pts; in 3 of those, THN-test has been performed and a paradoxical increase of GH (peak 206, 76 and 51.4±0.1, respectively) has been observed. It is conceivable that the fractionated radiation protocol and the low total dose might induce, in adult undergoing BMT, an early and/or late involvement of hypothalamo-pituitary axis which regulates GH secretion.

**P2.154**

**THE VALUE OF PLASMA IGF-I CONCENTRATION AND ORAL GLUCOSE TOLERANCE TEST IN THE DIAGNOSIS OF ACROMEGALY.**
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Plasma IGF-I level is considered as an indicator of the preceding 24-48 h overall GH secretion and is therefore recommended to assess the activity and the effect of therapy of acromegaly. Three years with acromegaly were studied, 13 before treatment. 20 after transsphenoidal surgery. In all patients basal plasma IGF-I levels (normal range 0.57-2.2 U/ml, Nichols RIA kit) and serum GH values were determined during an OGTT (normal suppression: GH decreases to <2 µg/l). All 9 patients with normal plasma IGF-I levels had normal GH suppression during OGTT. In 19 patients with elevated plasma IGF-I levels the serum GH values did not decrease to <2 µg/l. In 5 patients, despite the elevated plasma IGF-I levels (2.61-3.85 U/ml), the GH suppression during OGTT was normal. In active disease defined by the elevated IGF-I level, a significant positive correlation (p<0.05) was found between basal GH concentrations (mean of two morning fasting measurements), the area under the GH curve during OGTT, and plasma IGF-I levels. Conversely, when activity was defined by insufficient GH suppressibility, the above correlations could not be observed. In conclusion, we consider the plasma IGF-I level a screening test for acromegaly. Its use as an index of activity of the disease and to assess the exact efficacy of therapy is appropriate only in patients with normal values, in cases of still elevated plasma IGF-I concentrations it is necessary to perform also an OGTT.

**P2.155**

**PLASMA IGFBP3 MEASUREMENT IN THE ASSESSMENT OF ACTIVITY OF ACROMEGALY.**

The assessment of activity of any of the therapeutic modalities (surgery, radiotherapy, or medical therapy) remains difficult. IGF-I levels determination has proved to be the most reliable criterion for biochemical cure providing that the measurements are done cautiously following acid gel filtration. IGBP3, the major form of circulating IGF-binding protein, is positively regulated by GH. IGBP3 has been shown to be increased in acromegaly. In order to evaluate the potential interest of plasma IGBP3 measurements in the follow-up of treatment, 26 acromegalic patients (14 F, 12 M aged 18-65) previously treated by surgery (n=21), radiation (n=7), or somatostatin analogs (n=13) were studied. IGBP3 were measured by RIA using a commercially available kit (Ciba Corning). Normal levels (mean±SD) in 21 adults were 5.5±2 µg/ml. Mean plasma GH (IRMa) was calculated from a diurnal hourly profile and urinarian GH (uGH) levels, determined by IRMa (normal levels in adults are less than 10 ng/24h). IGBP3 was determined by RIA (following acid gel filtration (normal levels in adults are 303±60 µg/ml). While mean plasma GH and IGF-I levels were closely correlated as well as mean plasma GH and uGH (r=0.62 and 0.94, respectively), a weak correlation (r=0.36, p=0.025) was found between plasma IGBP3 and mean plasma GH levels. Eleven of the 15 (75%) acromegalic patients with normal IGBP3 levels (>17.7 µg/ml) had in fact mean plasma GH levels exceeding 3 µg/ml. A poor correlation was found between IGBP3 and uGH levels. Among the patients with normal IGBP3 levels, 75% had increased (> 422 µg/l) plasma IGF-I levels. Lastly, no significant correlation was found between IGBP3 and uGH. Sixty-five percent of the patients with normal IGBP3 levels had an uGH above 15 µg/ml.

The conclusion of the present study is that IGBP3 measurements using a commercial kit appeared much less reliable than evaluation of IGF-I levels in the assessment of activity of acromegaly.

**P2.156**

**PLASMA IGFBP3 AND IGF-I MEASUREMENTS REFLECT GH PRODUCTION RATES IN HUMANS EQUALLY WELL.**
1 Dept. Clinical Chemistry and Endocrinology, University Hospital, Leiden, the Netherlands. 2 Dept. Medicine, University of Virginia, Health Science Center, Charlottesville, Virginia, U.S.A.

GH secretion patterns of 11 patients with active acromegaly, 14 operated and cured patients and 17 age and gender matched controls were established by measuring GH concentrations in blood sampled every 10 min over 24 hr with highly sensitive time resolved immunofluorescent assay. Investigated persons were hospitalized for this time, lights off 23.00 - 08.00 hr, and got 3 meals at 08.00, 12.30 and 18.00 hr. By multipleparameter deconvolution analysis the total GH production rate (GHPR) in mU/L distribution volume/day was estimated. Plasma IGF-I and IGBP3 concentrations were measured in the first sample of each series. Measurement of IGF-I was by a RIA after extraction and purification on ODS-silica columns. Detection limit: 1.5 nmol/l, coefficient of variation (VC) for interassay results: 12.5 - 16.3% at different levels. Measurement of IGBP3 was by a RIA, after 1/100 dilution. Detection limit 0.025 mg/l, interassay VC = 4.8 - 6.1% at different levels. The regression analysis of the IGF-I levels versus the logarithm of the GHPR showed a correlation coefficient r = 0.749 (p<0.001), whereas for IGBP3 r = 0.776 (p<0.001). No significant difference was detected between these correlations. Conclusions: In humans there is a good correlation between plasma IGBP3 levels and the GH production rate. For technical reasons IGBP3 measurements can be preferred over IGF-I detections - performance is easier, VC's lower and the clinical information equals that of IGF-I.
**P2.157**

SYNERGISTIC ACTION OF GHRP-6 AND GHRH ON GROWTH HORMONE (GH) RELEASE IN PATIENTS WITH ACROMEGALY

Popović V., Damjanić S., Petakov M., Micic D., Djurović M., Dokić M., Dieguez C., Casanueva F., Institute of Endocrinology, University Clinical Center, Belgrade YU, Endocrine Section, Santiago de Compostela University, Spain.

Synergic action upon growth hormone (GH) release by GHRP-6 and GHRH has been shown in healthy subjects, in obese subjects and in eutopic acromegaly. We studied ten patients with newly diagnosed acromegaly due to pituitary adenoma, 6 male and 4 female aged 25-70 yrs (mean 45 yrs). GHRP-6 (Peninsula, UK) 100 mcg i.v. bolus was administered at 0 min. Another day patients received 100 mcg i.v. bolus of GHRH (GHRH 1-29, Serono Spain) and on another occasion GHRH 100 mcg + GHRP-6 100 mcg i.v. bolus. Plasma GH levels (Delfia Pharmacia, mU/L) were measured every 15 min for 2h. The data are presented as mean peak GH levels and as area under the secretory curve (AUC) and tested by Students t test. Mean basal GH level was 77 +/- 36 mU/L while mean peak GH level after GHRP-6 was 198 +/- 48 mU/L (p<0.05), after GHRH was 142 +/- 40 mU/L (p<0.05) and after GHRH + GHRP-6 was 259 +/- 45 mU/L (p<0.01). AUC after GHRH was 12638 mU/L/min. AUC after GHRP-6 was 15249 mU/L/min while after GHRH + GHRP was 19215 mU/L/min (p<0.05). In conclusion the greatest release of GH in our patients was after GHRP-6 + GHRH meaning that GHRP-6 and GHRH act synergistically upon GH release from somatotroph adenoma. Three acromegalics who did not respond after GHRH responded by further GH discharge after GHRP-6 (p<0.05) suggesting synergistic action upon maximally stimulated adenylate cyclase in the adenomas of these patients.

**P2.158**

GH SECRETION IN ACTIVE AND CURED ACROMEGALY

G. van den Berg, M. Fröhlich, J.D. Veldhuis and F. Roelofsma.

Department of Endocrinology, University Hospital, Leiden, The Netherlands and Department of Medicine, University of Virginia Health Science Center, Charlottesville, Virginia, USA.

GH secretion patterns were studied in 14 recently transphenoidally operated patients by measuring GH concentrations in blood sampled every 10 min over 24h with a highly sensitive time-resolved immunofluorescent assay. Plasma GH concentrations were analyzed with a multiparameter deconvolution technique. Since we found a highly significant difference in GH secretion between male and female controls, the results obtained in patients were compared with their gender- and age- matched controls. Patients with active acromegaly displayed a significantly larger number of deconvolution-estimated secretory bursts (30/24h in males and 28/24h in female patients). The estimated secretion rate per 24h was 25 times larger in female and 100 times larger in male acromegalics than in the controls. In patients with active acromegaly about 30% of GH was secreted in a non pulsatile fashion. In contrast, normal subjects and patients shortly after pituitary surgery secreted GH predominantly (>99%) in a pulsatile way. By deconvolution analysis, the mean plasma half-life of GH was 19.7 min in treated male patients and 19.5 min in treated female patients (NS vs controls), estimated mean total GH production/day 188 µg in males and 240 µg in females (NS vs controls) and the number of secretory bursts/24h 19.3 in male patients and 21.9 in female patients (NS vs controls). The present data suggest that the basic abnormality of acromegaly resides in the pituitary gland rather than in the hypothalamus.

**P2.159**

THE LEAN BODY MASS IS INCREASED WHILE TOTAL BODY CALCIUM IS NORMAL IN PATIENTS WITH ACTIVE ACROMEGALY.

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Departments of Endocrinology, Odense University Hospital and Aarhus AmtsSygehus, Denmark.

Dual X-ray absorptiometry (DEXA) is an accurate, simple and non-invasive method for simultaneous investigation of body composition and bone mineral content. Growth hormone affects bone turnover and has profound effects on body composition as assessed by other methods. DEXA-scans were performed in 12 patients with active acromegaly and 24 healthy age- and sex-matched controls using a Hologic QDR-1000W.

<table>
<thead>
<tr>
<th></th>
<th>Acrromegaly</th>
<th>Controls</th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (Yr)</td>
<td>52.2 (15.5)*</td>
<td>52.3 (15.2)*</td>
<td>NS</td>
</tr>
<tr>
<td>sex</td>
<td>SF: 7M</td>
<td>10F: 14M</td>
<td></td>
</tr>
<tr>
<td>fat mass, kg</td>
<td>12.2(5.71-30.93)</td>
<td>13.9(7.66-27.08)</td>
<td>NS</td>
</tr>
<tr>
<td>lean body mass, kg</td>
<td>63.00(42.95-79.83)</td>
<td>47.39(34.17-71.52)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>fat fraction</td>
<td>13.5%(8.7-37.1)</td>
<td>22.6%(9.5-38.1)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>lean fraction</td>
<td>83.2%(61.6-88.8)</td>
<td>74.58%(59.07-87.0)</td>
<td>p=0.03</td>
</tr>
<tr>
<td>total body mass, kg</td>
<td>81.81(50.32-95.98)</td>
<td>67.85(49.73-94.09)</td>
<td>p=0.03</td>
</tr>
<tr>
<td>bone mineral content, kg</td>
<td>2.31(1.66-3.32)</td>
<td>2.38(1.66-3.27)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Age: mean (SD). All other observations: median (range).

Conclusion: The total body mass is increased in acromegaly and this appears to be entirely due to a greater lean body mass. Absolute fat mass and bone mineral content do not differ from that of healthy controls.
P2.160

CIRCADIAN PROFILE OF BLOOD PRESSURE IN PATIENTS WITH ACTIVE ACROMEGALY.

C. Matrella, M. Terruso, S. Luceri, M. Borriero, A. Boccazzi, G. Raimundo, A. Pia, E. Rovero, **F. Cesario, A. Angeli. Department of Clinical and Biological Sciences, Chair of Internal Medicine, S. Luigi Hospital, University of Turin and *Endocrinology, S. Croce Hospital, Cuneo, Italy.

Circadian rhythm of blood pressure (BP) is not apparent in some endocrine hypertensions. Although acromegaly is frequently associated to hypertension, little is known about 24-h profile of BP in this condition. BP profiles were evaluated by a portable, automatic oscillometric device (Spacelabs monitor 90207, Kontron) in 17 patients (pts) with active acromegaly (9 F-8 M, aged 27-85 years, with a disease duration of 4-19 years), and in 30 healthy, normotensive, age-matched subjects. BP was measured every 30 minutes for 24 hours; circadian parameters were assessed by single Cosinor computation. 8/17 acromegalics had a mid-moderate hypertension. A family history of essential hypertension was found in 9/17 pts but it was not associated with actual hypertension. 5/17 (29%) pts with active acromegaly showed mean 24-h systolic BP >2 SDs from healthy subjects. This figure was 8/17 (47%) for diastolic BP. Circadian rhythmity of BP was normally apparent only in 5/17 (29%) pts with active acromegaly for systolic and in 7/17 (41%) for diastolic BP, respectively, in comparison to 83% and 93% in healthy subjects (p<0.005). The alteration of BP rhythmity consisted in an increased variability of BP values along the 24-h period or in a flattening of BP profile. No correlation was found between mean 24-h systolic or diastolic BP and demographic, hormonal (IGF-I), mean 24-h GH levels and clinical data of our pts. To conclude, BP profile in active acromegaly is often altered independently of the presence of hypertensive values. It may be hypothesized that GH/IGF-I hypersécrétion acts indirectly through the induction of factors able to regulate the circulating volume and/or sympatho-vagal balance.

P2.161

SIGNAL AVERAGED ELECTROCARDIOGRAM PATTERNS IN 22 PATIENTS WITH ACROMEGALY


INTRODUCTION: An increased prevalence of heart rhythm disturbances, correlated to acromegaly have been described. Signal-averaged electrocardiograms (SAECG) have been used to identify small signals in the terminal QRS called "late potentials". SAECG has been demonstrated to be a useful, non-invasive test to detect patients at risk for ventricular tachyarrhythmias. For this reason, we studied the prevalence of SAECG in acromegaly in relation to the disease's activity parameters. PATIENTS AND METHODS: 22 acromegalics patients were studied (50±2 yrs), of whom 11 untreated (U) and 11 treated (T). The parameters studied included: 1) the filtered QRS duration (QRS) 2) the duration that the filtered QRS complex remained below 40 µV (IAPS) and 3) the root-mean-square voltage of the terminal 40 ms of the filtered QRS (RMS). A t QRS >144 ms, a IAPS >32 ms and a RMS <20 µV defined a late potential. Late potentials were considered to be present (+) if any two or all three of the described criteria were met. The signal was studied in three different frequencies (25,40,80:250 Hz) and a score from 0 to 9 was obtained. RESULTS: 9/22 (41%) patients had abnormal SAECG.

<table>
<thead>
<tr>
<th>SAECG</th>
<th>(U)</th>
<th>(T)</th>
<th>Lean Mass % (LM)</th>
<th>Cellular Mass Kg (CM)</th>
<th>IGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentile</td>
<td>(1) (3)</td>
<td>(1) (3)</td>
<td>(U)</td>
<td>(T)</td>
<td>83±2</td>
</tr>
</tbody>
</table>

The score was significantly related with LM (p<0.04), water % (p<0.05), CM (p<0.002), IEG-1 (p<0.01). CONCLUSIONS: In conclusion we observed a higher prevalence of abnormal SAECG in acromegalic patients (41%) vs normal population (0.7% literature data). The appearance of SAECG seems related to the disease's activity (IGF-1). Treated patients present a less pathologic signal (score) and improvement of RMS values.
**P2.162**

**INSULIN RESISTANCE IN ACROMEGALY.**


AIM: Acromegaly is characterized by insulin resistance (IR) and frequently is associated with impaired glucose tolerance or non-insulin dependent diabetes mellitus. The aim was to study the peripheral insulin sensitivity (SI) in acromegaly and to assess the influence of long-term octreotide (SOM 201-959) treatment.

SUBJECTS AND METHODS: We studied 7 non-diabetic acromegalic patients and used 13 B.M.I.-matched subjects as controls. All of the controls had normal glucose tolerance and no high blood pressure nor family history of diabetes. All of the subjects underwent a frequently sampled intravenous glucose tolerance test (FSIVG). Six acromegalic patients were treated with octreotide (0.1 mg s.c. / 8h) for 4 weeks and the FSIVG was repeated. On the day of study octreotide was withdrawn. SI was estimated using the Minimal Model method. Glucose disappearance rate (Kg) and insulin-mediated glucose uptake (IMGU) were also calculated. Insulin secretion was calculated as the area under the curve (AUC). For comparisons we used the Mann-Whitney and Wilcoxon tests as appropriate. Data are shown as mean±SD.

RESULTS: The acromegalic patients had a significantly lower Kg (1.29 ± 0.36 vs. 2.03 ± 0.75 min⁻¹, p<0.02). SI (1.88 ± 1.23 vs. 3.72 ± 2.22 min⁻¹·(μU/ml)⁻¹, p<0.04) and IMGU (4.06 ± 1.70 vs. 8.29 ± 5.24 mg/Kg/min, p<0.03) than controls. We did not find any significant differences in AUC (9094±4452 vs. 7438±3551 (μU/ml)·min, NS). We found a positive and significantly correlation between fasting insulin and GH levels (Spearman's = 0.7143, p<0.05). SI increased significantly after octreotide treatment (1.91 ± 1.35 vs. 3.15 ± 2.08 min⁻¹·(μU/ml)⁻¹, p<0.05).

CONCLUSIONS: Our data suggest that IR is present in acromegaly and that octreotide improves this IR.

**P2.163**

**TISSUE SENSITIVITY IN PATIENTS WITH ACTIVE ACROMEGALY STUDIED BY BIOSTATOR EUGLYCEAMIC CLAMP TECHNIQUE.**

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The chronic growth hormone (GH) excess leads to peripheral insulin resistance in most patients with acromegaly. With a view to determining the influence of increased GH level on the peripheral tissue sensitivity, 10 patients with active Acromegaly and normal glucose tolerance were investigated using BioStator Euglycaemic clamp technique.

Material and method: 10 patients (mean ages 38.9, 30.3) and 8 normal subjects (mean ages 30.6, 11.9) were studied. The clamp study was carried at the BioStator using 6.1 program-fixed dextrose-infusion rate on a IV step (100-200-300-400 mg/min). The insulin infusion was used to estimate glucose metabolism.

Results: The mean insulin infusion rate in acromegalic patients was 123.4 91.6 - 107.5 86.2 - 193.5 122.4 - 287.1 102.0 mU/min vs 36.2 21.4 (p<0.01) ± 42.9 20.6 (p<0.05) - 95.36 61.09 - 85.44 55.0 (p<0.05) mU/ml in normal subjects. The mean GH level was 19.26 9.05 - 19.36 8.9 - 19.18 9.8 - 19.24 9.7 mU/ml during the clamp. The mean e-epidid level was 5.88 2.6 - 5.31 3.8 - 6.45 2.7 mU/ml during the clamp. We concluded that the tissue sensitivity was significantly decreased in patients with Acromegaly comparing to the sensitivity in the control group. The clamp technique used is a valuable method to detect early carbohydrate metabolism disturbance in acromegalic patients with no glucose tolerance impairments.

**P2.164**

**THE IMPROVEMENT OF PERIPHERAL INSULIN RESISTANCE AFTER PITUITARY ADENOMAS REMOVAL IN ACROMEGALIC PATIENTS WITH NORMAL GLUCOSE TOLERANCE.**

D.Babić, M. Sušić, Z. Metelko, Metabolism Department, Institute "Vuk Vrhovac", Zagreb, Croatia.

It has been previously described that growth hormone counters the effect of insulin on glucose metabolism. In order to investigate the chronic effect of human growth hormone (HGH) on plasma insulin concentrations (PIC) and peripheral insulin resistance (PIR), the euglycemic hyperinsulinemic clamp (by using BioStator with specially designed clamp algorithm, mode 911) was performed in 5 slightly obese, acromegalic patients with normal glucose tolerance (females, age 53.2 ± 5.4 years, body mass index (BMI) 31.1 ± 3.2 kg/m², fasting blood glucose 5.6 ± 0.5 mmol/L), before (HIGH basal plasma concentration 632.8 ± 249.3 pmol/L) and 6 months after (HIGH basal plasma concentration 544±12 pmol/L) the transphenoidal pituitary adenomas removal. Using the clamp at the constant low dose insulin infusion rate (40 mU/m²/min) the glucose disposal rate (GDR) was significantly higher after the treatment (4.2 ± 0.3 mg/kg/min vs 2.9 ± 0.2 mg/kg/min, p < 0.001). During the constant high dose insulin infusion rate (240 mU/m²/min), statistically significant difference in GDR was not noticed (9.3 ± 0.7 mg/kg/min., before vs. 10.0 ± 1.4 mg/kg/min., after the treatment, stat. n.s.). Fasting PIC was significantly lower after the transphenoidal adenectomy has been done (14.2 ± 3.2 mU/L vs. 21.7 ± 3.9 mU/L, p<0.05). Comparing the BMI (32.3 ± 3.1 kg/m²) but of no statistical significance. These results indicate that transphenoidal removal of pituitary adenomas improves peripheral insulin resistance (primarily by increasing insulin sensitivity) and fasting hyperinsulinemia in acromegalic patients with normal glucose tolerance.

**P2.165**

**LONG-TERM TREATMENT OF CLINICALLY NON-FUNCTIONING PITUITARY MACROADENOMAS WITH QUINAGOLIDE.**

W.W. de Herder, F.R.F.E. Nobels, D.J. Kwakkenbos, A.J. van der Lely, S.W.J. Lambertz, Department of Internal Medicine III, University Hospital Rotterdam, Rotterdam, the Netherlands.

Preliminary reports suggest a possible therapeutic role of dopamine-agonists in the therapy of clinically non-functioning pituitary adenomas (NIPA).

We studied the long-term effect of 0.225 - 0.300 mg quinagolide (CV 205-502), on the serum levels of gonadotropins (FSH or LH) and/or alpha-subunits as well as on tumor diameter as measured by CT or MR in 14 patients with NIPA, aged between 43 and 86 years (mean 68 years). In all patients, the tumor diameter was > 10 mm, but none of the patients experienced visual field defects, or a reduced vision due to compression of the optic chiasm or optic nerves. The treatment period amounted from 16 to 61 months (mean 36 months). None of the patients complained of progressive loss of vision, or deterioration of the visual fields during therapy. The patients did not complain of serious side effects of the therapy. In none of the patients there was an increase in adenoma size. However, in one patient there was a significant reduction of the tumor size from 12 to 5 mm. There was no significant change in serum FSH, LH, or alpha-subunit concentrations during therapy.

Conclusion: Quinagolide might prove to be a safe medical therapy in patients with clinically non-functioning pituitary adenomas, in whom there is no acute medical indications for transphenoidal surgery. This study suggests that it has a tumor growth inhibiting effect, as none of these 14 patients experienced tumor growth during a mean follow-up of 3 years.
P2.166

CHRONIC TREATMENT WITH CV 205-502 IN RESISTANT ACROMEGALIC PATIENTS.
D. Ferone, B. Merola, A. Colao, F. Sarnacchiari, P. Marzullo, S. Longobardi, C. Di Somma, G. Cerbone, G. Lombardi, L. Zarrilli, Department of Clinical and Molecular Endocrinology and Oncology, University "Federico II", Naples, Italy.

In the present study the results of chronic treatment with CV 205-502 alone or in association with octreotide are reported in 12 patients (4 M, 8 F; 24-62 yrs) with active acromegaly. All patients had previously undergone unsuccessful surgery and were subjected to a chronic treatment with octreotide without normalizing their GH and IGF-I levels CV 205-502 was administered at the initial dose of 0.3 subsequently increased to 0.6 mg/day. A normalization of GH and IGF-I levels was obtained in 5 patients after a 3 month-treatment with 0.3 mg/day. In the remaining 7, CV 205-502 dose was increased to 0.6 mg and was associated to combined octreotide at the dose of 0.6 mg t.i.d. for 3 months. Two out of 7 patients significantly reduced their GH and IGF-I levels with the combined treatment in comparison with the effect of each drug administered alone (GH from 50±28.3 to 4±0.3 µg/l, IGF-I from 542.5±123.7 to 162.4±46 µg/l).

In conclusion, CV 205-502 is able to normalize GH and IGF-I levels and to improve clinical symptoms in several acromegalic resistant to other therapeutic regimens CV 205-502 can, therefore, be considered an effective alternative in the medical management of acromegaly.

P2.167

OCTREOTIDE SCINTIGRAPHY VERSUS OTHER IMAGING MODALITIES IN PATIENTS WITH NEUROENDOCRINE TUMORS.

Octreotide scintigraphy (OS) (111In-DTPA-octreotide, Mallinckrodt) was carried out in 26 patients (7 men, 19 women; mean age: 52 yr) with neuro-endocrine tumors (NET) (gut (10 pts) and bronchial (5 pts) carcinoid; endocrine pancreas (6 pts), larynx (2 pts), uterine cervix (1 pt), and unknown primary (2 pts) tumors). Results were compared with all clinical and imaging data available. Positive uptake was found in 19 patients (73%). In 16 patients, all organ involvements were shown both by OS and by the other imaging procedures. In only 3 patients (15%), OS permitted the discovery of unknown tumor sites: visualization of a pancreatic tumor recurrence in one patient, hepatic metastasis in another patient with a pancreatic tumor, discovery of a pelvic uptake in a patient with a pancreatic tumor. Furthermore, in 2 patients, the positivity of OS demonstrated the metastatic nature of abnormalities already shown by other imaging modalities: osteo-medullary tumor involvement in one patient with a bronchial carcinoid, and a liver metastasis in a patient with an uterine cervix tumor.

In conclusion, OS can be useful for the work-up of patients with NET. However, to date, OS cannot replace the other imaging modalities.

P2.168

PITUITARY IMAGING WITH INDIUM-111 OCTREOTIDE (In-111-OCT) IN ACROMEGALICS CAN PREDICT THE RESPONSE TO CHRONIC OCT THERAPY.
A. Colao1, S. Lastoria2, D. Ferone1, E. Vergara2, P. Varrella2, S. Longobardi1, B. Merola1, M. Salvatore2, G. Lombardi1, 1Department of Clinical and Molecular Endocrinology and Oncology, University "Federico II" and 2Department of Nuclear Medicine, National Cancer Institute, Fondazione G. Pascale, Naples, Italy.

OCT normalizes GH and IGF-I secretion in most acromegalic patients whereas some patients do not respond satisfactorily, probably due to the presence of few somatostatin (SRIF) receptors on tumor cell membranes. In order to discriminate patients responder to OCT we evaluated the in vivo visualization of SRIF receptors by In-111 OCT in 15 patients with active acromegaly (10 F, 5 M, 14-52 yrs). OCT (Sandostatina, Sandoz) was administered acutely at the dose of 100 µg s.c. and subsequently at dose of 300 µg t.i.d., increased up to a maximum of 600 µg if serum GH and IGF-I were not normalized. The uptake of radiotracer was defined by a semiquantitative method as follows: 0=negative; 1=poor; 2=moderate; 3=intense. A significant correlation was found between tracer uptake and response after short- and long-term octreotide administration (r=0.6, p<0.05) but not after acute test (r=0.4).

In conclusion, the degree of In-111-OCT uptake within GH-secreting adenomas can predict the response to chronic OCT therapy.
P2.169

**Lp(a) AND CARBOHYDRATE METABOLISM AFTER OCTREOTIDE TREATMENT OF ACROMEGALY**

P. Madian, M. Mousapp, E. Ferretti, M. Pfeil, P. E. Fjaer, C. Martini, L. De Carlo, E. Pecorari, N. Scollo

**INTRODUCTION:** Increased Lp(a) levels, except for genetic reasons, are found 1) after administration of rGH in hypopituitary patients 2) in diabetic patients. Acromegaly is the ideal model to contemporarily study the influence of GH and carbohydrate metabolism on Lp(a) levels. Furthermore, while octreotide treatment reduces GH levels, it allows good glycemic tolerance in the same patients.

**MATERIALS AND METHODS:** 10 acromegalic patients were studied. In every single patient the following parameters have been studied: (A) and (B) Octreotide treatment for 30 days at 100 ìg x 3 s.c. (day 8-24 and 10-16/24) (sandostatin) LAR, (C) IRI (mmol/l), (D) total cholesterol(TC), (E) HDL-C, (F) triglycerides (TG) (mmol/l), (G) IGF-I (ng/ml), (H) Lp(a)(mg/dl), (I) APOA1, (J) APOB.

**RESULTS:** Parameters (A) and (B) are summarized in the following table (10 patients):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(A)</th>
<th>(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>8.00</td>
<td>4.78</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>18129±2245</td>
<td>8541±197</td>
</tr>
</tbody>
</table>

**CONCLUSIONS:** Our data show that in acromegaly, Lp(a) reduction is consensual to Octreotide-induced GH reduction and inversely correlated to carbohydrate tolerance. In conclusion, the hypothesis of a not exclusive genetic determination of Lp(a) is furthermore confirmed. Among conditioning factors, at least in acromegaly, the role of GH seems to be prevalent to carbohydrate tolerance.

P2.170

**PRELIMINARY RESULTS WITH SANDOSTATIN LAR IN ACROMEGALIC PATIENTS**


Sandostatin LAR is a new formulation of octreotide incorporated in microspheres of poly(D,L-lactide-co-glycolide). We report results obtained in 20 acromegalic patients (9 males and 11 females, aged 35-79 years) who, in the frame of a multicentre double-blind study, received either 20 or 30 mg single dose of Sandostatin LAR (10 patients/dose). The patients (pts) selected for the study were to be responsive to s.c. octreotide with a mean 12-hour GH concentrations of 5 ng/ml. Serum GH and octreotide concentrations were hourly assessed from 8.00 a.m. to 8.00 p.m. on days -14 (during the s.c. octreotide treatment), 0 (after at least 2-week wash-out period), 1 (day of the i.m. injection of Sandostatin LAR), 7, 14, 21, 28, 35, 42 and 60. After an initial octreotide peak (with no drug-burst) noted on day 1, day of the injection of SMS LAR, plateau octreotide concentrations were observed in all pts from days 7-14 to days 42-60. No fluctuation of octreotide concentration was noted during the 12-hour profile on the days of assessments. On day 1, GH secretion was markedly reduced in all but 3 pts and a consistent GH<2 ng/ml and long-lasting (28-60 days) suppression of GH secretion was achieved in 16/20 pts. Gastrin levels were recorded in 2/20 pts at the ultrasonographic examination made on day 60 after SMS LAR. Both pts had been already treated with s.c. octreotide for 1 and 48 months respectively and 1/2 pts had already developed stones during the s.c. octreotide. Both local and systemic tolerability was very good and no impairment of hematological and biochemistry safety tests was recorded. These results show that Sandostatin LAR is very effective in attaining a consistent and long-lasting suppression of GH secretion in acromegalic. The therapeutic efficacy of Sandostatin LAR (which could be administered every 4-6 weeks) compare very favourably with the chronic s.c. octreotide treatment.

P2.171

**SANDOSTATIN LAR: A NEW THERAPEUTIC TOOL IN ACROMEGALY (DATA IN 24 PATIENTS)**


Sandostatin (SMS) administered s.c. is widely used to successfully control acromegaly. SMS LAR - consisting of microspheres of DL-lactide-co-glycolide polymer containing octreotide - has been developed for monthly injections in acromegalic patients (pts) who require b.i.d. or t.i.d. s.c. inj. of SMS. Data collected in 24 acromegalic pts (16 men, 16 women, aged: 14-58 years) who received one year treatment with SMS LAR showed that at doses of 10, 20, 30 and 40 mg SMS LAR has a good local and systemic tolerability. No impairment on hematology and biochemistry safety tests was noted during 1 year follow-up. On repeated echographic examinations of the gallbladder region, biliary microlithiasis was diagnosed in 3/24 pts after 10-12 months' exposure to SMS LAR. A rapid, marked and long-lasting (6-6 weeks) suppression of the GH secretion was already noted after the first inj. Consistent suppression of GH secretion to below 5 µg/L (mean of 8 or 12 hours GH concentrations) was noted in 16/24 acromegalic pts. In 8/16 pts, this suppression was even below 2 µg/L. In 8/24 pts who were only partial responders to a previous s.c. SMS treatment, the monthly inj. of SMS LAR produced a more important and consistent suppression of GH secretion than the s.c. treatment. An important clinical improvement was noted in all pts. More than 50% reduction in tumor volume was documented by CT-scan in 3 pts who were assessed prior to and 11 months after the start of the SMS LAR therapy. The compliance of patients and the acceptability of the treatment with SMS LAR was very good. Based on the efficacy and tolerability data collected in our patients, we suggest that SMS LAR could be considered the long-term medical treatment of choice in acromegalic pts.
P2.173

**OCTREOTIDE NASAL POWDER: A PRELIMINARY STUDY IN ACROMEGALY**

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Repeated subcutaneous injections of Octreotide (OC) have to be given to maintain IGF-I normalization in responsive acromegalics.

A new octreotide formulation has been recently developed: the Sandostatin Nasal Powder (SNP). Six acromegalic patients were selected on the basis of a good responsiveness to the previous s.c. OC treatment. After a 15 day wash-out period, they were enrolled in a 6 month double blind study to assess the effects of different doses of SNP (as a part of a multicenter trial). Two pts received 0.25 mg tid, two 0.5 mg tid and two 1.0 mg tid. GH, IGF-I, IGFBP3, IGFBP3 were assessed at: 0, 14, 28, 56, 84, and 182 days. Results:

<table>
<thead>
<tr>
<th>Pt</th>
<th>GH</th>
<th>IGF-I</th>
<th>GH/IGF-I</th>
<th>GH/IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>-14</td>
<td>-14</td>
<td>0</td>
<td>0</td>
</tr>
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<td>22.2</td>
<td>616</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>225</td>
<td>0.5</td>
<td>490</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>124</td>
<td>9.4</td>
<td>362</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
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<td>13.3</td>
<td>437</td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>105</td>
<td>3.8</td>
<td>183</td>
</tr>
</tbody>
</table>

Legends: dose; ng; GH: ng/ml (mean of 8 hourly samples); IGF-I: mg/l (80-300); d.d.: drop out; -14: 10th OC at tid; 14: off therapy; 14 and 182: first and last control (day).

IGFBP3 increased as expected, after 3hrs in all patients (from 1.4 ± 0.3 ng/ml to 11.5 ± 8.2 ng/ml at 32nd day); IGFBP3 significantly decreased in parallel with IGF-I changes. Side effects: astrophic rhinitis and nasal itching were observed in all the patients. Conclusions: the intranasal route seems an effective way of administering OC, as GH and IGF-I levels remain suppressed even during long term treatment, however local tolerability in our patients was not fully satisfactory.

P2.174

**SANDOSTATIN NASAL POWDER: EFFICACY EVALUATION IN ACROMEGALY**

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Sandostatin nasal powder (SNP) consisting of octreotide and Avicel (microcrystalline cellulose powder, 16 mg/capsule) was administered intranasally t.i.d. to acromegalic patients (pts) responsive to s.c. Sandostatin. We report data on a double-blind, 6-month study which was carried out in 8 pts (4 men and 4 women, aged 35-85 years) to compare the effect of 0.1 mg t.i.d. s.c. octreotide with the intranasal application of 0.25 mg, 0.5 mg and 1.0 mg t.i.d. of SMS NP on serum GH and IGF-I concentrations. GH profile was assessed for 8 hours (by hourly sampling) on days -14 (during the s.c. Sandostatin treatment), 0 (after at least 2-week wash-out after s.c. treatment), 14, 28, 56, 84 and 182. IGF-I was determined at 08.00 a.m. at each visit. A marked and consistent suppression of GH secretion was observed in all but one patient, regardless of the dose. Mean 8-hour GH concentrations decreased to < 2 ng/ml in 6/8 pts and to 3.5 ng/ml in another pt. IGF-I was normalised in 2/8 pts. The local tolerability assessed by rhinocopy at each visit was good; 4/6 pts reported few episodes of sneezing at the beginning of the treatment. The systemic tolerability was very good. No newly occurring gallstones were recorded during the trial. No impairment of safety tests was noted.

The results show that SMS NP is effective in suppressing GH secretion and its efficacy compare favourably with the s.c. treatment.
SOMATOSTATIN ANALOG, TT2-32 INDUCES A BIPHASIC ACTIVATION OF PHOSPHOTYROSINE PHOSPHATASE IN HUMAN TUMOR CELL LINE, SW620
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Somatostatin has been demonstrated to activate phosphotyrosine phosphatase in the human pancreatic cell line MIA PaCa-2. In this work we have studied the effect of a somatostatin analog, TT2-32 on the phosphotyrosine phosphatase activity in SW620 human colon tumor cell line. TT2-32, a selective analog showing no effect on GH release, caused a strong inhibition of cell proliferation. In response to TT2-32 we found a rapid and sustained increase (5 to 30 min) in phosphotyrosine phosphatase activity showing two maxima at 0.1 and 30 uM concentrations, respectively. Tyrosine kinase activity was much less affected by TT2-32 during short term incubations. The TT2-32 induced activation of phosphotyrosine phosphatase may be an important early step in the inhibition of cell proliferation in colon carcinomas.