The subcellular defects in the androgen insensitivity syndrome

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ABSTRACT

The androgen insensitivity syndrome (AIS) was studied with consideration of the complexity of mechanisms involved on the intracellular level: testosterone (T) and dihydrotestosterone (DHT) receptors and the androgen-5α-reductase (A5R). Five children with "normal" female external genitalia (group A) and three patients with variable forms of ambiguity (group B), ages 1 to 18 years, were studied. Tissue specimens from genital skin were analysed for the Kd- and Nmax-values of the cytosolic and nuclear T- and DHT-receptors, as well as for the Km- and Vmax-data of the tissue specific A5R. The enzyme analyses were performed with a kinetic method. Results show that patients from group A mainly lack action of the nuclear DHT receptor, combined with reduces binding capacity in the cytosol. T binding was poor in both, cytosolic and nuclear fractions, respectively. Results of group B proved to be more inhomogenous, ranging from total absence of a DHT receptor to normal binding capacities in the nuclear fractions, accompanied by decreased cytosolic Nmax values for that ligand. T binding was poor in all patients of group B in the cytosolic and nuclear fractions, respectively. A5R was qualitatively normal in all patients examined, except one, but decreased enzyme activities could be observed in a wide range. In summary, the study confirms the complex mechanisms, presenting as AIS clinically. Moreover a close relationship between abnormalities of androgen receptor function and changes in A5R activity could be evaluated, thus confirming the recent theories about intracellular androgen action.

INTRODUCTION

The pathogenetic mechanisms, responsible for the development of male pseudohermaphroditism syndromes, are manyfold. When the endocrinological concept of so-called "endorgan defects" had been postulated this hypothesis was also applied to the action of androgens. The respective disease entities, originally denominated according to clinical signs and symptoms, like "hairless women with testes" (Wilkins 1950) or "testicular feminisation" (Morris 1953), were consequently given other names like "androgen resistance syndrome" (Griffin 1980) and, with still more exactness, "androgen insensitivity syndrome (AIS)" (Migeon et al. 1979). The latter term incorporates explicitly the dysfunction of the peripheral mechanism for the utilisation of androgen hormones, normally providing a correct masculinisation of the external genitalia. The postulated defect(s) of the androgen receptor system could be proved by laboratory methods in the meantime (Keenan et al. 1974, Amrhein et al. 1976). Not only quantitative deficiencies could be shown but also dysfunction in the quality of the receptor mechanism was found. However, not all aspects of the very complex problem are explained so far (Migeon et al. 1981).
The interdependency of the main androgen steroid testosterone (T), and the "terminal" effective metabolite dihydrottestosterone (DHT), as well as the enzyme, forming DHT out of the substrate T, the tissue specific androgen-5α-reductase (ASR), is an essential part of the relevant receptor mediated intracellular mechanisms. The complexity of actions, interactions and feed-back mechanisms is certainly not the final step responsible for the gene expression of androgens, but it is an important part for their efficiency on the cellular level. Therefore, we decided to study the mechanisms in their complexity as far as possible. In addition, normal control values, partly established in our own laboratory with new methods (Herkner et al. 1985), were used for comparison.

PATIENTS AND METHODS

Five children with "normal" female external genitalia (=group A) and three children with variable forms of ambiguity (=group B), age between 1 and 18 years, were studied. All of them had a normal male chromosome pattern 46XY, and defects in steroid hormone biosynthesis had been excluded previous to this study. 52 boys, age 1–14 years, who were brought to circumcision, without other genital anomalies, served as controls.

Examinations in genital skin:

Tissue specimens from genital skin, taken at operations or at diagnostic procedures (with parental consent), were used for the studies of receptor- and enzyme-dependent mechanisms, specific for androgen actions.

Receptor studies (Herkner et al. 1986)

Tissue specimens were homogenized in a 50 mM TRIS-buffer (pH=7.2 - 7.4), containing 20 nM sodium molybdate (Wright et al. 1981, Durrant & Durrant 1982), and the subcellular fractions were isolated by sucrose gradient centrifugation. Saturation analyses with the cytosolic and nuclear fractions, separated previously, were performed at 37 °C for 30 min. Tritiated T and DHT were used as ligands in increasing concentrations (0.1–10 nM). Parallel incubations with a 200 fold molar excess of radioinert ligands were done in order to evaluate specific binding. The separation of bound and free steroids was done by a dextrane coated charcoal technique. The specific binding data were plotted according to Scatchard (1949) and the calculation of results were made following Rosenthal (1967) and Rodbard (1973).

Androgen-5α-reductase assay (Herkner et al. 1985)

The tissue specimens were homogenized in a phosphate buffer (pH=6.8), 252 nM NADPH was added (Kaneyuki et al. 1959), followed by incubation with increasing concentrations (8-208 nM) of tritiated T for 1hr at 37 °C. Separation of reaction products was obtained by thin layer chromatography (TLC), and radio gas chromatography (RGC) was used for control of the specific radioactivities of the reaction products (Herkner et al. 1985). Calculation of the enzyme kinetic data was done by determination of the reaction velocities of processes forming the metabolites DHT, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol, respectively. Classification and quantitation of the enzyme was achieved by calculating the Km- and Vmax-values, according to Lineweaver-Burk, taking into consideration the classical biochemical parameters parameters by following the hypothesis of Michaelis-Menten. All calculations and evaluations of data, mentioned above, were performed computer aided (on line) by a laboratory computer system.

RESULTS

Receptors: The results are summarized in table 1. Taken as a whole, the laboratory findings are in agreement with the clinical separation of our patients in groups A and B. All patients of group A lack an
active nuclear receptor for DHT, except for pat.4 who has one, but with markedly reduced capacity. Though pat.1 presents with a Nmax of 158 (fmol/mg), this receptor cannot be classified as a specific androgen receptor, according to the Kd-value. In addition, the patient proved to have an atypical A5R (see below). The binding capacity of the cytosolic DHT receptor is significantly reduced in patients 1 to 5, too. The T receptor show subnormal binding capacity in the cytosolic as well as in the nuclear fractions. Pat.2 has a nuclear T receptor with a qualitative defect. In group B, pat.6 shows interesting results: the nuclear DHT receptor is absent and the T receptor has a Kd within the normal range, but Nmax values being even slightly above normal for his age. A5R in this patient proved to be extremely reduced. The results of pat.7 and 8 are similar: normal nuclear, but reduced cytosolic binding capacities for DHT. In contrast to pat.6, they both had no nuclear and very low cytosolic T binding, thus focusing a primary T receptor deficiency.

Androgen-5α-reductase: The results of the tissue specific values Km and Vmax are summarized in table 1, too. With the exception of pat.1, all the Km-values were within the normal range. This means that the enzyme was in fact substrate and tissue specific. But all Vmax-values were subnormal, compared with our large and and age-matched control group (Herkner et al. 1986). Out of the whole AIS-group A and B, only pat.6 showed significantly reduced enzyme activity.

Normal controls (Herkner et al. 1986): Our own normal control values are incorporated in table 1. Only the Nmax-values of our receptor studies are presented graphically in figure 1, due to age dependent fluctuations of data.

Figure 1: Normal values for the androgen (T/DHT) receptor binding capacity (Nmax). Age dependent distribution of data. Receptors were evaluated in the cytosolic and nuclear fractions of foreskin tissues of 52 boys, age 1-14 years. Bars represent mean values of age groups as indicated.
Table 1: Results of receptor analyses and A5R assays. Receptor evaluations were performed using T and DHT as ligands. Data for specific binding were obtained by parallel saturation analyses with a 200 fold molar excess of radioactive ligands. A5R determination was done by an enzyme kinetic method in order to obtain Km- and Vmax-values.

Group A: patients with "normal" female external genitalia. Group B: patients with variable forms of ambiguity.

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ad*: Nmax ranges under age dependent fluctuations. Data are therefore plotted in fig.1.
DISCUSSION

The concept of the action of androgens on the subcellular level, presented by Chan & O'Malley (1976), does not fully explain the variability of findings, obtained in the studies of peripheral androgen insensitivity and/or "resistance". In agreement with that concept, the following steps of action are essential: a) The binding of the "terminal" androgen DHT to a specific receptor protein in the cytoplasm, b) the translocation of this complex into the nucleus, mediated by another "receptor" at this site, and c) followed by an "acceptor" within the chromatin matrix. At this point the transcription to a messenger-RNA takes place, causing the synthesis of the new "virilizing protein", which will manifest the hormone-induced stimulus on the genital structure.

Our studies cannot give informations about those last steps in the chain of actions, of course. But in contrast to the concept of Chan & O'Malley (1976), they support the theory that not only a receptor system for DHT exists, but that also one, specific for binding T must be postulated in androgen dependent tissues (Mainwaring 1977, Liao et al. 1984). Though the mechanism for passing the cell membrane for T is not quite clear our findings support the existence of a specific receptor system, mediating the transport and localisation of T in the cytosol, where it serves as the substrate for the A5R to produce DHT. At the same time, it demonstrates very well the interdependency of receptor- and enzyme-mediated processes in such conditions.

The classification of patients with complete absence of androgen receptors, established as a clear heritable disease-entity (Keenan et al. 1974) as being the classical "complete type of AIS" is beyond discussion. The patients 1-5 of our group A fit into that concept. The difficulties begin with the interpretation of the so-called "receptor-positive" cases of the complete type of AIS (Brown et al. 1982, Kaufman et al. 1979, Grunstein et al. 1982), and they increase with the incomplete, mild or partial forms of the AIS (Chabab & Sultan 1985, Sultan et al. 1983, Migeon et al. 1985), with variable receptor findings. In the light of these complex and complicated mechanisms, outlined earlier, attempts for a subclassification of the AIS, according to quantitative, as well as to qualitative criteria of intracellular mechanisms are justified. But in addition to that, a special aspect of the defects related to androgen actions emerges from our study, and this is the role of the A5R within the whole system. The existence of a hereditary, primary defect of this enzyme, with the consequence of deficient virilisation of the external genitalia in the male is established. However earlier studies (DeLarminat et al. 1981), as well as our own presented here, demonstrate the possibility of secondary A5R-deficiency in cases of the incomplete AIS. "Down-regulations" of the DHT receptors as a consequence of deficient production of the ligand is quite understandable, of course, while the opposite "feed-back" is not. However, our observations of pat.7 and 8 would support a theory (Jukier et al. 1984), according to which the A5R activity is directly positively related with the number of nuclear DHT receptor binding sites. In addition, the results of our study suggest that also a significant reduction of the postulated T receptor should have a negative influence on the intracellular enzyme activity (see group B). The latter phenomenon could be interpreted that "in vivo" only a substrate which is correctly bound to a receptor will be accepted by the enzyme A5R.

CONCLUSION

An intracellular binding protein (cytosolic receptor) is an essential intermediate step for bringing the informations, contained in the hormone, to the geneexpressive target. Within the cell the receptor-
hormone complex is subjected to several mechanisms of activation and desactivation, involving specific enzyme(s), as well as to changes in conformation during translocalisation. In addition, the intracellular migration times and nuclear "stand-by" periods are further characteristics for a successful expression of the gene. Altogether these actions form parameters for the evaluation of the hormone effects as such, which still deserve clarification. This concept demonstrates that the analyses of endorgan defects is much more complicated than previously thought. An essential prerequisite for obtaining generally acceptable results is the reproducibility and comparability of this kind of data and of the very delicate laboratory methods. The analytical difficulties of tissue and cell culture examinations are numerous, and we wish to stress here only the need for considering very carefully the basic demand of biochemistry, e.g. in A5R-determinations (Herkner et al. 1985). Only on such grounds, comparable figures from one research group to the other will be obtained.

FINAL COMMENT

Androgen insensitivity in the periphery has grown out from a concept which primarily was thought to be rather simple. The ineffectivity of the hormone, total or incomplete, has split into a bundle of causative defects. Sophisticated laboratory methods have improved our insight into the machinisms involved, however new questions, still unanswered as yet, arose at the same time. From the practical point of view the unsolved questions certainly are of minor importance, as a non-response of the tissues to the androgens does not allow any other conclusion than to attribute all these variants of the "XY-females" to the female gender role, which includes the consequence of estrogen hormone substitution and possibly plastic surgery.

REFERENCES


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402