

INVITED REVIEW

Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions

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Abstract

Proopiomelanocortin (POMC) is the polypeptide precursor of ACTH. First discovered in anterior pituitary corticotroph cells, it has more recently been revealed to have many other physiological aspects. The fine molecular mechanisms of ACTH biosynthesis show that ACTH is but one piece of a puzzle which contains many other peptides. Present in various tissues, among which are pituitary, hypothalamus, central nervous system and skin, POMC undergoes extensive post-translational processing. This processing is tissue-specific and generates, depending on the case, various sets of peptides involved in completely diverse biological functions. POMC expressed in corticotroph cells of the pituitary is necessary for adrenal function. Recent developments have shown that POMC-expressing neurons in the brain play a major role in the control of pain and energy homeostasis. Local production of POMC-derived peptides in skin may influence melanogenesis. A still unknown function in the placenta is likely.

POMC has become a paradigmatic polypeptide precursor model illustrating the variable roles of a single gene and its various products in different localities.

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Discovering proopiomelanocortin (POMC)

The era of precursors of polypeptide hormones really started in the late 1960s with the discovery and characterisation of two prototypes: proinsulin, the precursor to insulin, by Steiner in Chicago (1), and the lipotrophins (β - and γ LPH), the precursors to β -melanocyte-stimulating hormone (β MSH) by Li and Chrétien in San Francisco (2). Clues that a precursor to adrenocorticotrophin (ACTH) might also exist came from the work of Yalow & Berson (3) who found a high molecular weight (HMW) immunoreactive (IR) ACTH material in the extracts of a human thymic tumour responsible for a case of ectopic ACTH syndrome. This biologically inactive molecule released bioactive ACTH under mild digestion by trypsin. From these premisses the search for an ACTH precursor was launched.

The major progress came from fine biosynthetic studies using labelled amino acids incorporated into newly translated proteins combined with pulse-chase strategies. This approach was feasible in a cell line which had a high rate of ACTH synthesis. The AtT-20

cell line, which is derived from an irradiation-induced mouse pituitary tumour and selectively produces and secretes large amounts of ACTH, proved to be an invaluable tool. The latter technique indeed established that the small peptides that were identified (the end-products of the precursor processing, i.e. ACTH) were not mere degradation products generated during the extraction procedure, since they were not obtained after the cells had been submitted to only short pulses of labelling. These studies therefore showed that the HMW ACTH precursor ultimately generated a material that was physically and immunologically indistinguishable from ACTH (4). Later, the same biochemical studies showed that labelled amino acids incorporated into the HMW ACTH precursor were immunoprecipitated by anti-ACTH but also by anti- β LPH and anti- β endorphin (β end) antibodies (5). Thus the precursor to ACTH also seemed to contain LPH sequences as well, an explanation for the previous observations reporting strictly parallel variations of ACTH and LPH plasma concentrations in a variety of pathological conditions (6).

The final step was carried out by Numa's group: the cDNA of the mRNA coding for the precursor

to ACTH was obtained and cloned (7). The sequence revealed – for the first time – the structure of the ACTH precursor: ACTH itself was right in the middle, flanked by new sequences on its N-terminal end and by LPH on its C-terminal end. Thus the puzzle was ultimately assembled, and established the molecular links between ACTH, the LPHs, β end and putative new peptides.

POMC expression and maturation in human beings

The POMC gene (Fig. 1)

There is a single POMC gene per haploid genome in man. It is located on chromosome 2p23. It comprises 7665 bp and consists of three exons and two introns.

Exon 1 (87 bp) only contains untranslated sequences. Exon 2 (152 bp) codes for the signal peptide and the first amino acids of the N-terminal peptide

(NT). Exon 3 (833 bp) codes for most of the translated mRNA, i.e. the C-terminal part of the NT, joining peptide (JP), ACTH, and β LPH (8, 9). Comparisons between species show a strong homology between the DNA sequences coding for the NT, ACTH, β MSH and β end, whereas the sequences coding for the JP and γ LPH have poor homologies (10).

The NT includes two intramolecular disulphide bridges, stabilising a loop structure composed of amphipathic amino acids. This conformational motif appears like a molecular sorting signal for POMC towards the regulated secretory pathway (11).

POMC gene expression in different tissues (Fig. 2)

POMC gene expression in the pituitary In normal human pituitary, the POMC gene is only expressed in corticotroph cells. Most mammals also possess an intermediate lobe with POMC-expressing melanotroph cells but this lobe is vestigial in adult man.

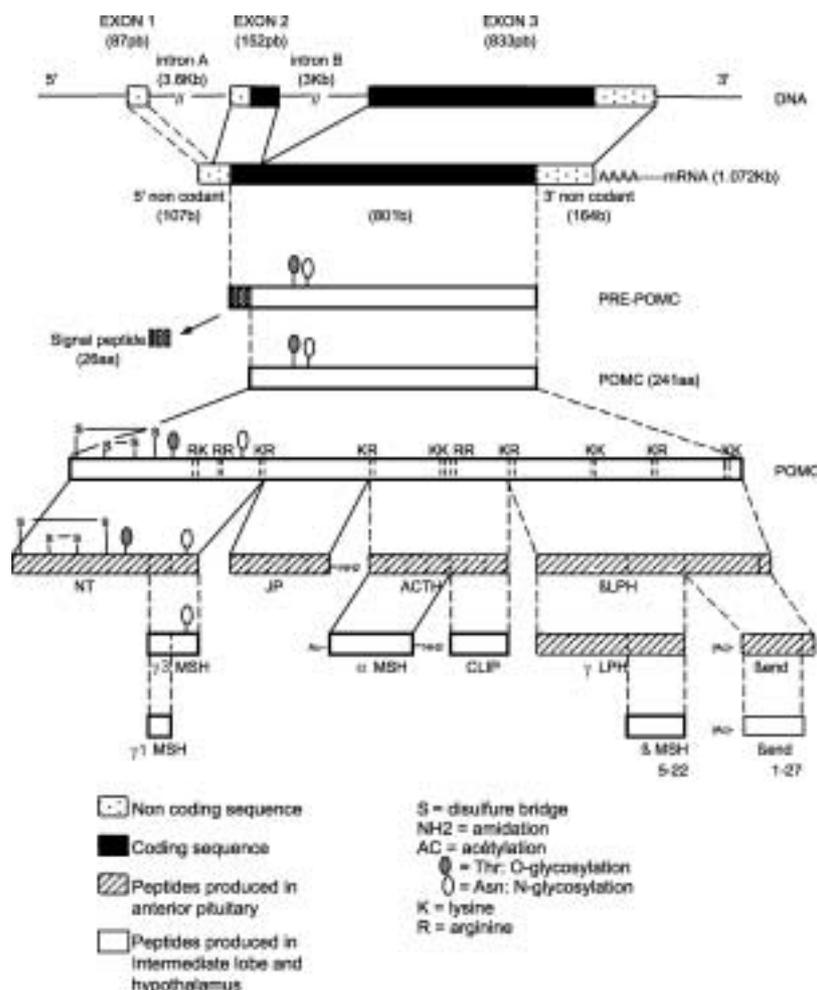


Figure 1 POMC gene structure and expression.

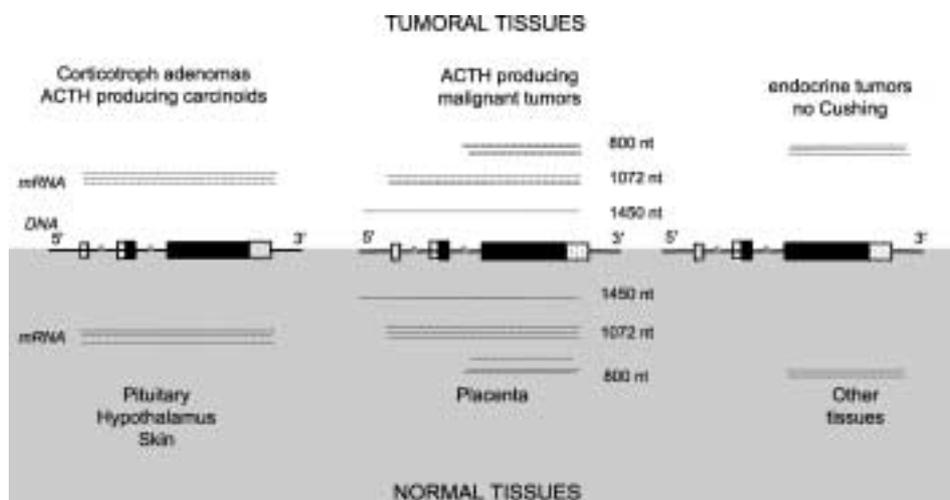


Figure 2 POMC gene expression in normal and tumoral tissues.

In corticotroph cells, a mature POMC mRNA of 1072 nucleotides (nt) is generated, after the splicing of the primary transcript and a poly A + tail of ~200 nt is added. The 801 nt of the coding region are translated into a prePOMC molecule starting with a 26 amino acid signal peptide necessary for the translocation of the nascent protein through the membrane of the rough endoplasmic reticulum (RER). The peptide signal is rapidly cleaved. The protein is then engaged into the secretory pathway: at that time, the 241 amino acid POMC molecule is made of the peptides NT, JP, ACTH and β LPH, and is ready for maturation (or processing).

POMC gene expression in non-pituitary tissues

POMC mRNA has been reported by different groups in many normal non-pituitary tissues, in animals and man.

In most of these tissues, POMC gene expression is quantitatively and qualitatively different from that in the pituitary: the tissue concentration of POMC mRNA is extremely low, and the generated mRNAs are essentially short, truncated, transcripts of ~800 nt. These transcripts result from heterogeneous transcription initiation at the 5' end of exon 3 (12–15). They are non-functional and cannot be efficiently translated into POMC.

In the brain, however, a well-defined population of neurons – in the arcuate nucleus – do express a POMC mRNA that is identical to that in the pituitary (16–18). In these neurons POMC is produced and serves as the precursor to cerebral β end, α MSH and other peptides which have biological functions in the brain. Recently the MSH system has gained attention as its role in energy homeostasis emerged (see below).

The placenta is another physiological site of POMC-derived peptide production. The short 800 nt

transcript is found by Northern blot as well as the 1072 nt pituitary mRNA and sometimes a larger 1450 nt transcript. POMC mRNAs are produced at a low rate but the placental mass explains why this expression can influence plasma concentration of POMC-derived peptides during pregnancy (see further) (19, 20).

More recently, POMC gene expression and the production of melanocyte-stimulating peptides have been demonstrated in keratinocytes, melanocytes and dermal microvascular endothelial cells, raising the possibility of an auto/paracrine role influencing skin pigmentation. The baseline expression of the 1072 nt transcript is upregulated by ultraviolet radiation (21–23).

POMC processing (Fig. 3)

Once POMC has reached the lumen of the RER, it follows the intracellular traffic of secreted protein through the Golgi apparatus and ultimately the secretory granules where the end-products of the processing are stored before being secreted by exocytosis. During this traffic the POMC molecule undergoes a series of proteolytic cleavages and chemical transformations, which altogether result in the maturation or processing of the precursor, yielding the various biologically active POMC-derived peptides.

POMC is a prototype polypeptide precursor which contains eight pairs, and one quadruplet, of basic amino acids, which are potential cleavage sites for processing enzymes (Fig. 1). In a given tissue the nature of the POMC end-products indicates which sites are cleaved in this particular tissue. Thus, in the corticotroph cells of the anterior pituitary, only four of these cleavage sites are used, which are all of the Lys-Arg type. Indeed, the following six peptides are generated: NT, JP, ACTH, β LPH, and a small

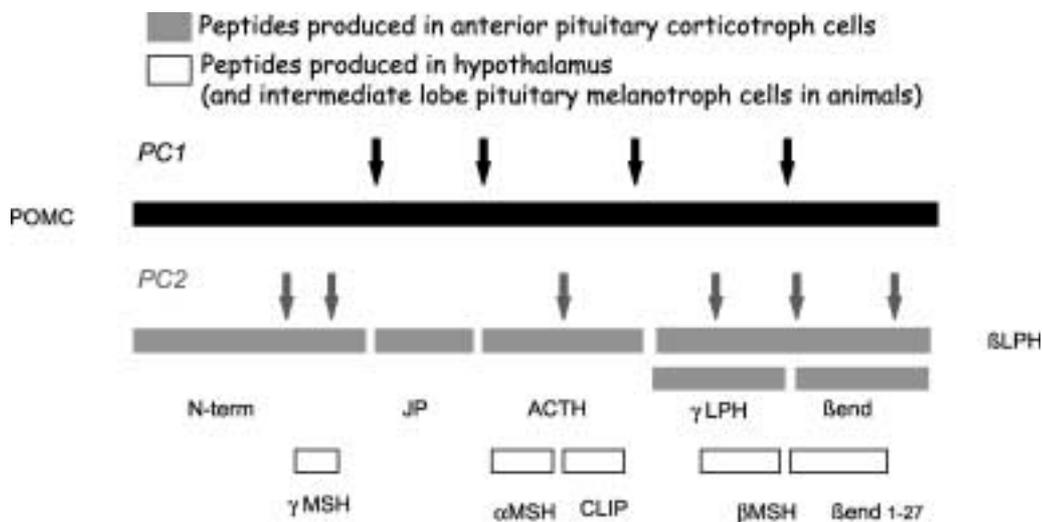


Figure 3 POMC processing by PC1 and PC2.

amount of γ LPH and β end, since the last cleavage site is only partially used (24–26). In the melanotroph cells of the intermediate lobe of the pituitary in rodents, and in hypothalamus and placenta in man, an alternate mode of POMC processing takes place. All the cleavage sites are used and smaller peptides are produced: the NT gives rise to the γ MSHs, ACTH to α MSH and CLIP (corticotrophin-like intermediate lobe peptide or ACTH_(18–39)), and β LPH to β MSH, β end_(1–31) and β end_(1–27) (25, 27–29).

The enzymes which specifically participate in the proteolysis of polypeptide hormone precursors have long been sought. They have been identified as a superfamily of homologous subtilisin-like enzymes, called prohormone convertases (PCs). The first two of them, PC1 (also called PC3) and PC2, were discovered almost simultaneously by the groups of Chrétien and Seidah in Montreal (30) and of Steiner in Chicago (31). Their isolation has allowed us to examine both their tissue distribution and their action on specific substrates. Elegant studies have unravelled the mechanisms which lead to alternate POMC processing in corticotroph and melanotroph cells of the pituitary (32–34): in the first cell type, PC1 only is present and its proteolytic action is limited, thus the overall processing only uses four sites and ACTH is a major end-product; in the second cell type, both PC1 and PC2 are present, and their coordinate or synergistic actions lead to a more pronounced proteolysis generating the smaller fragments already described (Fig. 3) (34, 35).

Chemical modifications such as glycosylation, amidation, phosphorylation, acetylation and sulphation also take place, some of them are cell-specific and alter the biological activity of the peptide (such as

acetylation of β end and α MSH). They contribute to the overall diversity of POMC products.

Biological effects of POMC-derived peptides (Fig. 4)

Three different POMC systems are schematically depicted in Fig. 5.

POMC products in blood

Because the peptides produced by corticotroph cells are derived from the same precursor molecule, they are secreted in equimolar amounts during the process of exocytosis (24, 36, 37). However, the circulating levels of the peptides are not identical because of their different half-lives. With the exception of pregnancy (see below), all POMC-derived peptides physiologically present in blood originate from the anterior pituitary. In those cells, POMC gene expression is stimulated by corticotrophin-releasing hormone (CRH) and vasopressin, whereas it is suppressed by glucocorticoids. The PC1 (38–40) is regulated in parallel.

ACTH and adrenal function Full length ACTH (ACTH_(1–39)) is the only POMC-derived peptide with a clear action on adrenocortical function (41). ACTH is the only known ligand of the melanocortin type 2 receptor (MC2-R) located on adrenal membranes (42). Binding of ACTH induces cAMP production, steroidogenesis resulting in secretion of glucocorticoids, androgenic steroids and, to a lesser extent, mineralocorticoids.

The acute effect of ACTH is to increase conversion of cholesterol into Δ^5 pregnenolone, the initial step

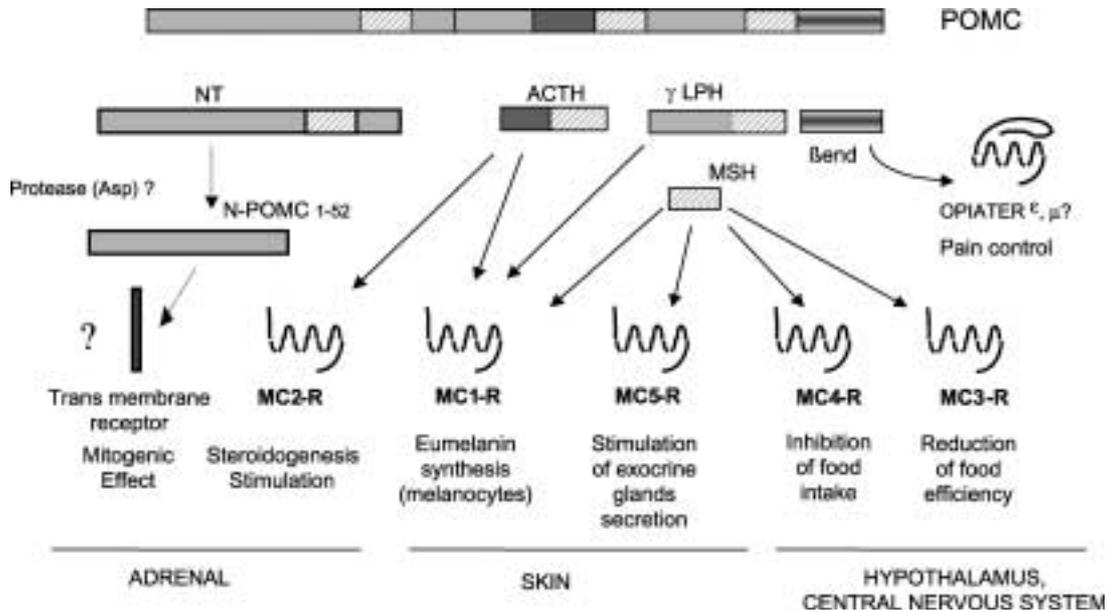


Figure 4 Physiological roles of POMC-derived peptides.

in cortisol biosynthesis. The chronic effects of ACTH include increased synthesis of most of the enzymes of the steroidogenic pathway.

When ACTH levels are low, such as during glucocorticoid therapy, the level of all CYP enzymes and of protein and RNA synthesis decline. The adrenal glands become small and atrophic. These changes are slowly reversed by ACTH administration. Thus, ACTH is essential for normal steroidogenesis and is required but not sufficient to maintain normal adrenal weight. It is likely that other factors participate in maintaining normal adrenal size and allowing

adrenal growth, for example after unilateral adrenalectomy (43, 44).

NTs and adrenal proliferation The mitogenic effect of the N-terminal sequences of the POMC molecule (N POMC) has been proposed for a long time by Lowry and colleagues (45, 46). This assumption is supported by *in vitro* studies (mitogenic effect of N POMC₍₁₋₂₈₎ on adrenal cells in culture), as well as *in vivo* argumentation: infusion of N POMC₍₁₋₂₈₎ partly prevents adrenocortical atrophy in hypophysectomised rats (45, 46).

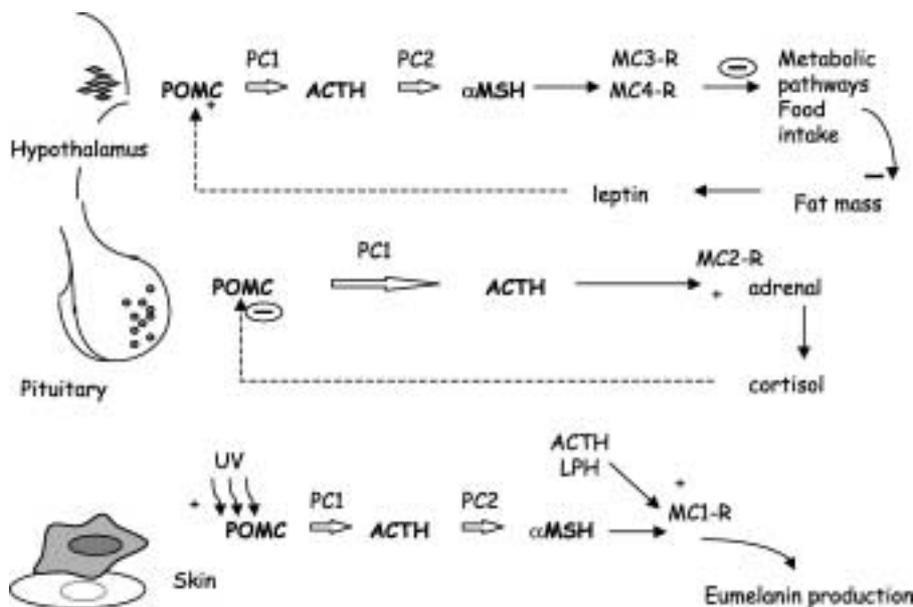


Figure 5 POMC processing and regulation in hypothalamus, pituitary and skin.

The adrenocortical growth induced by NT needs proteolysis: it is inhibited by the protease inhibitor trypsin. Lowry's group recently reported the cloning of a serine protease named Asp (47), specifically expressed in the adrenal cortex, located on cell membranes and upregulated during adrenal growth. This enzyme could cleave the biologically inactive pro γ MSH, a circulating peptide derived from the N POMC, into a shorter peptide, N POMC₍₁₋₅₂₎, possessing mitogenic properties (47).

ACTH, LPH, MSH and melanocyte stimulation

ACTH, β LPH and γ LPH, produced in corticotroph cells of human pituitary, all contain a common sequence (Met-Glu-His-Phe-Arg-Trp-Gly). This heptapeptide is responsible for the melanostimulating effect of these peptides through the activation of MC1-R (42). Other melanostimulating peptides, α MSH and β MSH, are not produced in human pituitary. However, they may be produced by some ACTH-secreting tumours responsible for the ectopic ACTH syndrome.

In patients with Addison's disease or Nelson's syndrome, overproduction of ACTH, β LPH and γ LPH contribute to skin pigmentation. Activation of MC1-R in melanocytes favours eumelanin synthesis and dispersion, resulting in a brown coloration of skin. In the absence of MC1-R activation, the red pigment, pheomelanin is produced, as observed in MC1-R-deficient subjects (48). In man, the contribution of POMC-derived peptides to physiological pigmentation was demonstrated by the phenotype of POMC-deficient children, who are red-haired. Furthermore, a local production of ACTH and α MSH has been shown in melanocytes, keratinocytes and human dermal microvascular endothelial cells. Production and release of these peptides is upregulated by cytokines and ultraviolet irradiation, in parallel with PC1 and PC2 expression. Thus, in addition to the endocrine effects of the pituitary peptides, an auto/paracrine role of POMC fragments is likely, influencing skin pigmentation and local immune responses (21–23).

α MSH and immune modulation α MSH may influence inflammatory processes by impairing important functions of both antigen-presenting cells and T cells. Several anti-inflammatory effects have been described for α MSH, such as suppression of fever induced by interleukin (IL)-1 or IL-6, induction of the anti-inflammatory mediator IL-10, and inhibition of macrophage function and leukocyte migration (49). The physiological importance of these actions remains to be determined.

POMC products in the central nervous system

Melanocortins Energy stores are maintained relatively constant in mammals, in spite of large variation in food availability and physical activity. This tight

regulation is achieved by an endocrine feedback loop initiated by leptin. Leptin signal triggers a neuroendocrine response involving neuropeptides that modulate appetite and energy expenditure. Among them, the central melanocortin system is an important mediator of leptin control on energy homeostasis (50).

POMC expression in the hypothalamic arcuate nucleus is induced by leptin. The precursor is processed by PC1 and PC2 into α MSH, the main agonist of MC3-R and MC4-R (50). Genetic or pharmacological disruption of this loop causes obesity in both human and rodents. POMC-deficient mice and humans are hyperphagic and obese. Conversely, intraventricular infusion of α MSH or synthetic agonists causes anorexia and weight loss (51, 52).

Both melanocortin receptors mediate the effect of the melanocortin system on energy stores. Many studies have established the importance of MC4-R in the control of energy homeostasis. Mutations of MC4-R have been demonstrated in up to 5% of severely obese children (53–55). Mutation carriers had increased fat and lean mass, increased linear growth, hyperphagia and severe hyperinsulinaemia, most pronounced in children younger than 10 years of age. Homozygotes were more severely affected than heterozygotes. The phenotype was less severe in subjects with mutations retaining residual signalling capacity (55). MC4-R-deficient mice are severely obese and hyperphagic (56). Overexpression of agouti-related transcript, a naturally occurring melanocortin receptor antagonist acting mostly on MC4-R, results in hyperphagia and maturity-onset obesity (57, 58). The role of MC3-R is less clear. Homozygous MC3-R-null mice (59, 60) were not (or only slightly) overweight, but fat mass of MC3-R^{-/-} mice is approximately twice that of wild type or heterozygous, whereas lean body mass is reduced. MC3-R^{-/-} mice did not exhibit increased food intake. Basic metabolic rate is normal. Increased feeding efficiency, not hyperphagia, contributes to fat mass gain in MC3-R^{-/-} mice. Thus the two receptors display complementary roles in weight control: while MC4-R mainly influences food intake MC3-R regulates fat stores by an exclusively metabolic pathway (59, 60).

The melanocortin system also mediates other hormonal effects of leptin. During fasting, a marked reduction in circulating thyroid hormone levels is observed. This action is largely orchestrated by the fall in leptin circulating levels. α MSH may have a role in leptin modulation of the hypothalamo–pituitary–thyroid axis as α MSH-producing neurons in the arcuate nucleus, which are a target of leptin, send monosynaptic projections to thyrotrophin-releasing hormone (TRH) neurons and because intraventricular infusion of α MSH can completely restore fasting levels of proTRH mRNA in the paraventricular nucleus to normal-fed levels (61). However, alternative pathways must exist as MC4-R knockout mice, as well as subjects with MC4-R deficiency, have normal thyroid function (55, 56).

The melanocortins also influence erectile function and sexual behaviour. α MSH or a specific MC4-R agonist increases erections and copulatory behaviour in mice, whereas this function is diminished in mice lacking MC4-R. MT II, a non-specific melanocortin agonist, could evoke spontaneous erections in men. MC4-R mRNA expression has been demonstrated in rat and human penis, rat spinal cord, hypothalamus and pelvic ganglion. The exact mechanism is unknown, but modulation of sexual function may derive from both peripheral and central actions (62). It is noteworthy that male subjects with MC4-R deficiency did not report decreased erectile function (55).

β end and analgesia β end is produced by neurons located in the arcuate nucleus and brain stem. These neurons project to most regions of the brain. β end exerts a potent analgesic effect through opiate receptors.

β end is also produced by the anterior pituitary lobe. Plasma concentrations may be very high, in Addison's disease or Nelson's syndrome for example. However, the circulating peptide does not cross the blood-brain barrier. These patients therefore have no particular analgesia or abnormal reaction to naloxone.

POMC-derived peptides during pregnancy

Normal gestation is associated with profound modifications of maternal corticotroph function. Plasma cortisol almost doubles by mid-gestation and ACTH also increases slightly, although it usually remains within the normal range. The full-length, pituitary-like POMC mRNA is present in human placenta

(19, 20). The pattern of POMC processing in the placenta is particular to that site: in addition to ACTH and LPHs, placental extracts contain small peptides such as β end and α MSH, as well as POMC itself, and this production is enhanced by CRH (28, 29, 63, 64). The HMW precursor is present in plasma during gestation. While POMC is not detected in non-pregnant women, POMC levels become measurable in early gestation, reaching 3–10 times the detection limit of the assay during the second trimester, and remaining rather constant thereafter (Fig. 6).

POMC plasma levels are higher in multiple pregnancies, display no diurnal variation, are unaffected by glucocorticoid administration, and return to normal within 3 days after delivery, consistent with its placental origin (19, 65). Its physiological function remains unknown. No correlation is observed with plasma ACTH or cortisol. Conversely, a positive correlation is observed with CRH. POMC is selectively concentrated at the fetomaternal interface, which raises the question of a potential role in materno-fetal exchanges (65).

POMC and pathology

Neuroendocrine tumours

In Cushing's syndrome, excessive or inappropriate ACTH secretion can originate from tumours of pituitary or non-pituitary origin, responsible for Cushing's disease in the first case and for the ectopic ACTH syndrome in the second.

In the vast majority of pituitary corticotroph adenomas, gene transcription shows no gross abnormality and the POMC transcripts in pituitary tumours are similar to those in the normal pituitary (66–68).

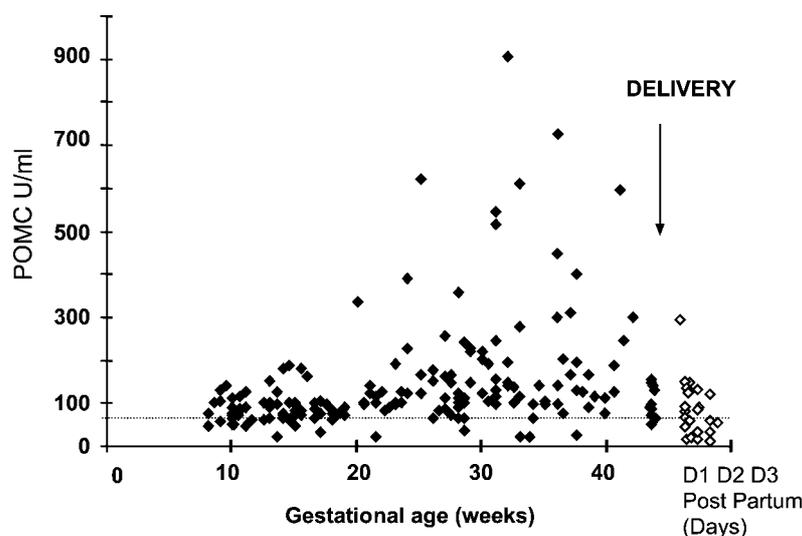


Figure 6 Plasma POMC levels during pregnancy and early post-partum.

In the same way, the products of POMC gene transcription and of POMC processing are identical to those in the normal human anterior pituitary. The NT, the JP, authentic ACTH_(1–39), β LPH and variable amounts of γ LPH and β end are the normal end-products of POMC processing found both in tumour extracts and in culture media (66). Yet the recruitment of proteolytic sites which are not normally activated in the normal pituitary does not occur.

In non-pituitary tumours, altered POMC gene expression is frequent. Most ACTH-secreting tumours contain various types of messengers: a mRNA of about 1450 nt is always associated with the normalized (pituitary-like) 1072 nt POMC mRNA, and with the small 800 nt messengers. The absolute amount and various molecular forms of POMC mRNA is different among the tumours. In bronchial carcinoid tumours associated with the ectopic ACTH syndrome, the pituitary messenger is highly predominant and present in high amounts. In endocrine tumours not associated with the ectopic ACTH syndrome, the short 800 nt mRNA is predominant and the total amount of POMC mRNA is low. The ectopic ACTH syndrome occurs with tumours capable of generating high amounts of the pituitary-like message (67–71).

In the same way, the maturation process is often altered in non-pituitary tumours, either because of the lack of PCs, or because – on the contrary – local PCs which operate in the tumour are appropriate for the resident hormone precursor of the given tissue but not for an ectopic precursor like POMC. Hence an abnormal maturation pattern of POMC is a classic feature of the ectopic ACTH syndrome (Fig. 3). In many cases intact POMC – or biosynthetic intermediates to ACTH – may be predominantly secreted (72–75); on the contrary, abnormal fragments such as CLIP and human β MSH_(5–22) may be generated (76). Recent studies have shown that PC2 was specifically present in these tumours which contained CLIP and not in those which contained predominantly ACTH (76).

In both cases these processing abnormalities diminish the tissue's ability to secrete authentic ACTH – the sole bioactive peptide in terms of steroidogenesis – and somehow protect the patients from the consequences of the tumour production; they also provide the investigator with subtle molecular clues that an ACTH-dependent Cushing's syndrome may originate from a non-pituitary source (Fig. 3).

Recent studies on large series demonstrated that aggressive – and poorly differentiated – tumours, like small-cell lung carcinomas, preferentially release intact POMC (73, 75), most likely because of a general defect in both PC1 and PC2 (77); in contrast carcinoids – which have a high degree of neuroendocrine differentiation – rather overprocess the precursor, releasing ACTH and smaller peptides like CLIP (76, 78), most likely because they are heavily loaded with various PCs including PC2 (77, 79).

It is noteworthy that these carcinoids, which also contain the pituitary-like 1072 nt POMC mRNA as the highly predominant if not sole transcript of the POMC gene, express the pituitary-specific V3 (V1b) vasopressin receptor. That both V3 receptor and POMC genes may be expressed in a pattern indistinguishable from that in pituitary corticotroph adenomas, strengthens the idea that a broad process of corticotroph differentiation is achieved in these non-pituitary tumours (80, 81).

Yet, abnormal processing of POMC is not specific to non-pituitary tumours and is found occasionally in rare pituitary macroadenomas and in some exceptional pituitary cancers. HMW IR-ACTH-like materials have been identified by gel exclusion chromatography in the plasma of such patients. In some cases a direct POMC IRMA in blood shows that the unprocessed precursor is directly secreted by the pituitary tumour (73).

Thus defective POMC processing actually indicates an impaired state of neuroendocrine differentiation in aggressive tumours, independently of their pituitary or non-pituitary origin. It is more often encountered in non-pituitary tumours.

POMC-defective patients

Two young children were described by Krude *et al.* (51) who had congenital corticotroph insufficiency, red hair and massive obesity. In both cases mutated POMC alleles were found.

The general phenotype of these children illustrates the pleomorphic role of POMC: the lack of ACTH induces the corticotroph insufficiency since no other ligand can stimulate the MC2-R at the adrenals; the red hair is secondary to the lack of stimulation of MC1-R at the melanocytes: as a consequence, pheomelanin (red pigment) is produced instead of eumelanin (black pigment). The pigmentation in these patients argues for a critical role of MC1-R signalling for human pigmentation as already shown in animals; obesity results from the lack of α MSH to act on MC3-R and MC4-R at the brain level and confirms the important role of melanocortin signalling in the regulation of energy stores in man. The lack of symptoms related to β end deficiency might be due to the redundancy of ligands in the opioid receptor network (enkephalins).

POMC-deficient mice (52) have a very similar phenotype to human POMC-deficient patients consisting of obesity, defective adrenal development and altered pigmentation. Corticosterone is very low, as well as aldosterone and epinephrine. Strikingly, the mutants survive and gain weight without any glucocorticoid supplementation. Like MC5-R-null mice, POMC^{-/-} animals have a reduced ability to repel water from their fur. When treated with a stable MSH agonist, mutant mice lost more than 40% of their excess weight after 2 weeks.

More subtle genetic variants in POMC sequence may contribute to early-onset obesity. Two children were found to be heterozygous for a missense mutation in the POMC sequence which disrupts the dibasic cleavage site between β MSH and β end (82). The abnormal fragment (β MSH linked to β end) bound to the human MC4-R with an affinity similar to its natural ligands, but had a markedly reduced ability to activate the receptor. This variant co-segregated with early-onset obesity over three generations in one family and was absent in 412 normal-weight Caucasian controls. These results suggest that the mutation may confer an inherited susceptibility to obesity through the production of an aberrant maturation product that has the capacity to interfere with central melanocortin signalling.

Congenital defect in *Tpit*

The group of Drouin in Montreal recently characterised a new transcription factor belonging to the family of Tbox proteins (83).

This factor, *Tpit*, plays a central role in the tissue-specific control of *POMC* gene expression. It is further emphasised by the finding of *Tpit* mutations – which presumably induced loss of function – in two patients with congenital isolated corticotrophic insufficiency (83).

***POMC* maturation defects**

A patient with a congenital defect in PC1 was reported by the group of O'Rahilly in Cambridge (84). This woman first presented with post-prandial hypoglycaemic episodes; she later had primary amenorrhoea after a normal puberty. But, most of all, she had always presented with a massive obesity since childhood, somewhat attenuated in adulthood.

Biological studies showed that this patient had low insulin plasma levels whereas proinsulin was high. Similarly we showed that she had extremely high early-morning POMC plasma levels (~ 7000 U/ml; normal < 60) (84). These results suggested that a general defect in prohormone processing was responsible. Indeed sequence analysis of the PC1 gene showed that the patient was a compound heterozygote, bearing two mutated alleles, one inherited from the father and one from the mother.

In this rather exceptional observation, increased POMC plasma levels are generated by highly stimulated corticotroph cells responding the negative feedback loop between cortisol and ACTH.

Although not proven, it is speculated that the general defect in PC1 induces a lack of α MSH generation in the hypothalamus, therefore inducing obesity. Yet, many other brain proneuropeptides might be affected by a PC1 defect and high proinsulin responsible for repeated hypoglycaemic episodes could also participate in abnormal weight gain.

PC1-deficient mice display a slightly different phenotype (85). These animals are normal at birth, but fail to grow normally. Growth hormone (GH)-releasing hormone (GHRH) processing is impaired resulting in low pituitary GH and hepatic insulin-like growth factor-I mRNA levels. Although GHRH processing was not examined in the human subject with PC1 defects, circulating GH levels were somewhat subnormal. This difference may be explained by a sequence difference between human and mouse proGHRH in the N-terminal cleavage site making GHRH production more dependent on PC1 activity in the mouse. Despite a severe defect in pituitary POMC processing to mature ACTH, blood corticosterone levels are essentially normal. The absence of PC1 disturbs POMC processing, leading to striking accumulations of unprocessed POMC, and to significant upregulation of POMC mRNA. However, blood corticosterone levels are nonetheless maintained at normal levels. This is also paralleled in the human subject, where circulating cortisol levels were normal but accompanied by large compensatory increases in circulating ACTH precursors (POMC or intermediates). In contrast, POMC-null mice lack adrenal tissue and have no detectable adrenal steroids. It is possible that some larger ACTH-containing intermediates retain some corticotrophic activity, or that small amounts of ACTH, not detected by chromatographic studies, are, however, generated in corticotroph cells by an alternative processing mechanism.

Interestingly, the heterozygote mice are moderately overweight, but severe early-onset obesity as observed in the human PC1/3-deficient subject was not evident in null mice. This emphasises the influence of species specificity in a such multifactorial phenotype.

Although PC2-null mice did not exhibit major alterations in glucocorticoid levels, growth, weight control or reproduction, they were chronically hypoglycaemic with severe defects in proglucagon, prosomatostatin (86) and proinsulin processing. They also have significant deficits in opioid peptide levels in the central nervous system (87).

PC2 requires interaction with the neuroendocrine protein 7B2 to generate an enzymatically active form. 7B2-null mice express no PC2 activity and release large quantities of uncleaved ACTH from the intermediate lobe, resulting in a lethal endocrine condition that resembles Cushing's syndrome (88) (Fig. 7). Both 7B2- and PC2-nulls contained highly elevated intermediate lobe pituitary ACTH; however, ACTH and corticosterone oversecretion occurs only in 7B2-deficient animals, indicating that 7B2 deficiency influences ACTH release by other mechanism(s). This situation cannot, however, constitute a model for human Cushing's disease as the intermediate lobe is vestigial in man. Interestingly, adrenalectomised 7B2-nulls exhibit a profound late-onset obesity (89). This phenotype may be related to a lack of the PC2-generated peptide α MSH. However, PC2-nulls, which also lack α MSH, do not

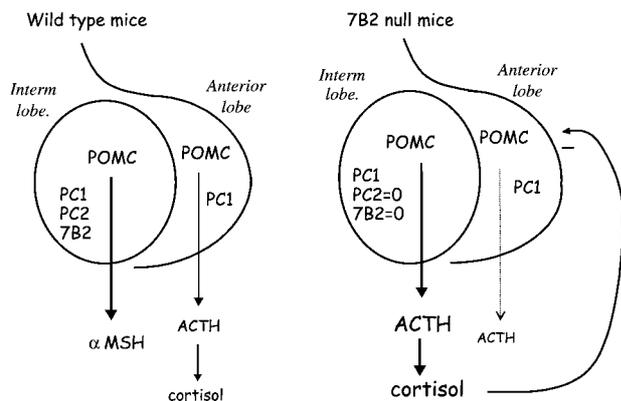


Figure 7 ACTH hypersecretion in 7B2-null mice.

exhibit significant obesity, suggesting that other factors also must play a role in the development of this condition.

Analysis of POMC gene expression mechanisms and POMC processing can help us to understand some pathological situations such as tumoural ACTH secretion and very rare conditions associated with obesity and/or adrenal insufficiency. Abnormal POMC maturation provides the clinician with new tools for the diagnosis and follow-up of patients with ACTH-secreting tumours. Further progress in the knowledge of POMC synthesis may offer in the future new therapeutic approaches in domains as different as tumoural development, pregnancy and obesity.

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