Functions of $\alpha$-melanotropin and other opiomelanocortin peptides in labour, intrauterine growth and brain development

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Abstract In a number of animals and in humans, factors from the fetal hypothalamus function in intrauterine growth, in labour and in brain development. Peptides of the opiomelanocortin family are produced by the pituitary, brain and placenta and are probably involved in these developmental processes.

In the rat, $\alpha$-MSH stimulates fetal growth, protein synthesis, wound healing and liver regeneration and it reduces periosteal bone resorption. In chick embryos, $\alpha$-MSH restores the corticosteroid-induced growth retardation. Thus $\alpha$-MSH seems to possess general trophic properties.

The fetal brain in humans is involved in timing the moment of birth. This process is probably mediated by peptides of the opiomelanocortin family as suggested from observations in anencephaly and other congenital brain anomalies and from the influence of corticosteroids or ACTH on labour. The high percentage of premature deliveries in heroin addicts is worth examining endocrinologically, in this respect. The exact nature of the peptides and mechanisms involved in labour is not yet known.

Some peptides of the opiomelanocortin family induce an acceleration of brain development. Neonatal treatment of rats with $\alpha$-MSH alters their later behaviour while ACTH fragments accelerate the onset of eye-opening. Opiates and methadone inhibit brain development, and neonatal administration of $\beta$-endorphin or naloxone causes permanent insensitivity to temperature stimuli.

The interrelated nature of the fetal pituitary, brain and placenta does not, at present, allow us to pin down which of these structures is primarily involved in the regulation of intrauterine growth, labour and brain development.

The fetal brain and birth

In sheep the fetal brain provides the primary stimulus for the onset of labour. After lesions in the brain or pituitary of the fetal sheep, parturition is delayed or does not even occur at all (Liggins et al 1977). However, important species differences are apparent in the degree to which the fetal brain is involved in parturition. Removal


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of the fetal brain in the rat does not change the gestation length (Swaab et al 1977). For human anencephalics (Honnebier & Swaab 1973) and in rhesus monkeys decapitated in utero (Novy 1977) the average length of gestation was normal; however, a strikingly high proportion of pre- and post-term labours was found. Hence, the fetal brain appears to adjust the time of birth around the species-specific mean. The degree to which the fetal brain is involved in the birth process in different species correlates with the degree of maturation of the fetal brain at term. According to a number of general measures of brain maturity, the sheep, of all the species examined, is the most mature at term and the rat the least mature while the human fetus is intermediate between the two (Swaab et al 1979). Correspondingly the fetal rat brain does not play a major role in determining gestation length. How the fetal sheep brain controls parturition is not yet clear, but one possibility is through its control of MSH-related peptides.

The opiomelanocortin peptide family and birth

Although many observations suggest a role for peptides of the opiomelanocortin family in birth, neither the actual compounds involved, nor the mechanism, is as yet established. In sheep, the work of Liggins et al (1977) points to a rise in fetal plasma ACTH as the cause of the increased cortisol secretion of the fetal adrenal gland and thus as the cause of labour. However, other results suggest that the rise in ACTH concentrations in sheep fetal plasma occurs after the rise in fetal plasma cortisol (Nathanielisz 1976). Honnebier et al (1974) proposed that α-MSH might be an adrenocorticotrophic hormone in the human fetus. Indeed, α-MSH causes a rise in plasma cortisol in fetal newborn lambs and also stimulates human and sheep fetal adrenal cells in culture (Glickman et al 1979, Llanos et al 1979). Furthermore, it stimulates fetal and neonatal cortisol production in the rabbit at a time when the adrenal responds only poorly to ACTH (Challis & Torosis 1977). Rudman et al (1980) recently found that α-MSH had a more potent stimulatory effect than ACTH on DNA and protein synthesis in the fetal adrenal gland of rat, rabbit and guinea-pig in early and mid-pregnancy. However, other experiments have revealed that α-MSH is less steroidogenic than ACTH on the human fetal adrenal gland (Giroud et al 1979) and that α-MSH even reduces the release of cortisol in response to ACTH in fetal sheep adrenal cells (Challis et al 1979). Consequently, the potential agonistic and antagonistic actions of peptides of the opiomelanocortin family on fetal adrenal function seem to be legion.

Opiomelanocortin peptides may also be involved in the timing mechanism of labour in women. After corticosteroid treatment in premature labour, a tendency towards prolongation of pregnancy is found, while corticosteroid or ACTH treatment is more likely to provoke labour if the patient is beyond term (Jensen & Wright 1977, and for additional references see Swaab et al 1978a). An interesting
observation in this respect is the high proportion of premature labours in narcotic addicts (Perlmutter 1967, Stone et al 1971). Hogerzeil (1980) found that the high frequency of premature deliveries could be attributed to those addicts that used only heroin. The 47% of premature labours in these patients appeared to be four times higher than normal, and this finding should be followed up by measurements of opiomelanocortin peptide concentrations, especially since Ratter et al (1981) found high basal levels of β-endorphin in heroin addicts.

α-MSH, a trophic hormone

The fetal brain plays an active role in intrauterine development. The protracted intrauterine growth observed in human congenital brain anomalies (for references see Swaab et al 1978a) and after experimental lesions in the fetal brain (Fujita et al 1970, Swaab et al 1978b, Swaab & Boer 1980) suggests that the fetal hypothalamus is involved in the production of a growth-promoting factor. In order to screen for such a factor, we developed a simple procedure for the experimental removal of the fetal brain in the rat (Swaab & Honnebier 1973). Of the many hypothalamic and hypophysial compounds injected directly into the fetus after removal of its brain, only α-MSH stimulated fetal growth (Honnebier & Swaab 1974, Swaab & Honnebier 1974). No stimulation of intrauterine growth was obtained on Day 21 of pregnancy in brainless fetuses after the injection on Day 19 of other fragments of the opiomelanocortin family: ACTH(1-24) (50 or 150 μg per fetus; Swaab & Honnebier 1974); γ-MSH (10 μg); ACTH(4-10) (15 μg, 75 μg); 4-Met(O2)8-D-Lys9-Phe-ACTH(4-9) (1 μg, 15 μg); β-LPH(61-91) (1 μg, 10 μg); β-LPH(61-77) (1 μg, 10 μg); and β-LPH(61-76) (1 μg, 10 μg), while the met-enkephalin analogue FK 33-824 C.H. (10 μg) induced a decrease in fetal body weight (D. F. Swaab, unpublished observations). Injection of purified anti-α-MSH directly into the intact fetus inhibited the growth of the fetus (Swaab et al 1976) and its brain (Swaab et al 1978b), arguing in favour of a physiological role of endogenous α-MSH in fetal development. The α-MSH effect on fetal body growth might be restricted to the fetal period since daily injections of either α-MSH or anti-α-MSH did not affect growth in the first two postnatal weeks (Swaab et al 1978b). When endogenous fetal α-MSH was neutralized by means of antibodies, brain weight, protein and lipid content were diminished while brain DNA content remained unchanged, indicating that α-MSH might have a role in nerve cell maturation rather than in cell multiplication. Whether this effect of α-MSH on the fetal brain is similar to that observed in epidermal melanocytes of newborn mice, where this hormone stimulates dendrite formation (Hirobe 1978), is not yet known. Since α-MSH was also found to stimulate protein synthesis, wound healing and liver regeneration (Rudman et al 1974, Vándor et al 1975) and to reduce periosteal bone resorption in hypophysectomized animals (Stenström et al 1979), it seems to have general trophic properties.
α-MSH and somatic development in chick embryos

Evidence from chick embryos suggests that α-MSH is involved in somatic development in this species also. This evidence comes from experiments on corticosteroid-induced inhibition of growth in chick embryos. Corticosteroids cause dose-dependent inhibition of increases in body weight and growth of the skeleton in developing chick embryos (Hall & Kalliecharan 1976, Karnofsky et al 1951). The inhibitory effects of corticosteroids on bone growth are well documented and include effects on the proliferation of chondrocytes and chondroblasts in vitro (Buno & Goyena 1955, Badran & Provenza 1969), effects on osteogenesis (Moscona & Karnofsky 1960, Ornoy 1971) and on collagen deposition and degradation (Reynolds 1972, Silberberg & Silberberg 1972). Since these are direct effects of corticosteroids on growing tissue it was assumed that the general somatic growth retardation was caused directly by the steroid. However, administration of α-MSH to chick embryos significantly reversed the reduction in body weight caused by the steroid (Fig. 1a). α-MSH did not reverse the steroid-induced retardation of bone growth (Fig. 1b), which agrees with the known direct actions of corticosteroids on development. Since pituitary regulation of the embryonic chick adrenal starts on Day 12 or earlier (Betz 1971, Jozsa et al 1979), it seems reasonable that high levels of corticosteroids may exert a feedback inhibition of α-MSH release. Corticosteroid-induced growth retardation may then result from a failure of the pituitary to secrete adequate amounts of α-MSH-related peptides. During development the fetal corticotrophic hormones might thus simultaneously stimulate the production of corticosteroid from the fetal adrenal and counteract its growth-inhibiting effects. Corticosteroid treatment also inhibits fetal growth in rats (Frank & Roberts 1979) and in humans (Reinisch et al 1978) and a similar peptide mechanism may be operating here. Since corticosteroids influence not only the birth weight but also the development of rat brain (Balázs et al 1975, Erskine et al 1979, Frank & Roberts 1979), and since they impair motor development in children exposed during pregnancy (Marton et al 1979), a study of the possible role of fetal opiomelanocortins in the clinical side-effects of glucocorticoid therapy seems worthwhile.

The opiomelanocortin family and brain development

Aminergic and amino acid neurotransmitters are present early in brain development. They may influence brain development by actions involving spontaneous electrical activity and also by their actions on the cell cycle, cell death and cell metabolism. Early alterations in the balance of these neurotransmitters, for example by the administration of psychotrophic drugs, often induce permanent structural and behavioural changes (Culver & Vernadakis 1979, Kellogg et al 1980, Lewis et al 1977a,b, Mirmiran et al 1980). The third and most recently described group of
FIG. 1a,b Reversal by α-MSH of the growth-inhibiting effects of corticosterone on the chick embryo. (J. T. Martin, unpublished results) Chicken eggs from a local hatchery were incubated in the laboratory in an automatic incubator, candled at 10 days of incubation and randomly assigned to five treatment groups (n = 10), including a non-injected control group, vehicle control, corticosterone (B), corticosterone plus α-MSH, and corticosterone plus arginine vasotocin (AVT). Corticosterone was injected (50 μg in 5 μl ethyl alcohol) on Days 10, 14 and 18 of incubation, whereas α-MSH (1 μg in 10 μl 0.005 M acetic acid) and AVT (1 μg in 10 μl 0.005 M acetic acid) were injected on Days 10, 12, 14, 16 and 18. The vehicle group was injected with an equivalent amount of the ethyl alcohol or acetic acid on the same schedule. Injections on Days 10, 12 and 14 were made under the chorioallantoic membrane; later injections were placed on the membrane. The embryos were sacrificed on Day 20.5 just before hatching. Body weights were obtained after removal of the egg membranes and the yolk sac. The tibias were removed, cleaned of connective tissue and muscle, and allowed to dry before the narrow diameter of the diaphysis and the length were measured. Mortality was not significantly altered by the injection procedure or by any of the treatments. Corticosterone treatment produced a significant 12% reduction in mean body weight from 33.5 g to 29.4 g (Student’s t test: P < 0.001). (Legend continued on p. 201.)
putative neurotransmitters, the peptides, appear to have similar effects on brain development. However, in contrast to the detrimental effects of drugs which alter the first two groups of neurotransmitters, the administration of some peptides seems to accelerate brain development. Neonatal treatment with α-MSH alters later social behaviour and improves the performance on learning, memory and attention tasks in rats (Beckwith et al 1977); neonatal injection of an ACTH(4-9) analogue enhances attention in adult rats (Champney et al 1976) and ACTH improves 'following' behaviour during imprinting in ducklings (Martin 1978). One subcutaneous injection of ACTH(1-39), (1-24) or (1-16) to neonatal female rats was sufficient to accelerate the onset of eye opening, while injections of ACTH(1-39), (1-24), (1-10) or α-MSH accelerated motor behaviour (van der Helm-Hylkema & de Wied 1976, Jakoubek 1978). These results may represent a direct effect of the peptide on brain development since injection of α-MSH antibodies subcutaneously into 19-day-old rat fetuses not only retarded body growth on Day 21, but also impaired brain maturation (Swaab et al 1978b). These results support the idea that endogenous fetal α-MSH stimulates brain development in the rat. The relationship found by Prechtl et al (1977) between age-inadequate EEG patterns in newborns and low oestrogen excretion by the mother certainly supports this possibility. Since the oestrogen secretion by the mother is largely determined by fetal hormones of the opiomelanocortin family (cf. Honnebier et al 1974), these hormones might be the common factor in both the disturbed oestrogen excretion and in brain development. However, negative effects of opiomelanocortin peptides on brain development may also exist. When ACTH is given to children with petit mal epilepsy reversible enlargement of the cerebral vesicles and subarachnoidal space, apathy, drowsiness and pseudodementia have been reported (Lagenstein et al 1979), while the inhibiting or deleterious actions of opiates and methadone on fetal brain development (Hutchings 1978, Slotkin et al 1979) might also be explained by an action via this peptide family. In addition, neonatal administration of β-endorphin or naloxone to the rat causes a permanent insensitivity to temperature stimuli (Sandman et al 1979).

1a Addition of α-MSH to the corticosterone-treated embryos significantly increased the body weight (P<0.05). The mean weight (32.1 g) of this α-MSH + corticosterone group was not significantly (P>0.20) different from vehicle-injected controls (33.5 g). AVT did not significantly improve the growth rate.

1b In contrast to the effect of α-MSH in restoring the body weight of corticosterone-treated embryos, it failed to overcome the corticosterone-induced inhibition of bone growth. Corticosterone treatment caused an 8.6% reduction in tibial diameter and a 5.7% reduction in tibial length. All corticosterone-treated groups were significantly (P<0.01) different from controls, and the addition of α-MSH or AVT did not restore the bone growth to normal (P>0.20).
FIG. 2. Content and concentration of radioimmunoassayable α-MSH in rat pituitary (a) and rat brain (b) during intrauterine development (Days 16-21) and on the first postnatal day (0), as measured by radioimmunoassay. (P = parturition). The day on which spermatozoa were found in the vaginal smear was taken as Day 0 of pregnancy. Note the rapid rise in pituitary α-MSH content on Day 19 of pregnancy and the relatively high content and concentrations of radioimmunoassayable α-MSH in the fetal brain early in development. Measurements for male adult pituitary and brain are given at the top of the figures (M. Visser & D. F. Swaab, unpublished observations).
The pituitary and fetal brain as production sites for α-MSH

Using an immunofluorescence approach we have found α-MSH in the fetal rat pituitary in the intermediate lobe and in a few scattered cells in the pars distalis (anterior lobe) from Day 18 of pregnancy, while radioimmunoassayable α-MSH was even present two days earlier. On Day 19 of pregnancy, the day of the intrauterine growth spurt in the rat, the fetal pituitary content of α-MSH as determined by both bioassay and radioimmunoassay rises rapidly. Since the absolute amount of bioassayable α-MSH measured in the fetal pituitary was much higher than the values found by radioimmunoassay, and since the drop in the amount of bioassayable α-MSH after fetal Day 20 was not revealed by radioimmunoassay, compounds which are related to α-MSH will contribute considerably to the bioassayable values (Swaab et al. 1976; Fig. 2). Radioimmunoassayable α-MSH is also present at an early stage in the fetal rat brain. The content and concentration in the brain showed a U-shaped curve between Day 16 of pregnancy and birth. The high levels at Day 16 of pregnancy were within the adult range, while the total amount in the brain was about the same as that in the pituitary on Day 16 (Fig. 2). The possibility that a compound related to but different from α-MSH is responsible for the early fetal radioimmunoassayable α-MSH values has to be investigated. According to Bayon et al. (1979) endorphin concentrations are also high in the fetal rat brain during early development, while enkephalin systems, in contrast, appear to be at a much less advanced stage. A fall was found in endorphin levels in the telencephalon, hippocampus and striatum after Day 16 of pregnancy (Bayon et al. 1979). This suggests a connection with the observed levels of α-MSH in whole brain, which also decrease after Day 16. In view of this and the possible function of peptides of the opio-melanocortin family in brain development (see below), and since opiate receptor binding has been found in the fetal rat brain as early as Day 14 of pregnancy (Clendeninn et al. 1976), knowledge of the developmental course of the other members of this family would be of great interest.

The existence of a distinct pars intermedia in the human fetal pituitary suggests the possible presence of the ‘typical’ intermediate lobe hormone, α-MSH, during human development. Using immunohistochemical techniques we have examined a series of human fetal pituitaries. In all of them the pars intermedia contained mainly α-MSH cells with only a few cells staining for ACTH (Fig. 3). In the pars distalis the opposite was observed. In the youngest fetus studied (15 weeks) the pars intermedia contained many α-MSH cells and no ACTH cells, while ACTH cells were already present in the pars distalis (Fig. 3, for details see Visser & Swaab 1979). These observations agree with results from bioassays showing melanocyte-stimulating activity in the human fetal pituitary from 11 weeks of pregnancy onwards (Kastin et al. 1968, Levina 1968). During the course of development the pars intermedia is gradually transferred into a zona intermedia which is punctuated by cysts. α-MSH immunoreactive cells were observed in our study in the zona intermedia and pars
FIG. 3a and b. Cells containing ACTH (a) and α-MSH (b) in a 28-week-old human fetus (see 'HEY' in Table 1 of Visser & Swaab 1979). Staining was performed with the indirect immunofluorescence procedure. In (a) a purified anti-ACTH(1-24) (14 13/12) was used; in (b) a purified anti-α-MSH (4394 9/4) was used. Note the predominant staining of α-MSH-containing cells and the distinct pars intermedia (PI) in the human fetus. NH = neurophysin, PD = pars distalis.
FIG. 4a and b. Cells containing ACTH (a) and α-MSH (b) in an adult human pituitary. The pituitary was obtained from a 35-year-old woman (Z.K.; S65/537) who died from a haemorrhage in the left hemisphere during early pregnancy: (fetus: 7.5 cm). The tissue had been stored for 13 years in paraffin. This staining is representative for human adults (see Visser & Swaab 1979). Staining was performed by means of the unlabelled antibody—enzyme technique. In (a) a purified anti-ACTH(1-24) (14 13/12) was used; in (b) a purified anti-α-MSH (4394 9/4) was used. ZI = 'zona intermedia', NH = neurohypophysis, PD = pars distalis. Note the high ratio of ACTH: α-MSH cells and the localization of the cells in the 'zona intermedia'. Some cells in the walls of the cysts (C) are also stained.
distalis of all pituitaries throughout life, irrespective of age, sex, pregnancy, therapy or cause of death. However, from birth up to 19 years of age progressively fewer α-MSH cells and more ACTH cells were detected in the pars distalis and zona intermedia (Fig. 4, Visser & Swaab 1979). The gradually decreasing ratio of α-MSH cells: ACTH cells in the human pituitary during development agrees fully with the biochemical work of Silman et al (1976). The few α-MSH-positive cells found in adulthood seem consistent with the low levels of α-MSH detectable by radioimmunoassay in the adult human pituitary (Abe et al 1967). The earlier claim that in human adults each ACTH cell also contained α-MSH (Phifer et al 1974) may be attributable to antibody cross-reactivity (Swaab et al 1976) since the reaction is not seen in our study where the antibodies were purified before use (Visser & Swaab 1979).

In the human fetal hypothalamus, neurons stained with antibodies to β-endorphin or ACTH(17-39) from the 11th week of pregnancy onwards (Bugnon et al 1979).

**Synthesis of α-MSH by the brain**

During intrauterine development there are at least three potential sources of peptides of the opiomelanocortin family: the fetal pituitary, the fetal brain (see above), and the placenta (see Genazzani et al 1979). Although it is now half a century since Zondek & Krohn (1932) proposed that 'intermedin', which they found in the brain, would be produced by the pituitary, and nearly 20 years since Guillemin et al (1962) suggested that 'MSH-like' peptides might also be produced by the brain, the relative contributions of the first two potential sources are still a matter of dispute.

The fact that α-MSH and ACTH remain present in similar amounts in the neurons in the various brain sites after hypophysectomy (for references see Swaab et al 1981) has not been accepted as proof of their central origin by Moldow & Yalow (1978). They argue that pituitary remnants found in the **sella turcica** after commercial hypophysectomy are responsible for this phenomenon. Since, in addition, the concentration of ACTH and MSH in the various brain sites was found to be related directly to the distance of these areas from the hypophysis, they suggested that the pituitary is the sole site of synthesis of these hormones. This idea is, of course, consistent with the observations that behavioural deficits after hypophysectomy could be restored by peripheral injections of ACTH and MSH (e.g., de Wied 1964, 1965). Although others found much less ACTH in the sella after hypophysectomy, and the concentrations in the blood were not affected by this remaining source (van Dijk 1979), one could add to Moldow's arguments that the pars tuberalis (superior lobe) might remain functional after hypophysectomy (Ordonneau 1979), while the presence of pituitary tissue in the pharyngeal canal, and even the pharynx itself (McGrath 1976, 1978), might be an additional source of hormones after hypophysectomy. Retrograde transport of peptides from the pituitary to the brain
– proposed by Popa as early as 1938 – is also possible (Mezey et al 1979), while transport via the cerebrospinal fluid, also proposed by Popa (1938), remains an additional possibility (Mezy et al 1979). There is, however, growing evidence for the production of 'pituitary' hormones by the nervous system itself:

(1) α-MSH is present in the arcuate nucleus of the hypothalamus and it disappears from the brain sites that receive fibres from the arcuate nucleus after electrolytic, knife, or monosodium glutamate lesioning of this area, while the pituitary store of α-MSH does not decrease (Eskay et al 1979a,b, O'Donohue et al 1979).

(2) α-MSH-like material can be stained immunocytochemically in explants of dorsal root ganglia (F. W. van Leeuwen, unpublished observation) and in organ cultures of the pineal gland, using synthetic culture medium which precludes uptake of exogenous peptides (Pévet et al 1980).

(3) The localization of 'pituitary' peptides in the nervous system is certainly not restricted to sites that are immediately connected to the hypophysial portal vessels as claimed by Moldow & Yalow (1978); α-MSH immunoreactivity occurs in neurons of the spinal cord and dorsal root ganglia and in the pineal gland (Swaab & Fisscher 1978, Pévet et al 1980, O'Donohue et al 1980).

(4) Immunoreactive α-MSH and related peptides have been found in nerve cells of invertebrates including the pond snail *Lymnaea stagnalis*, none of which have pituitary glands (Boer et al 1979, Schot et al 1980).

(5) Immunoelectronmicroscopical localization of α-MSH-like material on the endoplasmic reticulum of dorsal root ganglion cells (van Leeuwen 1980) and of α-MSH in vesicles of arcuate nucleus neurons (Pelletier & Dubé 1977, Pelletier & Leclerc 1979) also supports their central site of origin.

(6) Release of α-MSH from hypothalamic synaptosomes is potassium-dependent (Warberg et al 1979).

From these observations, and the fact that a significant transfer of peptides from the blood to the brain is unlikely in view of the effective blood–brain barrier for such peptides, there seems to be little doubt that opiomelanocortin peptides are also produced by the nervous system. The additional contribution of pituitary peptides to the peptide content and the physiology of various brain sites remains to be determined.

Problems in studying functions of the opiomelanocortin peptides in fetal growth and development

It is difficult to define the relative importance of the various opiomelanocortin peptides in normal human fetal growth and development for several reasons.

First, the number of known active peptides in the opiomelanocortin family has increased to such an extent that simple observations seem no longer meaningful. Second, the interrelated nature of the fetal pituitary, brain and placenta makes
interpretation of experimental intervention difficult. For example, it is unclear whether the fetal brain, the fetal pituitary or the placenta produces an intrauterine growth factor. Placental size depends on the integrity of the fetal brain, the fetal pituitary and the circulating pituitary hormones (Honnebier & Swaab 1973, 1974). Furthermore it is technically not feasible to hypophysectomize a fetal rat without concomitant damage to its hypothalamus; hence, it is difficult to determine the source of intrauterine growth factors. We have to rely on several inferential lines of evidence for a tentative answer. For example, brain aspiration in the rat fetus causes a growth reduction which is rather reproducible regardless of whether the pituitary remains intact or not (Swaab et al 1978a); hypothalamic lesions induce growth retardation in the rat fetus (Fujita et al 1970); microcephalic children who do not have a hypothalamus show growth retardation, while a case of hydro-encephaly (absent cerebrum but intact brain stem) had normal birth weight (Swaab et al 1978b). All these observations might mean either that the fetal hypothalamus produces a compound that is essential, e.g., for the release of the pituitary growth factor, or that the fetal hypothalamus produces the fetal growth factor itself. The latter possibility is emphasized by the observation that the five collected cases of children with congenital pituitary aplasia had normal birth weights and were born at term (Swaab & Honnebier 1974).

A third reason why the function of these peptides in fetal development is difficult to define is because some peptides of this group may have trophic effects on various organs. ACTH and, in some instances, α-MSH stimulate the fetal adrenal to release corticosteroids which may themselves influence embryonic growth. Controlling this source of variation is necessary for meaningful conclusions. It remains possible that α-MSH will prove to be a trophic hormone for other organs, such as the brain (Swaab et al 1978b), liver (Vándor et al 1975) and bone (Stenström et al 1979).

A fourth reason lies in the rapidly changing or dynamic nature of physiological processes in the fetus. Since the tissues are being transformed more rapidly during this stage of life than at any other time, the experimenter must deal with the problem of interaction between the treatments and a non-homeostatic, temporally unstable system. For example, Rychter & Šlastný (1979) found that corticosteroid treatment may either stimulate or inhibit the development of the choroid plexus of the embryonic chick telencephalon, depending on the developmental stage of treatment. Van der Helm-Hylkema (1973) also reported that the same peptides given at two different developmental stages to neonatal rats may not have the same effects on development.

These problems are further exacerbated by difficulties in analysing the small amounts of tissue available in the fetuses of most laboratory animals. Patience and attention to new ways to solve these problems will, we hope, eventually allow us to define more satisfactorily the relative importance of opiomelanocortin peptides in fetal development.
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DISCUSSION

Loh: Could you remind me about the day of pregnancy on which you observed the increase in α-MSH in the pituitaries of fetal rats?

Swaab: We found a rapid increase in radioimmunoassayable α-MSH in the rat pituitary between Day 18 and 20 of pregnancy so it occurred around the moment of the intrauterine growth spurt at Day 19. We also found a similar rapid increase when we used a bioassay (Swaab et al 1976), but the concentrations of α-MSH detectable by bioassay were much higher.

Loh: Did the concentrations fall after birth?

Swaab: When the amounts were measured by bioassay they did fall after birth (Swaab et al 1976), but when measured by radioimmunoassay they did not fall detectably, when expressed as total pituitary content.

Loh: Can you explain why there is a difference between results from the two methods?

Swaab: Presumably we are measuring more than just α-MSH in the bioassay.

Loh: Have you treated the bioassayable material with pronase to see if you are really measuring an α-MSH-related peptide?

Swaab: No.

Loh: We have used the same antibody that you used (specific for lysine-proline-valine amide; i.e. the α-MSH antibody), to study brain extracts of fetal rats. We did
not observe the increase that you found in brain α-MSH in the early stages (Day 16 of pregnancy) unless we used a bioassay (Loh et al 1980).

_swaab_: Your results again illustrate that the bioassay for α-MSH probably identifies other compounds as well.

_lowry_: Have you used high-pressure liquid chromatography to separate the various compounds extracted from the rat fetal brain?

_swaab_: No.

_tilders_: If MSH induces developmental changes by a direct action, it presumably has to reach the peripheral organs in order to produce those growth effects. Do you know the α-MSH concentrations in the general circulation?

_swaab_: We have measured hundreds of samples of human maternal blood, human fetal blood and amniotic fluid but we are not yet satisfied with our extraction and radioimmunoassay technique. However, the concentrations that we have estimated so far are very much lower than those reported by Clark et al (1978). If α-MSH is present at all in the fetal circulation (and I am not yet at all convinced that it is) it is only in picogram/ml amounts.

_silman_: There is also doubt about the presence of circulating α-MSH in the fetal rat (Wilson & Morgan 1980), even at the time of the intrauterine growth spurt, when the pituitary content of α-MSH is at its maximum.

_thody_: We have found high plasma α-MSH concentrations during the first few days of postnatal life in the rat. The concentrations then fall and remain low until about the onset of sexual maturity (Thody et al 1980).

_swaab_: We are not sure about our analyses of blood samples any more since we separated the α-MSH tracer (which was obtained by Thody’s procedure) on a pH gradient and found several peaks. So it is clear that there is more than just α-MSH that is labelled. We are not attempting to obtain a pure label which is of course necessary before we can be conclusive about the presence of the small amounts of α-MSH in fetal blood.

_besser_: Have you studied the effects on growth of any conventional hypothalamo-pituitary-regulating hormones?

_swaab_: We tried oxytocin but it did not have any effect (Swaab & Honnebier 1974); vasopressin might indeed be a growth factor (Boer et al 1980), but we did not try any of the conventional hypothalamic hormones further.

_hadley_: In children with congenital growth defects α-MSH stimulated the release of growth hormone (Bernasconi et al 1975). How good is the evidence that later in life α-MSH is a growth hormone-releasing factor?

_swaab_: α-MSH and ACTH have indeed growth hormone-releasing properties both in vivo and in vitro (Zahnd & Vecsey 1977). The physiological meaning of this effect remains unknown at present. But growth hormone itself did not stimulate rat fetal growth and is also supposed not to be essential for human fetal growth (Swaab & Honnebier 1974). We could find no effect on growth per se of α-MSH or anti-α-MSH injected into neonatal rats during the first 14 days post-
natally, so the \( \alpha \)-MSH seems to be a growth stimulant only during the fetal period (Swaab et al 1978). This idea is reinforced because the fetal growth-promoting effect we observed was described in fetal rats from which both the brain and the pituitary were removed (Honnebier & Swaab 1974). There is no indication whatsoever that this growth-promoting effect of \( \alpha \)-MSH is executed via growth hormone.

**Eberle:** Zahnd (1978) showed that \( \alpha \)-MSH stimulates the spontaneous secretion of human growth hormone *in vivo* and *in vitro* and of rat growth hormone in preparations of isolated pituitary tissue and cell cultures. Structure—activity studies revealed that addition of \( 10^{-5} \text{M} \) to \( 10^{-6} \text{M} \) \( \alpha \)-MSH or \( \alpha \)-MSH(5-10) produced a dose-related (\( 10^{-9} \text{M} \) to \( 10^{-6} \text{M} \)) increment of growth hormone release (Zahnd et al 1979); the NH\(_2\) and COOH-terminal fragments of \( \alpha \)-MSH were without effect. Addition of anti-\( \alpha \)-MSH antiserum to the test system suppressed the effects of either \( \alpha \)-MSH or the pituitary extracts. So far, our anti-\( \alpha \)-MSH antiserum has not been injected into neonatal rats.

**Lowry:** We have examined growth hormone release from cells in culture. Spontaneous release occurs sometimes. *In vivo* the control of release is under both positive and negative hypothalamic control, which probably makes release from cells in culture unstable. However, we have examined hypothalamic extracts, semi-purified by HPLC (J. Sykes, personal communication), and the released material is unrelated to \( \alpha \)-MSH. The amount of growth hormone-releasing activity in the fractions from the HPLC run that contained \( \alpha \)-MSH was very small.

**Tilders:** In view of the observations from Dr Porter's laboratory, we have to keep in mind that the anterior lobe of the pituitary gland might be exposed to extremely high concentrations of \( \alpha \)-MSH. In a study on male rats Oliver et al (1977) reported \( \alpha \)-MSH concentrations in portal blood of 103 ng/ml, whereas peripheral blood samples showed only 253 pg/ml.

**Lowry:** When \( \alpha \)-MSH is given *in vitro* in the nanogram range the magnitude of growth hormone release is not at all like that observed when a semi-pure hypothalamic extract of growth hormone-releasing factor is used.

**Edwardson:** We heard earlier that human prolactin in large doses causes skin darkening in hypophysectomized fish. Since large concentrations of \( \alpha \)-MSH release growth hormone, which is not unrelated to prolactin, I wondered whether cross-reactivity has been taken into consideration in these assays. If a substance such as \( \alpha \)-MSH is injected or used *in vitro* at a concentration of \( 10^{-5} \text{M} \) and one observes release of a substance such as growth hormone at a concentration of \( 10^{-9} \text{ or } 10^{-10} \text{M} \), then the cross-reactivity of \( \alpha \)-MSH in the immunoassay for growth hormone should be checked at 0.001%. If there was some structural resemblance between \( \alpha \)-MSH, growth hormone and prolactin this could explain the skin-darkening effects of prolactin and the apparent growth hormone-releasing effects of high doses of \( \alpha \)-MSH.

**Swaab:** I must say we have never put prolactin in the \( \alpha \)-MSH assay!

**Lowry:** Of course, a dose—response curve to trypsin can be produced for every peptide that is immunoassayed!
Swaab: Or to albumin.

Lowry: It's not significant; it happens because the radiolabelled peptide used in the radioimmunoassay is metabolized and so a dose—response curve can be produced. People who do peptide radioimmunoassays in plasma have to take proteolytic degradation into account; when they think they are detecting peptides they are probably seeing the result of label that has been degraded in their assay tubes during the 24-hour incubation.

Swaab: The only thing that I can add is that in immunocytochemistry, with antibodies to α-MSH or ACTH, one does not stain the prolactin cells in the pituitary. We have assayed fetal brains for α-MSH after vycor extraction, which probably does not extract the prolactin efficiently.

Lerner: What about the placenta as a source of these peptides?

Swaab: It is the third possible source of the entire family of opioid peptides (Genazzani et al 1979) and it complicates the matter so that if the fetal brain is removed, the placental weight decreases (Swaab & Honnebier 1973); we also found smaller placental weights in human anencephalics (Honnebier & Swaab 1973). If growth hormone or α-MSH is injected into the rat fetus the placental weight increases (Honnebier & Swaab 1974). So there is a correlation between the function of the hypothalamo-pituitary axis and the placental size and, consequently, possibly also the placental function.

Lerner: What amounts of peptides are present in the placenta?

Swaab: I don't know.

Besser: Presumably anencephaly is not an all-or-none phenomenon; there must be some fetuses that have a little bit of brain tissue and others that have none. Can you relate the onset of parturition to the amount of brain tissue present?

Swaab: The only available measurement we have is adrenal weight; we found no correlation between the onset of labour and the adrenal weight.

Silman: Anderson et al (1969, 1971) showed that anencephalics that were delivered post-term had small adrenal weights, whereas fetuses that were delivered at term had at least some fetal zone.

Swaab: Yes. We tried to confirm that and collected the adrenal weights (Honnebier & Swaab 1973) but we could not find any consistent trend.

REFERENCES


