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HUMAN FETAL CORTICOTROPHIN AND RELATED PITUITARY PEPTIDES

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SUMMARY

In an attempt to distinguish a family of closely related pituitary peptides, human fetal and adult pituitaries were extracted, chromatographed and the fractions subjected to at least 3 systems of radioimmunoassay. From the 12th week to the 38th week of gestation the dominant peptides of the human fetal pituitary were found to resemble corticotrophin-like intermidiate lobe peptide (CLIP) and α-MSH rather than ACTH. At term the profile was reversed and ACTH became the dominant peptide. It was not possible to investigate these findings in plasma.

We advance the hypothesis that it is CLIP or α-MSH which will prove to be the trophic hormone for the fetal zone of the adrenal cortex and suggest that the switch of peptide synthesis in the human fetal pituitary at term is functionally related to the metamorphosis of the human fetal adrenal gland.

INTRODUCTION

At birth the massive fetal zone of the human adrenal gland involutes and the thin definitive cortex hypertrophies. Liggins has shown that, in the sheep, the surge of fetal cortisol is the signal that triggers labour [1]. It has been suggested that in man there is a similar surge of fetal cortisol at term [2, 3]. Since the fetal zone of the human fetal adrenal cortex is incapable of synthesizing cortisol, the increased production is thought to derive from the definitive zone. Thus it may be that the metamorphosis of the gland is functionally related to the onset of labour.

Although it is undisputed that adrenocorticotrophin (ACTH) is the trophic hormone for the definitive zone, there has been no satisfactory explanation to account for the hypertrophy and subsequent involution of the fetal zone. The intention, therefore, of the present work was to investigate the hypothesis that the change in human fetal adrenal function at term was not an autonomous event but represented a direct effect of a change in the trophic stimulus from the pituitary.

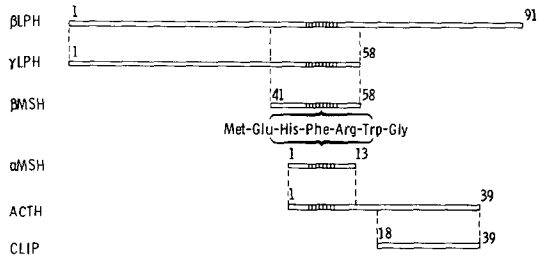

Fig. 1. Corticotrophin, lipotrophin and melanocyte stimulating hormones. β-LPH is a 91 amino acid peptide whose first 58 amino acids are identical in sequence to γ-LPH. β-MSH shares its 18 amino acid sequence with both β and γ-LPH. ACTH is a 39 amino acid peptide whose first 13 amino acids are identical in sequence with α-MSH, and whose 18–39 sequence is identical to CLIP. The hatched portion represents the common sequence of seven amino acids responsible for melanocyte stimulating activity in β-LPH, γ-LPH, β-MSH α-MSH and ACTH.

ACTH is one member of a family of related pituitary peptides (Fig. 1). It is synthesized in the anterior lobe together with β-lipotrophin (β-LPH) and possibly γ-lipotrophin (γ-LPH). α-melanocyte stimulating hormone (α-MSH) and β-melanocyte stimulating hormone (β-MSH), however, are formed in the pars intermedia. Corticotrophin-like intermediate lobe peptide (CLIP) which does not possess melanocyte stimulating activity, is also thought to be formed in the pars intermedia as an accompaniment to the synthesis of α-MSH [4].

It is well known that the pituitary content of these various peptides differs between species. Likewise it is possible that there is a different peptide profile in fetal and adult glands of the same species. Certainly the human fetal pituitary has a well-defined pars intermedia which involutes after birth [5], and from the 12th week of gestation it has melanocyte stimulating activity which cannot be correlated with ACTH bioactivity [6]. Moreover, many biological assays performed on human pregnancy blood and urine have demonstrated elevated levels of melanocyte stimulating activity [7, 8, 9]. Unfortunately in all such investigations the active compound has not been fully identified. This problem has therefore been examined afresh in an attempt to trace the transformations that might occur from fetal to post-natal life and to establish whether any such changes can be correlated with fetal adrenal function.

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EXPERIMENTAL

Plasma

Blood samples of approximately 40 ml were collected from women in the third trimester of pregnancy between 09.00 and 12.00 h, and from control non-pregnant women. The blood was placed in heparinized tubes and separated as rapidly as possible. The plasma was decanted and stored at −20°C until assayed.

Assays were carried out on plasma extracts prepared by adsorption on to leached silica glass (Florisil for bioassay; Vycor glass for immunoassay).

Pituitaries

a. *Hysterotomy.* Human fetal pituitaries up to the 24th week of gestation were obtained following hysterotomy for therapeutic abortion. The pituitaries were freshly dissected and stored at −20°C until extracted.

b. *Post-mortem.* After the 24th week of gestation fetal pituitaries were obtained at post-mortem. In some this followed stillbirth, in others neonatal death. The delay between death and post-mortem was usually about two days. Adult human pituitaries were also obtained following post-mortem, the delay after death being approximately two days. All pituitaries dissected at post-mortem were stored at −20°C until extracted.

c. *Extraction.* The pituitaries were sliced into portions of approximately 10 mg and each piece individually homogenized using a glass rod in an LP3 tube in 0.5 ml of 0.02 M HCl. A few grains of acid washed sand were included to assist homogenization. Diisopropylphosphofluoridate was added at a concentration of 10^{-3} M to inhibit enzyme action. The homogenate was centrifuged and the supernatant stored at −20°C until chromatography. All extraction procedures were carried out in the cold.

d. *Chromatography.* Pituitary extracts were chromatographed on a column (1.6 × 100 cm) packed with Sephadex G-100 or with Biogel P-6. Samples of 0.5 ml to 2 ml were applied and eluted in 1 M acetic acid at a rate of 3 ml/h. Fifteen minute fractions were collected and alternate fractions assayed by radioimmunoassay.

Radioimmunoassay

Synthetic α-MSH and synthetic 'human' β-MSH obtained from Ciba Ltd., Horsham, Sussex, and natural human ACTH, prepared in the Department of Chemical Pathology, St. Bartholomew's Hospital, were used as standards and for iodinated tracers.

The *β-MSH assay* [10] used rabbit antiserum raised against an impure ACTH preparation as antibody, synthetic 'human' β-MSH as standard and [^{125}I]-synthetic 'human' β-MSH as tracer. The assay showed no cross-reaction with ACTH or α-MSH but both β-LPH and γ-LPH gave parallel inhibition.

The *C-terminal ACTH assay* [11] used rabbit antiserum raised against synthetic 1-39 human ACTH as antibody, natural human ACTH as standard and [^{125}I]-natural human ACTH as tracer. The assay showed no cross-reaction with β-MSH, β-LPH, γ-LPH, and α-MSH but gave parallel inhibition with CLIP.

The *N-terminal ACTH assay* [12] used rabbit antiserum raised against 1–24 ACTH as antibody, natural human ACTH as standard and [^{125}I]-natural human ACTH as tracer. The assay showed no-cross reaction with β-MSH, β-LPH, γ-LPH and CLIP but gave parallel inhibition with α-MSH.

The *α-MSH assay* used rabbit antiserum raised against synthetic α-MSH as antibody, synthetic α-MSH as standard and [^{125}I]-synthetic α-MSH as tracer. The assay showed no cross-reaction with β-MSH, β-LPH, γ-LPH, ACTH and CLIP.

In vitro frog skin bioassay [13].

Light adapted *Rana temporaria* were employed. For each assay sixteen pieces of skin, obtained from the hind legs of four frogs, were secured on rings and arranged in a Latin square. The skins were bathed in Ringer solution and a reflectance meter was used to determine baseline readings. A high and low standard and a high and low unknown were each applied to a set of four rings. After one hour, reflectance readings were taken again and skin darkening recorded.

In this system the family of peptides α-MSH, β-MSH, γ-LPH, ACTH and β-LPH have, on a weight basis, relative potencies of 1.00:0.25:0.005: 0.003:0.002 respectively. For the plasma assays α-MSH was used as standard. For the pituitary fraction assays, ACTH was used as standard.

RESULTS

Plasma

No MSH activity was detected by bioassay in plasma from normal control subjects. In samples from 42 pregnant women, activity was detected in 24 (57%), the levels ranging from 6–200 pg α-MSH/ml (Fig. 2). No relationship to gestational age was apparent.

Immunoassay on plasma extracts did not show any difference between pregnant women and normal controls (Fig. 3). Using the N-terminal ACTH assay and the β-MSH assay, values for the pregnant subjects fell within the normal range. With the comparatively insensitive α-MSH assay, however, both groups fell below the minimum detection limit for the assay.

Pituitaries

a. *Adult.* The chromatographic profile was similar in all subjects and an example is illustrated in Fig. 4.

The *β-MSH assay* demonstrated two peaks of high molecular weight activity corresponding to the expected positions for β-LPH and γ-LPH. There was no activity in the expected position for β-MSH. The *C-terminal ACTH assay* demonstrated a peak of ac-

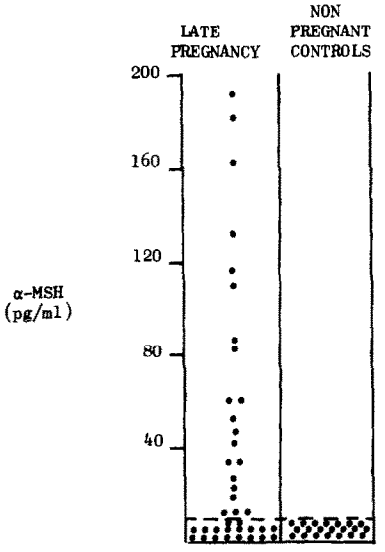

Fig. 2. Melanocyte stimulating activity in extracted human plasma. ---- represents the minimum detection limit of the assay. ● represents the assay results for individual plasma samples.

tivity in the expected position for ACTH and this was confirmed by a peak of activity in the same position using the *N-terminal ACTH assay*. The melanocyte stimulating activity of this peak was also consistent with it being ACTH.

Apart from the three major peaks representing β-LPH, γ-LPH and ACTH, there was a shoulder of C-terminal activity on the trailing edge of the ACTH peak which could represent a small amount of material resembling CLIP. Likewise there was a very small peak of low molecular weight N-terminal activity that could represent an α-MSH-like peptide. The biological activity of this last peak, though far greater than could be accounted for by ACTH, was in fact only

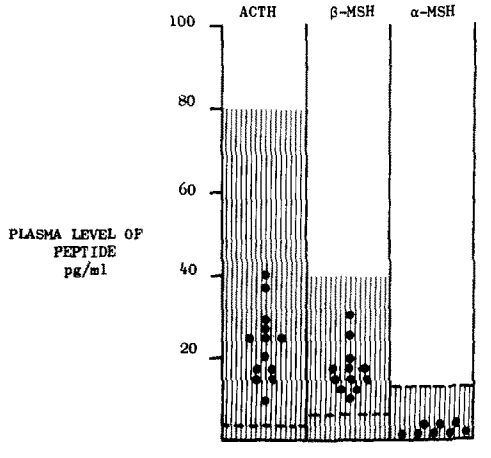

Fig. 3. Immunoassay of ACTH and MSH-like peptides in extracted human plasma. ACTH was measured by the *N-terminal ACTH assay*. The hatched portion represents the normal range for each assay. ---- represents the minimum detection limit for each assay. ● represents the results in each assay for plasma samples taken in the third trimester of pregnancy.

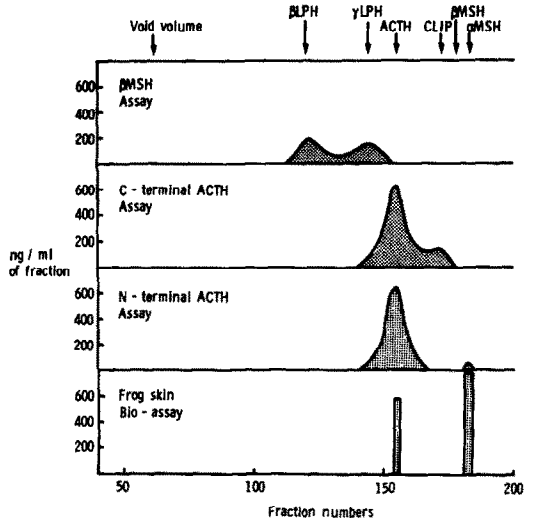

Fig. 4. Chromatography of a pituitary extract on Sephadex G-100 from an adult pituitary. The expected positions of β-LPH, γ-LPH, ACTH, CLIP, β-MSH and α-MSH are indicated by the arrows. The fractions were examined in 3 systems of radioimmunoassay and those encompassing the peaks of ACTH and α-MSH were examined by frog skin bioassay.

about 5% of the potency expected for authentic α-MSH.

b. *Fetal.* The pituitary from a female fetus of 24 weeks gestational age was extracted and chromatographed on a Sephadex G-100 column. The fractions were analysed and the results illustrated in Fig. 5.

The *β-MSH assay* gave a similar profile to that found with the adult pituitaries. There were two peaks of high molecular weight activity corresponding to the expected positions for β-LPH and γ-LPH and again there was no evidence for β-MSH.

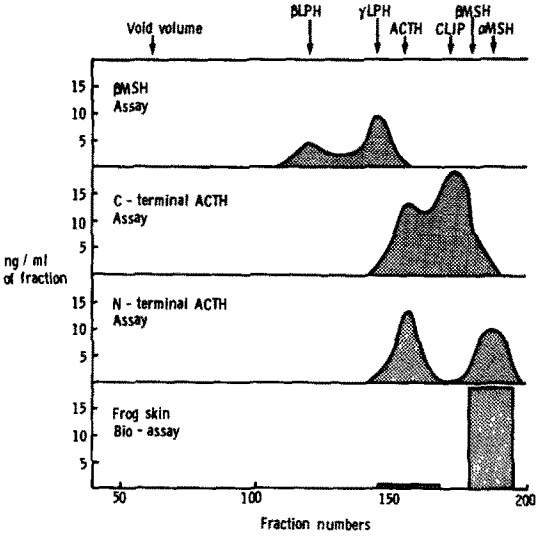

Fig. 5. Chromatography of a pituitary extract on Sephadex G-100 from a fetus of 24 weeks gestation delivered by hysterotomy. The expected positions of β-LPH, γ-LPH, ACTH, CLIP, β-MSH and α-MSH are indicated by the arrows. The fractions were examined in 3 systems of radioimmunoassay and those encompassing the peaks of ACTH and α-MSH were examined by frog skin bioassay.

The *C-terminal ACTH assay*, while it demonstrated a peak of activity in the expected position for ACTH, showed it to be dominated by a peak of activity corresponding to the expected position for CLIP. The *N-terminal ACTH assay* demonstrated a peak of activity in the expected position for ACTH but showed no activity in the expected position for CLIP confirming, therefore, that this peak was of solely C-terminal activity. A substantial peak of small molecular weight N-terminal activity was demonstrated. Its biological activity was far more than could be accounted for by ACTH, though less than would be expected for α-MSH.

A 12-week, 13-week, 14-week and two 15-week fetal pituitaries were extracted. The supernatants were pooled and chromatographed on a G-100 Sephadex column. The immunoassay results are illustrated in Fig. 6. There were peaks of activity, as with the adult pituitaries, corresponding to β-LPH, γ-LPH and ACTH. There was no evidence for β-MSH. However, as with the 24-week fetal pituitary, there were in addition two dominant peaks of activity corresponding to material resembling CLIP and α-MSH.

c. *Fetal to adult.* Fetal pituitaries of 33-weeks and 38-weeks gestational age were extracted and chromatographed on a Biogel P-6 column which permitted the complete separation of CLIP and ACTH. The fractions were analysed by the *C-terminal ACTH assay* and the ratio of ACTH to material resembling CLIP determined. The results showed a dominance of material resembling CLIP over ACTH and were similar to those of early gestation.

A 40-week fetal pituitary treated in the same manner had approximately equal proportions of ACTH and material resembling CLIP. The pituitary from a

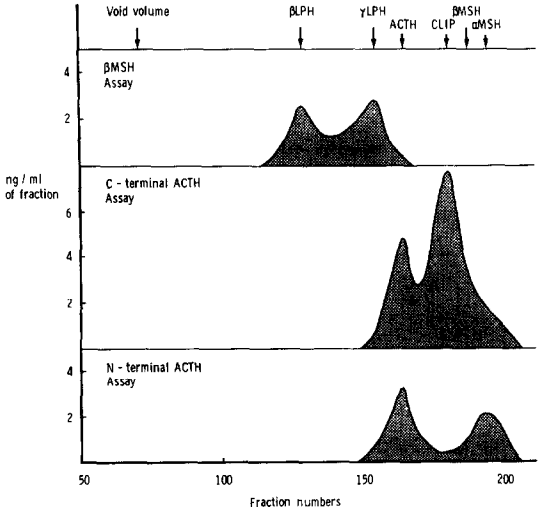

Fig. 6. Chromatography of a pituitary extract on Sephadex G-100 from fetuses of 12, 13, 14 and 15 weeks gestation. All fetuses were delivered by hysterotomy and their pituitary extracts pooled. The expected positions of β-LPH, γ-LPH, ACTH, CLIP, β-MSH and α-MSH are indicated by the arrows. The fractions were examined in 3 systems of radioimmunoassay.

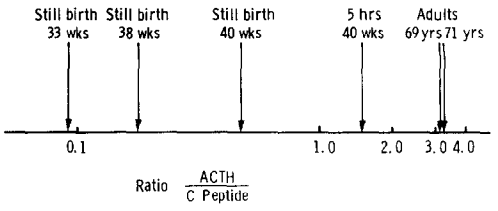

Fig. 7. Ratio of ACTH activity to small C-terminal peptide activity in adult and fetal pituitaries as assessed by chromatography on Biogel P-6. The fetal pituitaries were obtained from a 33-week stillbirth, a 38-week stillbirth, a 40-week stillbirth and a full-term infant who survived 5 hours. The adult pituitaries were obtained post-mortem from patients aged 69 and 71 years.
(From Silman *et al.* [16])

full-term infant who survived 5 h showed slightly more ACTH than material resembling CLIP. As can be seen in Fig. 7, the results from these term pituitaries lie between those of fetus and adult.

DISCUSSION

The results have demonstrated that the adult human pituitary produces β-LPH, γ-LPH and ACTH. These peptides are also produced by the human fetal pituitary but here they are dominated by the production of peptides resembling CLIP and α-MSH. At no stage in human development is there any evidence for the existence of β-MSH.

The presence of peptides resembling α-MSH and CLIP in the human fetal pituitary may relate to some specific physiological function. Much work has been done to demonstrate that pars intermedia peptides, in particular α-MSH [14], stimulate sebum secretion in the rat. The heavy secretion of the sebaceous vernix which covers the human fetus, and which is a specific feature of the fetus, might therefore be stimulated by the peptides described. Alternatively, or additionally, the fetal growth promoting role of α-MSH in the rat, which has been described by Swaab and his colleagues [15] might also play a part in the human fetus, though it is uncertain that the peptide described is indeed α-MSH.

Although the results presented are clear for the pituitary extracts, this is not the case for plasma. It has been shown here that there is increased melanocyte stimulating activity in pregnancy plasma but it was not possible to identify the substance or to relate it to other findings. However, it is clear that because of the similarities between the peptides, a single assay system or even a multiple assay system will be unable to distinguish the type of changes noted in pituitary extracts. It is only possible to distinguish ACTH from peptides resembling α-MSH and CLIP by a combination of the *N-terminal and C-terminal ACTH assays* with column chromatography. Unfortunately the concentration of these peptides in plasma is too low to permit this. Thus if there is a change from one species of peptide to another in the plasma, it will be undetectable in the present systems.

It has been demonstrated here that the change from fetal to adult profile of the ACTH-like peptides in the human pituitary is not a gradual transformation with increasing gestational age but rather appears to occur abruptly at term. Parturition is accompanied by a switch in production from material resembling CLIP to ACTH. Consequently, we would propose the hypothesis that it is the material resembling CLIP or α-MSH which is the trophic stimulus for the fetal zone of the human fetal adrenal cortex while ACTH is the trophic stimulus for the definitive zone, and we suggest that it is the switch in the trophic stimulus from the pituitary which is responsible for the metamorphosis of the human fetal adrenal gland and the onset of labour.

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DISCUSSION

Grumbach. This is really potentially exceedingly important in terms of the fetal adrenal cortex and some of the evidence that we've heard earlier about ACTH. You've certainly cleared up something, and that is if this appears, if the same patterns holds through on the circulation, which remains to be demonstrated, and I think it would be important to do this, then it would explain why there is not a better correlation between the growth of the fetal adrenal cortex and the pattern of ACTH as measured by radioimmunoassay both by the Portland group and the Dallas group.

Silman. That's right and I was trying to suggest just this to Dr. Challis yesterday. We have undertaken a pilot study in the sheep and it appears that at parturition there is also a switch in the fetal pituitary from 'fetal' ACTH to 'adult' ACTH. But, employing a simple RIA system, both forms cross-react equally. So, if a rise in cortisol consequent upon an increase in 'adult' ACTH were itself accompanied by a decline in the secretion of 'fetal' ACTH, this would be missed by our present methods of measurement and it would appear as if nothing at all had happened. Our problem now is to try and develop a system which will distinguish these peptides in plasma.