reductions in glucose utilisation and to a greater degree. At the highest dosage (1 mg per kg per min) glucose utilisation was significantly depressed in all but 2 of the 26 structures examined (Table 1). Although the magnitude of the effects was substantial in many structures, the greatest depressions were in all the components of the auditory system, (cochlear nucleus, superior olive, nuclei of lateral lemniscus, inferior colliculus, medial geniculate body, and auditory cortex, Table 1). Normally, these structures have relatively high rates of glucose utilisation in the brain and, therefore, clearly stand out in the autoradiographs as areas of high optical density (Fig. 1*b*). Propranolol reduced the rates of metabolism of the auditory structures more than those of other structures which caused their representation in the autoradiographs to fade into the background of the other structures (Fig. 1*a*).

The α-adrenergic blocker phentolamine also produced systemic physiological effects. Body temperature tended to fall by as much as 3 °C, and blood pressure and pulse rate fell significantly with all doses used. Most of the animals exhibited progressive decreases in alertness and remained quiet with the eyes closed, but they responded to tactile and auditory stimuli. The penetrability of phentolamine through the blood–brain barrier has been reported to be limited[9], but, nevertheless, the drug at the concentrations used did exert effects on the glucose utilisation of several structures of the brain. Metabolic rate tended to be reduced in most of the structures examined, but these effects were of lesser magnitude than those seen with propranolol. The most striking effects, however, were also in the components of the auditory system, but in contrast to the effects of propranolol, glucose utilisation in these structures was stimulated by phentolamine (Table 1). The effects on the auditory system were sufficiently pronounced that they could readily be visualised in the autoradiograms by the markedly increased densities in the regions corresponding to these structures (Fig. 1*c*).

The results of these studies demonstrate that the α- and β-adrenergic blocking agents, phentolamine and propranolol, respectively, have striking and opposite effects on glucose utilisation in the auditory system. These effects on energy metabolism seem to reflect alterations in functional activity. Furlow *et al.*[10] found that doses of propranolol and phentolamine like those used in the present studies produce corresponding effects on auditory evoked responses in the rat; propranolol depresses and phentolamine enhances the amplitude of all components of the evoked auditory responses.

The stimulation of glucose utilisation by phentolamine seems to be dependent on the presence of auditory input. In rats deafened bilaterally by rupture of the tympanic membranes and occlusion of the external auditory canals with wax, phentolamine failed to increase glucose utilisation in the auditory structures significantly above the low levels seen in deafened animals. These results indicate that the effect of phentolamine is manifested only during auditory input and suggest that its effects in the auditory system may be the result of blockade of an α-adrenergic negative feedback pathway in the central auditory system. α-Adrenergic mechanisms have previously been implicated in the auditory system, and noradrenaline-containing nerve terminals have been localised in most of the structural components of the auditory system[11–16].

DL-Propranolol is not only a β-adrenergic blocking agent, but it also has membrane-stabilising properties. Experiments with sotalol, a more specific β-adrenergic blocking agent lacking membrane-stabilising properties[17], indicate that it does not affect LCGU of the auditory system like DL-propranolol, but it also failed to produce the same behavioural effects, possibly because of its limited ability to cross the blood–brain barrier[17,18]. L-Alprenolol, another specific β-adrenergic blocker, has effects similar to those seen with propranolol, but this drug also has the membrane-stabilising property. Preliminary studies with the separated isomers of propranolol indicate that they have almost equal effects on the glucose utilisation of the auditory system, despite the fact that L-propranolol is twice as potent a β-adrenergic blocking agent as the D-isomer in the central nervous system[19]. Both, however, are approximately equally potent in membrane stabilisation[20], and these results suggest that the membrane-stabilising properties might be responsible for the effects in the auditory system.

Propranolol has recently been proposed as a therapeutic agent for treatment of psychoses[7,8] and is particularly effective in suppressing the auditory hallucinations in schizophrenia[7,21]. Atsmon *et al.*[22] have reported a case study in which the administration of an α-adrenergic blocking agent reversed the beneficial effects of propranolol in a psychotic patient. The effects of propranolol on the auditory system observed in the present studies might be related to its effects in the control of auditory hallucinations.

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Received 10 July; accepted 11 October 1978.

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High-affinity enkephalin-degrading peptidase in brain is increased after morphine

CONSIDERABLE evidence now exists to suggest that the endogenous opioid pentapeptides Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) function as neurotransmitters in the central nervous system. A correlate of this hypothesis is that a specific inactivation mechanism must operate in the vicinity of opiate receptors to turn off rapidly the enkephalin signal. Indeed recent studies have shown that the pentapeptides are subject to extremely rapid inactivation in various tissues, occurring primarily by cleavage of the Tyr–Gly amide bond[1–5]. This feature accounts for the short-lasting biological activity of these peptides, contrasting with the potent activity of synthetic analogues such as (D-Ala^2)-Met-enkephalinamide in which this bond is protected[6]. However, many tissues including brain contain a spectrum of peptidases with low specificities and affinities[7] and no evidence has been yet provided for the involvement of a specific

0028–0836/78/0276—0523$01.00

enzyme in the regulation of enkephalinergic transmission. We now report the presence of a high-affinity peptidase in a particulate fraction of mouse striatum splitting the Leu-enkephalin molecule with release of a tripeptide fragment (Tyr-Gly-Gly) and exhibiting definite substrate specificity. The marked and selective increase in the activity of this peptidase in the striatum of mice chronically treated with morphine suggests that it might be associated with enkephalinergic transmission.

Enkephalin-hydrolysing activity exhibits a considerable heterogeneity in rat brain subfractions[8]. Starting from the hypothesis that a specific peptidase involved in the control of enkephalin concentration in the synaptic cleft should be membrane-bound and should exhibit a high affinity for the pentapeptides, we have compared the saturation kinetics of Leu-enkephalin-hydrolysing enzymes in a soluble and a particulate fraction from mouse striatum, a region containing a high concentration of enkephalins and opiate receptors. As shown in Fig. 1 the shape of the curves representing the fraction of ^{3}H-Leu-enkephalin hydrolysed as a function of total substrate concentration is monophasic in the soluble fraction but clearly biphasic in the particulate fraction. In the particulate, at least two classes of enkephalin-hydrolysing activities are present; one seems to correspond to that found in the soluble fraction (IC_{50} ~ 30 μM in both cases), whereas the other, representing 20–25% of the total hydrolysing activity at low enkephalin concentrations, is restricted to the particulate fraction. This component, represented in Lineweaver–Burk plots (Fig. 1*b*) exhibits a high affinity (K_m = 90 nM) as compared to most peptidases for which the K_m is in the range 10–100 μM (ref. 9).

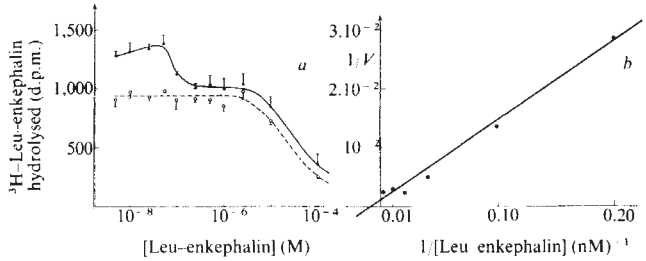

Fig. 1 ^{3}H-Leu-enkephalin hydrolysed by particulate and soluble fractions from mouse striatum incubated in the presence of increasing concentrations of non-radioactive Leu-enkephalin. Striata of three male Swiss mice (18–20 g) were homogenised into 10 ml of 0.05 M Tris-HCl buffer *p*H 7.4 (3×5 s at 1,500 r.p.m. in a glass–Teflon Potter homogeniser, clearance 0.1–0.15 mm). A pellet was obtained after two successive centrifugations at 4°C (1,000 *g* × 1 min and 200,000*g* × 1 min) and the supernatant of the second centrifugation was kept as 'soluble fraction'. The pellet was resuspended using a Dounce homogeniser (clearance 20–55 μm) into 10 ml of cold buffer and centrifuged (200,000*g* × 1 min). The resulting pellet, after a wash without resuspension with 15 ml of buffer (which was then discarded), was resuspended into 5 ml of buffer: this suspension was used as 'particulate fraction'. After a 15-min preincubation at 20°C, incubations (15 min, 20°C) of the particulate or soluble fractions were started by addition of 100 μl of buffer containing ^{3}H-Leu-enkephalin (1 nM final concentration; the Radiochemical Centre, 41Ci mmol^{-1}, previously purified by chromatography on Porapak beads microcolumns) and stopped by the addition of 50 μl 0.1 M HCl followed by heat deproteinisation (95°C, 15 min). ^{3}H-metabolites were separated from intact ^{3}H-Leu-enkephalin by chromatography[5] on 80 mg Porapak Q (100-120 mesh, Waters Assoc.) contained in Pasteur pipettes. The metabolites were eluted from column with 2×1 ml distilled water directly collected into scintillation vials; Leu-enkephalin was retained on the column. *a*, Effects of increasing concentrations of non-radioactive Leu-enkephalin on the formation of hydrolysis ^{3}H-products by the particulate fraction (●) or the soluble fraction (○). *b*, Lineweaver–Burk plot of the high-affinity Leu-enkephalin hydrolysing activity (corresponding to substrate concentration below 5 μM, that is, up to the plateau) of the particulate fraction (same data as in *a*). The rate of hydrolysis (fmol per mg protein per min) was evaluated, taking into account the specific activities of the substrate. K_m = 90 nM, V_{max} = 850 fmol per mg protein per min. Means ±s.e.m. of three determinations.

Table 1 Inhibitory potencies of various compounds on the hydrolysis of ^{3}H-Leu-enkephalin by particulate and soluble fractions of mouse striatum

	IC_{50} (nM) for ^{3}H-Leu-enkephalin hydrolysis by Particulate fraction		Soluble fraction
	High affinity peptidase	Low affinity peptidase	
Leu-enkephalin	70	20,000	35,000
Met-enkephalin	350	17,000	39,400
(D-Phe^4)Met-enkephalin	>1,000	25,000	66,000
(L-Ala^2)Met-enkephalin	10	1,000	2,700
(D-Ala^2)Met-enkephalin	>10,000	500,000	520,000
(L-Ala^3)Met-enkephalin	97	9,700	16,500
(D-Ala^3)Met-enkephalin	>1,000	29,000	24,200
Tyr	>100,000	>100,000	>100,000
Tyr-Gly	>100,000	>100,000	>100,000
Tyr-Gly-Gly	>100,000	>100,000	>100,000
Tyr-Gly-Gly-Phe	>100,000	>100,000	>100,000
Gly-Gly-Phe-Met	1,500	>100,000	>100,000
Gly-Gly-Phe-Leu	2,100	>100,000	>100,000
Phenylmethylsulphonyl-fluoride	>100,000	>100,000	>100,000
Bacitracin	20,000	20,000	20,000
p-Chloromercuriphenyl-sulphonic acid	>100,000	1,000*	300†
Substance P	>1,000	2,000	1,800
Bradykinin	>1,000	9,000	7,500
Glutathione	>100,000	>100,000	>100,000
Carnosine	>100,000	>100,000	>100,000
LHRH	>100,000	>100,000	>100,000
TRH	>100,000	>100,000	>100,000
α-Endorphin	>5,000	54,000	56,000
β-Endorphin	>5,000	62,000	32,000
Morphine	>100,000	>100,000	>100,000
Naloxone	>100,000	>100,000	>100,000

Particulate and soluble fractions from mouse striata were prepared as described in Fig. 1. Incubations (20°C, 15 min) in the presence of 20 nM ^{3}H-Leu-enkephalin and increasing concentrations (6–8) of the various compounds. ^{3}H-Leu-enkephalin hydrolysis evaluated by column chromatography on Porapak Q beads (see Fig. 1). IC_{50} values were determined by iterative analysis of the concentration-inhibition curves, analogous to that reported in Fig. 1, by a computer program based on the least squares (variance-covariance matrix)[13]. This analysis provided an accurate determination of IC_{50} for the inhibition of the two peptidasic activities in the particulate fraction in the case of a clear biphasic curve, that is, when the IC_{50} differed by more than 50-fold. This was not the case for several compounds: (D-Phe^4)Met-enkephalin, (D-Ala^3) Met-enkephalin, bradykinin and substance P, for which a clear plateau was not present and therefore the IC_{50} on the high-affinity peptidase could not be estimated with accuracy.

* Maximal inhibition ~70%.

† Maximal inhibition ~90%.

In six independent experiments the presence of this high-affinity component was confirmed and the analysis of the data by a computer program (see Table 1 legend) led to mean K_m values (±s.e.m.) of 41 ± 10 nM and of 55,000 ± 17,000 nM for the high- and low-affinity peptidase activities, respectively. Both peptidase activities in the particulate fraction vary linearly as a function of protein concentration or time.

The high-affinity of a class of peptidase activity found in the particulate fraction suggested that it might exhibit substrate specificity. This was indirectly investigated by determining the inhibitory potency of a variety of compounds towards the different classes of peptidase activities (Table 1). The specificity of the high-affinity peptidase activity is demonstrated by the enormous range in inhibitory potency of the various compounds tested (IC_{50} between 10 and >100,000 nM). Although the low-affinity peptidase activities in both particulate and soluble fractions also exhibit some specificity, the range in IC_{50} variation is much less pronounced. The main conclusions which can be drawn from the data of Table 1 can be summarised as follows:

(1) Stereochemical requirements are more stringent in the case of the high-affinity peptidase as evidenced for the pair of (Ala^3)-Met-enkephalin stereoisomers.

(2) The integrity of the enkephalin molecule is important for the inhibitory potency as shown by the dramatic loss of activity towards all classes of peptidases in the fragments Tyr, Tyr-Gly, Tyr-Gly-Gly and Tyr-Gly-Gly-Phe.

(3) The C-terminal moiety of the enkephalin molecule seems to be more important for inhibition of the high-affinity peptidase

Table 2 Identification by thin-layer chromatography of the radioactive compounds present after incubation of ^{3}H-Leu-enkephalin with a particulate fraction of mouse striatum

Solvent system	Incubation conditions	^{3}H-compounds (d.p.m.) Before column chromatography: Tyr	Before column chromatography: Tyr-Gly-Gly	Before column chromatography: Leu-enkephalin	After column chromatography: Tyr	After column chromatography: Tyr-Gly-Gly
(1)	Control[(a)]	1,526±101	628±91	14,840±122		
	+Leu-enkephalin[(a)] (5 μM)	1,217±171	291±42**	15,524±188*		
	Control	418±90	465±18	15,880±693	500±55	705±58
	+Gly-Gly-Phe-Met (10 μM)	303±43	118±30***	16,503±518	575±65	265±83*
(2)	Control	838±110	530±33	16,305±478	998±45	955±65
	+Gly-Gly-Phe-Met (10 μM)	743±15	100±10***	17,545±555	895±15	293±18***
(3)	Control				570±50	660±53
	+Gly-Gly-Phe-Met (10 μM)				555±88	215±18***

Incubation of samples (30 μg proteins per 200 μl) of the particulate fraction (15 min, 20°C) in the presence of 250 nM of purified ^{3}H-Leu-enkephalin and 1 μM *p*-chloromercuriphenylsulphonic acid (except in conditions (a)) and in the presence or absence of either Leu-enkephalin or Gly-Gly-Phe-Met. A 10 μl aliquot of the deproteinised supernatant or of the Porapak column eluate was applied on a TLC plate (plastic sheets Silicagel 60, Merck, thickness 0.2 mm). Solvent systems and R_f values of the reference compounds were: (1) $CHCl_3$:CH_3OH:AcOH:H_2O (45:30:6:9); Tyr = 0.61, Tyr-Gly-Gly = 0.47, Leu-enkephalin = 0.88 (2) BuOH:AcOH:H_2O (4:1:1); Tyr = 0.47, Tyr-Gly-Gly = 0.31, Leu-enkephalin = 0.58 (3) isopropanol:AcOEt:5% AcOH (2:2:1); Tyr = 0.70, Tyr-Gly-Gly = 0.41, Leu-enkephalin = 0.80. Markers visualised with a ninhydrin spray and radioactive peaks at their R_f isolated by cutting the plates and counted by liquid scintillation (40% efficiency). Means ±s.e.m. of 3–6 determinations * $P<0.025$. ** $P<0.01$. *** $P<0.001$. No other radioactive peak could be detected when the entire plate was counted by fractions of 0.5 cm. In particular no significant radioactivity was detected at the R_fs of Tyr-Gly-Gly-Phe (0.80 in system (1) and 0.45 in system (2)).

Table 3 Effect of chronic morphine treatment on ^{3}H-enkephalin-hydrolysing activity of particulate and soluble fractions of mouse striatum

	^{3}H-enkephalin-hydrolysing activity (d.p.m. per mg protein per min) Particulate fraction: High affinity peptidase	Particulate fraction: Low affinity peptidase	Particulate fraction: Total peptidase	Soluble fraction
Controls	7,858±977	33,344±2,309	41,203±2,620	120,990±20,468
Morphine	12,521±932** (+59%)	33,255±1,998[NS]	45,776±2,042* (+12%)	125,297± 9,590[NS]

Groups of mice were implanted under light ether anaesthesia with a morphine pellet (75 mg of base, s.c.) which was removed 3 days later, 16 h before death. Controls were sham-operated in a parallel manner. ^{3}H-enkephalin-hydrolysing activity measured after incubations of fractions prepared as described in Fig. 1 (15 min at 20°C) with 20 nM ^{3}H-Leu-enkephalin. The low-affinity hydrolysing activity of the particulate fraction represents the activity measured in the presence of 5 μM Leu-enkephalin whereas the high-affinity activity was estimated as the difference between total (in the absence of 5 μM Leu-enkephalin) and low-affinity activities. Degraded ^{3}H-Leu-enkephalin was measured by Porapak column chromatography. Means ±s.e.m. of 12–16 experiments on pooled striatal fractions from two mice. * $P<0.05$. ** $P<0.0005$. NS, not significant.

than the N-terminal moiety as shown by the much greater potency of the tetrapeptides Gly-Gly-Phe-Met or Gly-Gly-Phe-Leu when compared to the tetrapeptide Tyr-Gly-Gly-Phe; this conclusion does not hold true for the two classes of low-affinity peptidases.

(4) Among classical peptidase inhibitors, only bacitracin has significant potency towards the high-affinity peptidase, whereas *p*-chloromercuriphenylsulphonic acid, a potent inhibitor of the low-affinity peptidases, is practically inactive on the high-affinity enzyme; this indicates that the latter does not belong to the group of thiol-peptidases. The heterogeneity of enkephalin-hydrolysing activity as shown by response to thiol-blocking agents has been already suggested[8].

(5) Structural requirements for recognition of the opiate receptors and the high-affinity peptidase are unrelated as demonstrated by the lack of potency of α- and β-endorphin as well as opiates.

From these data it seems that three compounds are useful to differentiate the high- and low-affinity peptidase activities in the particulate fraction: Leu-enkephalin, Gly-Gly-Phe-Met and *p*-chloromercuriphenylsulphonic acid. Therefore, these compounds were used as tools in the identification of the ^{3}H-fragments released under the action of each class of peptidases in the particulate fraction.

Thin-layer chromatography of extracts (obtained after incubation of ^{3}H-Leu-enkephalin with the particulate fraction) yields three radioactive peaks with R_f values closely corresponding to ^{3}H-Leu-enkephalin, ^{3}H-Tyr and ^{3}H-Tyr-Gly-Gly (Table 2). In contrast, only ^{3}H-Leu-enkephalin and ^{3}H-Tyr were found after incubation of the soluble fraction. Whereas the presence of ^{3}H-Tyr is in agreement with a number of reports[1–5], ^{3}H-Tyr-Gly-Gly was an unexpected component. The presence of ^{3}H-Tyr-Gly-Gly was confirmed first by unidimensional TLC in three different solvent systems (Table 2), second by bidimensional co-chromatography in solvent systems (2) and (3), and third by high-performance liquid co-chromatography on a 205 U Waters apparatus (reversed phase on standard microbondapak C 18; solvent CH_3CN:10 mM NH_4Ac buffer, 3.125:96.875; 1 ml min^{-1}; retention times Tyr = 290 s, Tyr-Gly-Gly = 350 s, Leu-enkephalin > 1 h).

The fact that Tyr-Gly-Gly was not previously identified by others as a breakdown product of enkephalin can easily be explained by its low level relative to total metabolites (~25% in our washed particulate fraction but only 2–3% in the whole homogenate as used by most workers). Nevertheless, the occurrence of a minor unidentified peak with R_f corresponding to Tyr-Gly-Gly has been reported in brain extracts[2], and formation of this tripeptide by cultured human endothelial cells has been reported[10].

That ^{3}H-Tyr-Gly-Gly is specifically produced by the high-affinity peptidase is clearly demonstrated by first the marked decrease observed after incubations in the presence of 5 μM Leu-enkephalin or 10 μM Gly-Gly-Phe-Met contrasting with the unchanged ^{3}H-Tyr peak in the same conditions; and second the lack of effect of *p*-chloromercuriphenylsulphonic acid on ^{3}H-Tyr-Gly-Gly formation, contrasting with the strong reduction in the ^{3}H-Tyr peak (solvent system (1)). These results indicate that the high-affinity peptidase is probably a C-terminal

dipeptidase splitting the Gly–Phe bond of enkephalin, a conclusion consistent with the high inhibitory potency of the C-terminal tetrapeptide as compared to the N-terminal tetrapeptide (Table 1). As we have not yet characterised the formation of Phe-Leu or Phe-Met (because they are not labelled) we cannot exclude the possibility that ^{3}H-Tyr-Gly-Gly formation results from the sequential action of carboxypeptidases. However, this is unlikely because ^{3}H-Tyr-Gly-Gly-Phe formation was never observed in our experiments.

In mice chronically treated with morphine we observe a dramatic increase in the activity of the high-affinity peptidase in the particulate fraction (Table 3). A similar effect is also observed in rats implanted with a morphine pellet (data not shown). This effect seems to be selective because the low-affinity peptidase activity in either the particulate or the soluble fractions is unchanged. The increase in activity reflects an elevated V_{max} without significant change in K_m value (data not shown), suggesting that it corresponds to an increased number of enzyme molecules. The effect of morphine might result from a feedback mechanism operating at the target cells bearing the opiate receptors: conceivably these cells, to compensate the overstimulation of opiate receptors, could develop a state of hyposensitivity to released enkephalins, through an increased rate of inactivation of these peptides. In this respect, changes in acetylcholinesterase activity in brain have been already reported to mediate the modified responsiveness of target cells to the transmitter, developing in response to sustained changes in cholinergic transmission[11]. Although not direct proof, the effect of morphine strongly suggests that the high-affinity peptidase is involved in the control of enkephalinergic transmission. This hypothesis is consistent with the heterogeneous distribution of this 'enkephalinase' in mouse brain regions (striatum > hypothalamus > cortex > brainstem ≥ hippocampus > cerebellum, with a five-fold variation between striatum and cerebellum) which is highly correlated ($r = 0.94$) with that of opiate receptors (manuscript in preparation). If true, this would imply that a new class of therapeutic agents (enkephalinase inhibitors) exhibiting opiate-like activity could be developed. In addition the increased peptidase activity after chronic morphine treatment could be involved, with other mechanisms[12], in the development of dependence on opiates.

We thank X. Delamare and R. Barbes (Waters Assoc., France) for HPLC analysis. This work was supported by a grant from the DGRST (78.7.0593).

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The ACTH 'family tree' of the rhesus monkey changes with development

THE pituitary hormone adrenocorticotrophin (ACTH) belongs to a 'family' of peptides derived from a common 'stem hormone' (Fig. 1). Depending on the way in which the stem hormone is cleaved, the various peptides are expressed and it is possible that qualitative changes in the expression of the peptide 'family tree' may be responsible for the alteration in fetal adrenal function which precedes birth[1]. We now report evidence for such changes in the rhesus monkey, which has a fetal adrenal comparable with that of man.

After delivery the fetal zone of the human adrenal cortex involutes, while the definitive zone or adult cortex, consisting of a thin layer of cells covering the large fetal zone, hypertrophies.

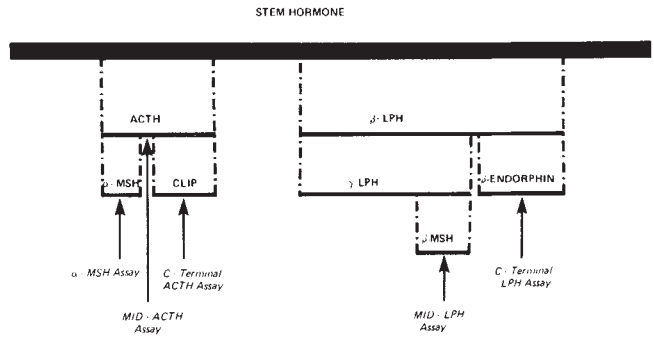

Fig. 1 The stem hormone family tree gives rise to two major branches—ACTH and β-LPH. ACTH is a 39-amino acid peptide whose first 13 amino acids are identical in sequence with α-MSH and whose 18–39 sequence is identical to CLIP. β-LPH is a 91-amino acid peptide whose first 58 amino acids are identical in sequence with γ-LPH and whose 61–91 sequence is identical to β-endorphin. The sequence 41–58 is identical to the 18-amino acid peptide β-MSH. The five radioimmunoassays are directed against different antigenic sites of this family tree.

This change may be triggered by a switch in the production of trophic hormones in the fetus, from peptides resembling α-melanocyte stimulating hormone (α-MSH) and corticotrophin-like intermediate lobe peptide (CLIP) to ACTH[2,3]. The idea is supported by a report[4] that α-MSH stimulated the fetal adrenal gland of the rabbit when synacthen (α-1–24 ACTH) was relatively ineffective, whereas synacthen was potent in the newborn and α-MSH was ineffective. Others have shown that although ACTH stimulated the definitive zone *in vitro* its effect on the fetal zone was inconsistent[5]. The metamorphosis of the adrenal at birth is but one aspect of the change in adrenal function. In the sheep, for example, in which there is no specific fetal zone, there are nevertheless important changes in steroid output, notably a prepartum rise of cortisol which prepares the fetus for delivery and initiates parturition[6]. As with the human, there are differences in the composition of pituitary hormones between fetus and adult[1]. Unlike the human, however, it is the large molecular weight forms of ACTH which predominate in the fetal sheep. There are three such forms, two of which contain sequences common to β-lipotrophic hormone (β-LPH), γ-LPH,

0028-0836/78/0276—0526$01.00