

## Immunohistochemical localization of enkephalin- and ACTH-related substances in the pituitary of the lamprey

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**Summary.** The distributions of ACTH-,  $\alpha$ MSH-,  $\beta$ LPH-, and enkephalin-related substances were determined immunohistochemically in the pituitary of the brook lamprey, *Lampetra lamoteni*. An antiserum directed against the middle region of ACTH reacted chiefly with cells in the pro-adenohypophysis. An antiserum specific for  $\alpha$ MSH reacted with all of the cells of the meta-adenohypophysis, but did not react with any of the middle ACTH-positive cells in the pro-adenohypophysis. Several antisera which crossreact with both  $\beta$ LPH and  $\beta$ -endorphin did not react with any region of the lamprey pituitary. However, an antiserum directed against  $\gamma$ LPH did react with a small population of cells in the meso-adenohypophysis. This reactivity could be blocked following pre-absorption with mouse  $\beta$ LPH but was not blocked by synthetic  $\beta$ -endorphin(1–31).

Antisera directed against either met-enkephalin or leu-enkephalin reacted with fibers in the anterior neurohypophysis, cells in the pro-adenohypophysis, and all the cells of the meta-adenohypophysis. This crossreactivity could be blocked following pre-absorption with the appropriate enkephalin, but not by pre-absorption with synthetic  $\beta$ -endorphin(1–31) or dynorphin(1–13). In addition, the enkephalin-like reactivity in the adenohypophysis of the lamprey was coincident with middle ACTH-like immunoreactivity in the pro-adenohypophysis and with  $\alpha$ MSH-like immunoreactivity in the meta-adenohypophysis.

The absence of  $\beta$ LPH/ $\beta$ -endorphin immunoreactivity coincident with ACTH immunoreactivity, and the presence of enkephalin-like material in the adenohypophysis are unique to the lamprey.

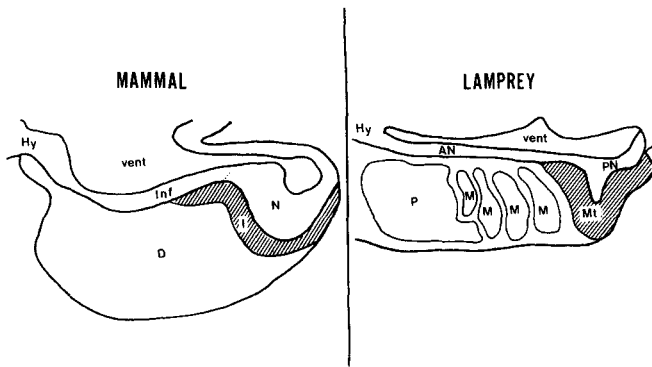
**Key words:** ACTH –  $\alpha$ MSH – Enkephalin – Adenohypophysis – Lamprey

The ACTH/ $\beta$ LPH family of adenohypophyseal hormones is a collection of polypeptides related by sequence homology (Baker 1980) and a common biosynthetic pathway (Eipper and Mains 1980). In mammals, ACTH (adrenocorticotropin) and  $\beta$ LPH (beta lipotropin) are synthesized as elements of a larger (30 K) common precursor protein (pro-ACTH/endorphin or proopioidmelanocortin) both in corticotropic cells of the pars distalis and in melanotropic cells

of the pars intermedia (Eipper and Mains 1978; Nakanishi et al. 1979; Herbert et al. 1980; Krieger et al. 1980). Post-translational processing of the precursor in the pars distalis yields 16 K fragment (the NH<sub>2</sub>-terminal third of pro-ACTH/endorphin), ACTH,  $\beta$ LPH,  $\gamma$ LPH (the NH<sub>2</sub>-terminal two thirds of  $\beta$ LPH), and  $\beta$ -endorphin (the COOH terminal third of  $\beta$ LPH) as end products (Mains et al. 1978; Roberts and Herbert 1977; Eipper and Mains 1978; Mains and Eipper 1978). The initial proteolytic processing of pro-ACTH/endorphin in the pars intermedia is identical to that in the pars distalis. However, ACTH is processed further to yield  $\alpha$ MSH-sized molecules ( $\alpha$ -melanotropin) and CLIP (corticotropin-like intermediate lobe peptide), while nearly all of the  $\beta$ LPH is cleaved to yield  $\gamma$ LPH and  $\beta$ -endorphin-sized molecules (Eipper and Mains 1978; Gianoulakis et al. 1979; Mains and Eipper 1979, 1981; Eipper and Mains 1981). In some species  $\gamma$ LPH and 16 K fragment may be cleaved to smaller peptides (Pederson and Brownie 1979; Brownie et al. 1981; Estivariz et al. 1982).

Among non-mammalian vertebrates a similar biosynthetic pathway has been reported in amphibians (Loh and Gainer 1977; Loh 1979; Pezella et al. 1978) and reptiles (Dores 1982a, b). Furthermore, in birds, bony fishes, and cartilaginous fishes, end products of the ACTH/ $\beta$ LPH system have been isolated and, in some instances, fully characterized (Kawauchi and Muramoto 1979; Baker 1980; Kawauchi et al. 1980a, b, c; McLean and Lowry 1981; Kawauchi et al. 1982). However, little is known about the distribution of peptides related to ACTH and  $\beta$ LPH in agnathans (lampreys and hagfish), the most ancient lineage of extant vertebrates. The present study will focus on the distribution of these peptides in lampreys.

The pituitary of the lamprey is similar in organization to the pituitary of other vertebrates (Hardisty 1981). The adenohypophysis is divisible into the pro- and meso-adenohypophyses (homologs of pars distalis) and the meta-adenohypophysis (homolog of the pars intermedia; Fig. 1). The neurohypophysis is divided into two regions: the anterior neurohypophysis, the homolog of the infundibulum, and the posterior neurohypophysis, the homolog of the pars nervosa. The latter region is the storage site for a vasotocin-like peptide (Rurak and Perks 1976, 1977). With respect to the ACTH/ $\beta$ LPH system, ACTH-like bioactivity and ACTH-related immunoreactivity have been detected in whole pituitary homogenates (Scott et al. 1971; Eastman and Portanova 1982). Furthermore, MSH-like bioactivity has been localized in the meta-adenohypophysis (Lanzing



**Fig. 1.** Comparison of idealized sagittal sections through the pituitaries of typical mammal (left) and of brook lamprey (right). Pro-(P) and meso-(M)adenohypophysis in lamprey supposedly equivalent to part of mammalian pars distalis (D); metaadenohypophysis (Mt) of lampreys (hatched area) similar to mammalian pars intermedia (I) (hatched area)

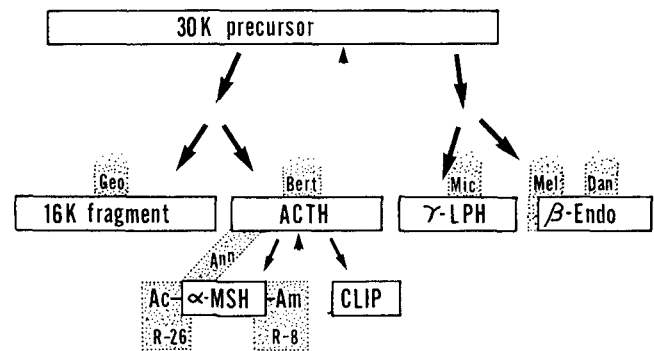
1954). Thus some elements of the ACTH/ $\beta$ LPH family are present in the pituitary of lampreys. Based on these findings, this study was undertaken to determine the distribution of ACTH-,  $\alpha$ MSH-,  $\beta$ LPH-,  $\beta$ -endorphin-, and 16 K fragment-like substances in the pituitary of the brook lamprey, *Lampetra lamottenii*. In the course of this study the distribution of enkephalin-like material in the pituitary of this species was also determined. A preliminary report of some of these results has appeared previously (Dores et al. 1982).

### Materials and methods

The immunohistochemical studies were carried out on 11 sexually mature brook lampreys, *Lampetra lamottenii*, approximately 11 cm in length. The brain, with pituitary attached, was removed from the animal and placed in a fixing solution for 2–6 h. Various fixatives were used (4% paraformaldehyde in buffer, Bouin's, or paraformaldehyde at two different pH values; see Berod et al. 1981), but optimal, consistent staining was obtained with the periodate-lysine-paraformaldehyde solution of McLean and Nakane (1974). Following fixation, the tissue was transferred to a solution of 10% sucrose in 0.1 M phosphate buffer (pH 7.2) and allowed to equilibrate overnight. The brain and pituitary were sectioned on a cryostat at 10–15  $\mu$ m in the sagittal plane. For each case, 4 to 6 parallel series of sections were prepared to facilitate comparisons between antisera. Cryostat sections were thaw mounted on gelatin coated slides and dried under a cool airstream for at least 30 min. The sections were preincubated in 1% normal goat serum (NGS) prior to application of the primary antiserum.

The primary antisera were diluted with 1% normal goat serum and 0.3% Triton X-100 in 0.1 M phosphate buffer

**Abbreviations:** Ac acetyl group; ACTH adrenocorticotropin; Am amide group; AN anterior neurohypophysis; Bert Antiserum Bertha; CLIP corticotropin-like intermediate lobe peptide; D pars distalis of the mammalian pituitary; Dan Antiserum Danielle; Endo endorphin; Enk enkephalin; Geo Antiserum Georgie; Hy hypothalamus; I pars intermedia of the mammalian pituitary; Inf infundibulum; LPH lipotropin; M meso-adenohypophysis; Mel Antiserum Melinda; Mic Antiserum Michelle; MSH melanotropin; Mt meta-adenohypophysis; N pars nervosa of the mammalian pituitary; P pro-adenohypophysis; PN posterior neurohypophysis; vent hypothalamic recess of the third ventricle



**Fig. 2.** Diagram of antibody specificity (stippled areas) superimposed biosynthetic scheme of mammalian ACTH-, MSH- and LPH-related compounds. All antisera except R-26 and R-8 (directed against modified ends of  $\alpha$ MSH) crossreact with all larger precursor forms, e.g., antiserum Ann crossreacts with non-acetylated  $\alpha$ MSH, ACTH(1–13), intact ACTH, ACTH biosynthetic intermediate (not shown) and 30 K precursor (Pro-ACTH/Endorphin)

to a final concentration of 1:100–1:2000 as determined empirically to produce optimal staining for each antiserum. Each primary antiserum was applied to the tissue sections and allowed to react overnight at 4° C. All specific immunoreactivity was blocked by preabsorption of the antiserum with 10  $\mu$ M of the appropriate peptide. The antisera used in this study were all raised to mammalian peptides.

The peroxidase-anti-peroxidase (PAP) technique as specified by Sternberger et al. (1979) was used. After incubation with the primary antiserum, the tissue sections were washed three times with 1% NGS in 0.1 M phosphate buffer. Unlabeled goat-anti-rabbit serum (Cappel) was then applied at a dilution of 1:50 for 30–60 min at room temperature. Following three washes in 0.1 M phosphate buffer, the sections were incubated with rabbit PAP (Cappel) at a dilution of 1:100 for 1 h at room temperature. The peroxidase was reacted with diaminobenzidine (25 mg/50 ml) and the reaction product amplified by the metal intensification method as described by Adams (1981). The specificity of some of the antisera tested is shown in Fig. 2.

### ACTH-related antisera

Five ACTH-related antisera were tested. Affinity purified antiserum Bertha is specific for the middle region of ACTH and has less than 0.1% crossreactivity with  $\alpha$ MSH [ $\alpha$ -N-acetyl-ACTH(1–13)NH<sub>2</sub>] and CLIP [ACTH(18–39)] (Mains and Eipper 1979). Affinity purified antiserum Ann is directed against the NH<sub>2</sub>-terminal region of ACTH and crossreacts fully with  $\alpha$ MSH (Glombotski 1982), as determined by radioimmunoassay. Antiserum Patti and antiserum R-26 (kindly provided by Drs. E. Weber and K.H. Voigt; Univ. of Ulm, West Germany) are specific for the acetylated NH<sub>2</sub>-terminal of  $\alpha$ MSH (Eipper et al. 1983). Antiserum R-8 (kindly provided by Drs. S. Watson and H. Akil; Univ. of Michigan, Ann Arbor) is specific for the amidated COOH-terminal of  $\alpha$ MSH.

### $\beta$ -Endorphin-related antisera

Four  $\beta$ -endorphin directed antisera were tested. Affinity purified antiserum Melinda is specific for the NH<sub>2</sub>-terminal

region of  $\beta$ -endorphin and crossreacts fully with  $\beta$ LPH (Eipper and Mains 1978). Antisera Danielle and R-21 are specific for the middle region of  $\beta$ -endorphin and crossreact fully with  $\beta$ LPH (Mains and Eipper 1979). Antiserum R-19 is specific for the COOH-terminal of  $\alpha$ -endorphin [ $\beta$ -endorphin(1-16)] and does not crossreact with either synthetic  $\beta$ -endorphin(1-31) or mouse  $\beta$ LPH.

#### $\beta$ LPH antiserum

Antiserum Michelle was affinity purified on a Sepharose 4B column with mouse  $\beta$ LPH linked to the column (Eipper and Mains 1978), and is specific for the  $\gamma$ LPH region of mouse  $\beta$ LPH. This antiserum crossreacts fully with mouse  $\beta$ LPH, but has less than 1% molar crossreactivity with synthetic  $\beta$ -endorphin(1-31).

#### 16 K fragment antisera

Antisera Georgie and Gertrude are specific for the  $\gamma$ MSH region of 16 K fragment (Eipper and Mains 1978; Eipper et al. 1983). Both antisera crossreact fully with 16 K fragment, but neither antiserum crossreacts with other end products of the ACTH/ $\beta$ LPH biosynthetic pathway.

#### Enkephalin antisera

Antiserum A-206, generously supplied by Dr. R. Miller (Univ. of Chicago), was raised against leu-enkephalin, but crossreacts slightly with met-enkephalin (Miller et al. 1978). Another enkephalin antiserum (lot 49279), obtained from Immunonuclear Corp. was raised against met-enkephalin and has 5% crossreactivity with leu-enkephalin.

#### Arginine vasopressin antiserum

Arginine vasopressin antiserum, kindly provided by Dr. W. Vale (Salk Institute, La Jolla), crossreacts with vasotocin in the brains of goldfish and bullhead catfish (T. Finger, unpublished observations).

#### Peptides

Synthetic camel  $\beta$ -endorphin(1-31), and synthetic  $\alpha$ MSH were purchased from Bachem. Synthetic met- and leu-enkephalin were purchased from Peninsula Laboratories. Mouse  $\beta$ LPH was kindly provided by Dr. B. Eipper (Univ. Colorado). Synthetic ACTH(1-24) was provided by Organon. Dynorphin(1-13) was purchased from Bachem.

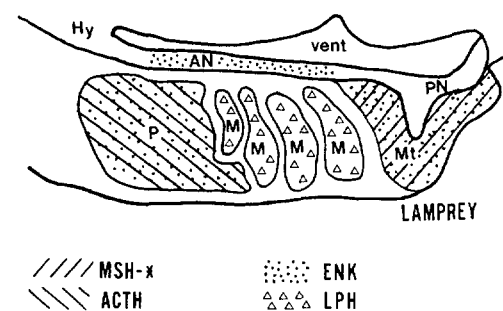
#### Results

A striking feature of the antisera tested (Table 1) is the absence of either  $\beta$ -endorphin-like or 16 K fragment-like immunoreactivity. Since antisera R-19, R-21, and Gertrude have not been used for immunohistochemical studies in species other than lamprey it is difficult to interpret the significance of the negative results for these antisera. However, antiserum Danielle reacts immunohistochemically with  $\beta$ -endorphin-related material in the rat pars intermedia (S. Watson, personal communication). Furthermore antiserum Melinda crossreacts immunohistochemically with  $\beta$ -endorphin-related material in the pars intermedia and hypothalamus of the rat (Watson et al. 1978) and the pars intermedia

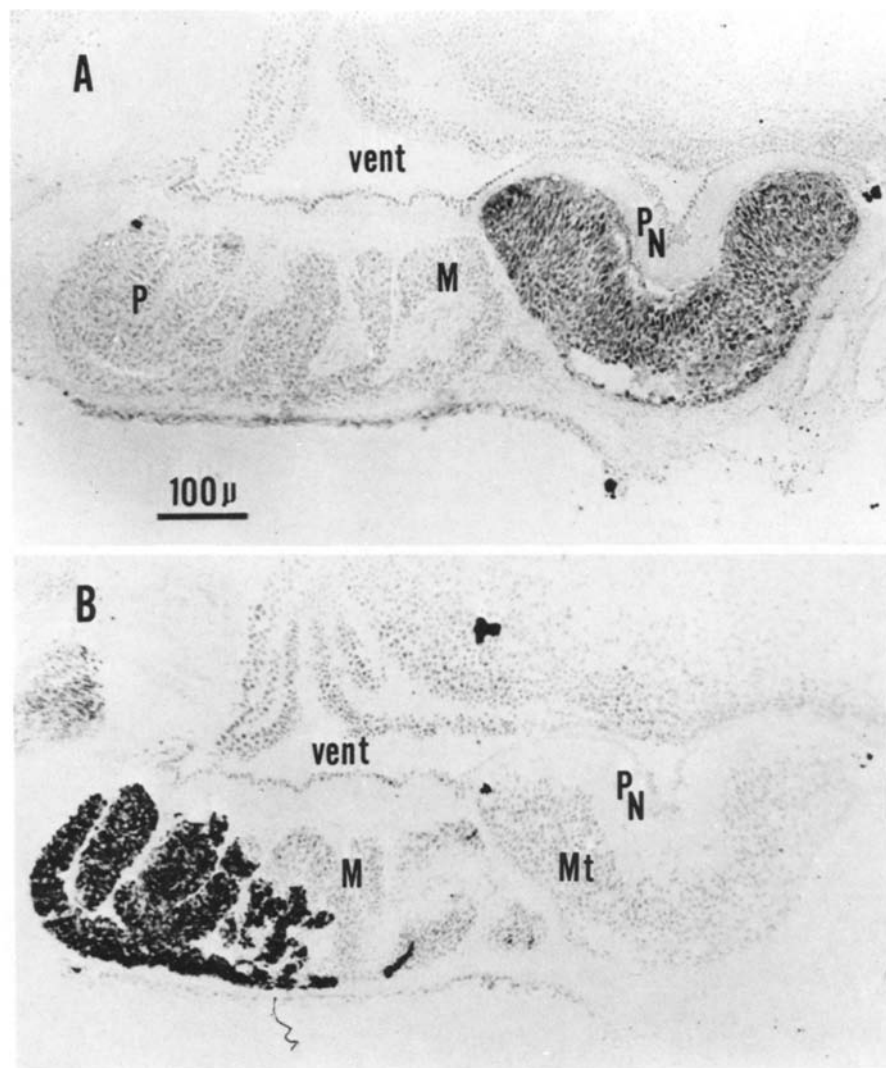
**Table 1.** Results of antisera tested

Antisera	Specificity	Dilution	Staining <sup>a</sup>
<i>1. ACTH-related</i>			
Bertha	Middle region of ACTH	1:1000	+P
Ann	NH <sub>2</sub> -terminal of ACTH	1:2000	+Mt
Patti	Acetylated NH <sub>2</sub> -terminal of $\alpha$ MSH	1:100	-
R-26	Acetylated NH <sub>2</sub> -terminal of $\alpha$ MSH	1:100	-
R-8	Amidated COOH-terminal of $\alpha$ MSH	1:100	-
<i>2. <math>\beta</math>-Endorphin-related</i>			
Melinda	NH <sub>2</sub> -terminal of $\beta$ -endorphin	1:100	-
Danielle	Middle region of $\beta$ -endorphin	1:100	-
R-21	Middle region of $\beta$ -endorphin	1:100	-
R-19	COOH-terminal of $\alpha$ -endorphin	1:100	-
<i>3. <math>\beta</math>-Lipotropin-related</i>			
Michelle	Crossreacts with mouse LPH and $\beta$ LPH, but not with mouse $\beta$ -endorphin	1:500	+M
<i>4. 16K Fragment-related</i>			
Georgie	Middle region of 16 K fragment	1:100	-
Gertrude	$\gamma$ MSH	1:100	-
<i>5. Enkephalin-related</i>			
Met-Enk, (49279)	Methionine enkephalin	1:500	+AN, Mt, P
Leu-Enk, (A-206)	Leucine enkephalin	1:500	+AN, Mt, P
<i>6. Vasopressin-related</i>			
Arg-vasopressin	Crossreacts with arginine vasopressin and vasotocin	1:500	+AN, PN

<sup>a</sup> See list of abbreviations



**Fig. 3.** Schematic sagittal section through pituitary of lamprey to summarize results with *ACTH*-related antisera. Pro-adenohypophysis shows immunoreactivity to *ACTH* and enkephalin antisera; meso-adenohypophysis to an LPH-related antiserum; meta-adenohypophysis to *MSH* and enkephalin antisera; and anterior neurohypophysis to enkephalin antisera. Both anterior and posterior parts of neurohypophysis exhibit immunoreactivity (not illustrated) to arg-vasopressin antiserum that crossreacts with vasotocin in many fish species



**Fig. 4A, B.** Parasagittal sections through hypothalamus and pituitary of brook lamprey (rostral, left).

**A** Ann (MSH-like) immunoreactivity. Reactive cells only in meta-adenohypophysis (*Mt*).

**B** Bertha (middle-ACTH-like) immunoreactivity. Reactive cells located in pro-adenohypophysis (*P*). Note few scattered cells and islands of immunoreactive cells ventral to meso-adenohypophysis (*M*).  $\times 120$

of the reptile *Anolis carolinensis* (Dores 1982a). Antiserum Georgie also crossreacts with material in the adenohypophysis of the reptile (Dores 1982a) and 16 K fragment-related material in the pituitary and hypothalamus of the rat (Pelletier et al. 1977).

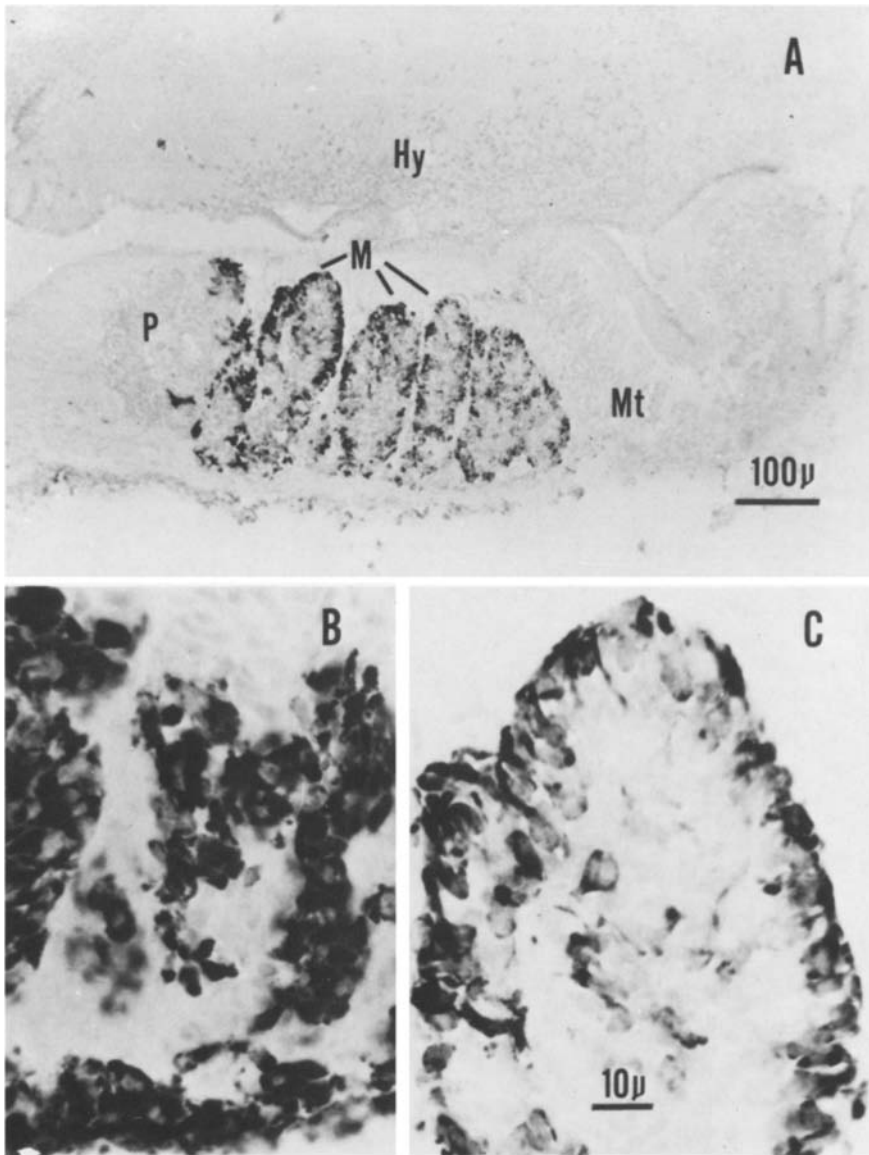
#### *ACTH-related immunoreactivity*

An antiserum specific for the middle region of ACTH reveals ACTH-related immunoreactivity in the pro-adenohypophysis and in clusters of cells ventral to the rostral part of the meso-adenohypophysis (Figs. 3, 4b, 5b). This reactivity was blocked by preincubation of the antiserum with  $10 \mu\text{M}$  of synthetic ACTH(1–24). However, immunohistochemical staining with a  $\text{NH}_2$ -terminal ACTH antiserum (Ann) that crossreacts fully with ACTH and  $\alpha\text{MSH}$  was restricted to the cells of the meta-adenohypophysis (Fig. 4A), and did not overlap with immunostaining by the middle ACTH antiserum (Bertha). Pre-absorption of antiserum Ann with  $10 \mu\text{M}$  synthetic  $\alpha\text{MSH}$  blocked this immunostaining. These results are consistent with those of earlier studies which showed that the meta-adenohypophysis of the lamprey is the source of MSH bioactivity (Lanzing

1954). In an attempt to characterize further the  $\alpha\text{MSH}$ -like immunoreactivity in the meta-adenohypophysis, sections were incubated with antisera directed against either the acetylated  $\text{NH}_2$ -terminal of  $\alpha\text{MSH}$  (antisera Patti and R-26) or the amidated  $\text{COOH}$ -terminal of  $\alpha\text{MSH}$  (antiserum R-8). None of these antisera reacted with the cells of the meta-adenohypophysis. Both antisera R-26 and R-8 provide consistent immunostaining of cells of the pars intermedia of a lizard (Dores 1982a) and the goldfish, *Carassius auratus* (Finger, unpublished observations). Thus these data suggest that the MSH-like substance in the lamprey pituitary may not be  $\alpha$ , N-acetylated, or  $\text{COOH}$ -terminally amidated. The failure of antiserum Ann to crossreact with the cells of the pro-adenohypophysis, which exhibit middle ACTH-like immunoreactivity, indicates that the  $\text{NH}_2$  terminal portion of the ACTH-like form in lampreys is significantly different from its mammalian and even teleostean counterparts.

#### *$\beta\text{LPH}$ -related immunoreactivity*

As presented above (Table 1), antisera known to crossreact with  $\beta$ -endorphin and the  $\text{COOH}$ -terminal of  $\beta\text{LPH}$  did not crossreact with any region of the lamprey adeno-



**Fig. 5.** A Parasagittal section through hypothalamus and pituitary (rostral, left). Michelle ( $\gamma$ LPH-like) immunoreactivity in cells of meso-adenohypophysis.  $\times 120$   
**B** Bertha (ACTH-like) reactivity in the cells of pro-adenohypophysis ventral to anterior part of meso-adenohypophysis. Note that this pattern of reactivity is quite different from  $\gamma$ LPH-like reactivity of meso-adenohypophysis shown in C. Same scale as panel C.  $\times 800$   
**C** Michelle (LPH-like) immunoreactive cells of meso-adenohypophysis. Note immunoreactivity mostly around margin of each cell island.  $\times 800$

physis. An antiserum specific for the  $\gamma$ LPH region of mouse  $\beta$ LPH (the  $\text{NH}_2$ -terminal two thirds of  $\beta$ LPH) did react with some cells in the meso-adenohypophysis (Figs. 3, 5A, C). These  $\beta$ LPH-immunoreactive cells are located around the periphery of the cell islands that compose the meso-adenohypophysis. This immunoreactivity was blocked by pre-absorption with mouse  $\beta$ LPH, but not by synthetic  $\beta$ -endorphin(1-31).

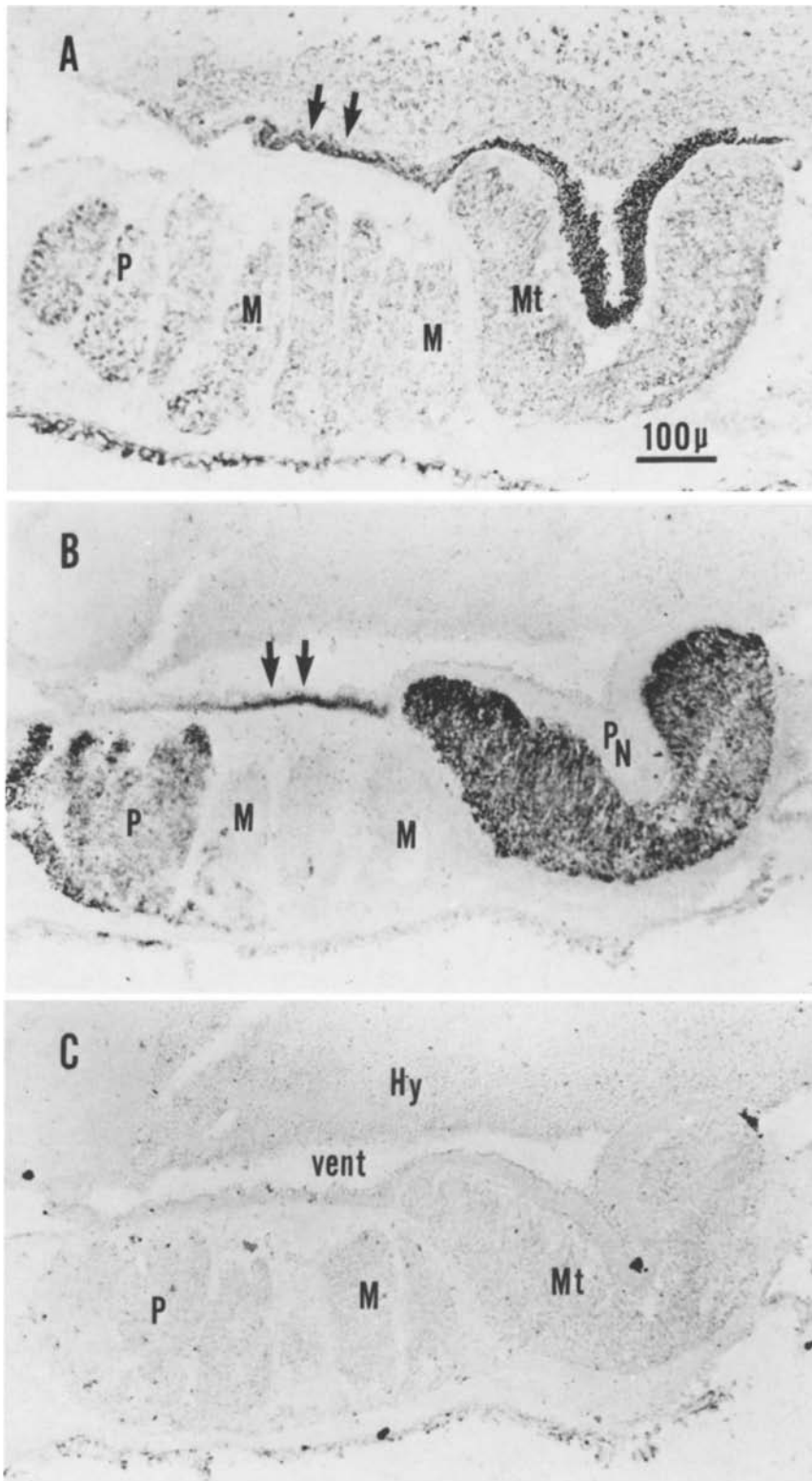
#### *Enkephalin-related immunoreactivity*

To further our understanding of the phylogenetic distribution of opioid peptides in vertebrates, the localization of enkephalin related material was also studied. Enkephalin-size material has been detected by radioimmunoassay in the brain of the hagfish (Simantov et al. 1976), and enkephalin immunoreactivity has been described in the brain of the brook lamprey (Gold and Finger 1981); however, the presence of enkephalins in the pituitary of lampreys has not been established. Our immunohistochemical analyses detected enkephalin-like immunoreactivity in both the

neurohypophysis and adenohypophysis of the brook lamprey (Figs. 3, 6b).

Immunoreactive met-enkephalin fibers were observed in the anterior part of the neurohypophysis. The extent of the neurohypophysis in this species was determined by the immunohistochemical distribution of vasotocin-like substances (Fig. 6A; see Goossens et al. 1977). Based on these results, the posterior neurohypophysis, the homolog of the pars nervosa, does not contain enkephalin-like material.

The most intense met-enkephalin-like immunoreactivity was detected in the cells of the meta-adenohypophysis. This is the same region of the lamprey adenohypophysis that contains  $\alpha$  MSH-related material (Figs. 3, 4A). In addition, met-enkephalin-like immunoreactivity was detected in the same region of the pro-adenohypophysis where ACTH-like immunoreactivity was observed (Figs. 3, 4B). Pre-absorption of the met-enkephalin antiserum with synthetic met-enkephalin blocked the reaction (Fig. 6c); however, pre-absorption with synthetic  $\beta$ -endorphin(1-31) or dynorphin(1-31) did not block the enkephalin-like staining. A similar staining pattern was obtained with the leu-enkephalin antiserum (data not shown).



**Fig. 6A–C.** Parasagittal sections through hypothalamus and pituitary of brook lamprey (rostral, left).  
**A** Vasotocin (arg-vasopressin-like) immunoreactivity in neurohypophysis. Note reactivity in both anterior (*double arrows*) and posterior portions.  
**B** Enkephalin-like immunoreactivity in anterior neurohypophysis (*double arrows*) as well as pro- and meta-adenohypophyses.  
**C** Blocked control: immunoreactivity in section adjacent to that in B but primary antiserum had been preabsorbed with  $10 \mu\text{m}$  L-enkephalin prior to being applied to tissue. Preabsorption of the antiserum with dynorphin or  $\beta$ -endorphin did not block immunostaining.  $\times 100$

## Discussion

The pituitary appeared early in the vertebrate lineage (Holmes and Ball 1974). In lampreys, the pituitary is anatomically similar to that of gnathostome vertebrates (Fig. 1) and consists of a three-part adenohypophysis and a two-part neurohypophysis. A key difference between the pitui-

tary of lampreys, and those of other classes of vertebrates is the degree of contact between regions of the adenohypophysis and the hypothalamus. In most vertebrates, with the exception of teleosts, the pars distalis is in communication with the hypothalamus via a portal system. In teleosts, neurosecretory axons originating in the hypothalamus terminate directly in the pars distalis (Holmes and Ball 1974).

In some species, the pars intermedia may share a common capillary network with the pars nervosa. In lampreys, the meta-adenohypophysis, the source of MSH-like biological activity, shares a capillary plexus with the posterior neurohypophysis (Gorbman 1965). However, the pro- and meso-adenohypophyses of the lamprey do not appear to be linked directly to the hypothalamus by either a portal system or innervation (Tsuneki and Gorbman 1975a, b). In fact the status of the pro- and meso-adenohypophyses as endocrine glands is unclear. Although a number of morphologically distinct cell types are present in these regions of the lamprey pituitary (Hardisty 1981), there has not been a definitive identification of any of the adenohypophysal hormone families (i.e., growth hormone-prolactin, FSH-LH/TSH, ACTH/ $\beta$ LPH) found in other vertebrates (Holmes and Ball 1974). Since ACTH-like bioactivity and immunoreactivity were detected in extracts of whole pituitary (Scott et al. 1971; Eastman and Portonova 1982), this study was undertaken to localize the ACTH-like substances immunohistochemically and to determine whether other elements of the ACTH/ $\beta$ LPH system could be detected.

In gnathostome vertebrates, ACTH- and  $\beta$ LPH-related substances are present in the same cells and are packaged in the same granules (Baker 1980). This distribution is due to the biosynthesis of ACTH,  $\beta$ LPH, and 16 K fragment as parts of a common precursor protein (Eipper and Mains 1980). In the lamprey pituitary a number of deviations from this model were observed. To begin with neither 16 K fragment-like nor  $\beta$ -endorphin-like immunoreactivity was observed. The absence of the former may reflect significant sequence modifications in the putative 16 K fragment of the lamprey. This would not be surprising as the sequence of 16 K fragment is variable across phylogeny. For example, the sequence of  $\gamma$ MSH (the middle portion of 16 K fragment) in the dogfish differs considerably from the sequence of mammalian  $\gamma$ MSH (McLean and Lowry 1981); in the salmon pituitary the  $\gamma$ MSH sequence is absent from the  $\text{NH}_2$ -terminal region of pro-ACTH/endorphin (Kawachi et al. 1982). The absence of  $\beta$ -endorphin reactivity in lamprey is more difficult to interpret;  $\beta$ -endorphin-like and  $\alpha$ -endorphin-like [ $\beta$ -endorphin(1-16)] immunoreactivity has been reported in a number of species of fish (Baker 1980). As three of the  $\beta$ -endorphin antisera used in this study react fully with  $\beta$ LPH, it was assumed that a  $\beta$ LPH-like form would be detected even if  $\beta$ -endorphin were not an end product of the ACTH biosynthetic pathway in the lamprey. This was not observed. In fact, when an antiserum directed against  $\gamma$ LPH (the  $\text{NH}_2$ -terminal region of  $\beta$ LPH) was tested, none of the ACTH-related immunoreactive cells in either the pro-adenohypophysis or the meta-adenohypophysis reacted. However, a population of cells in the meso-adenohypophysis did exhibit immunoreactivity. Based on these results it is unclear if ACTH-related and  $\beta$ LPH-related material in the lamprey pituitary are synthesized from a common precursor. Alternatively, posttranslational processing of a common precursor in the lamprey may differ radically from that in mammals.

The distribution of ACTH-related substances was more consistent with the patterns observed in other vertebrates (Fig. 6). The meta-adenohypophysis, a region known to contain MSH-like biological activity, reacted with an antiserum directed against the  $\text{NH}_2$ -terminal ( $\alpha$ MSH region) of ACTH. This  $\alpha$ MSH-like material probably does not undergo post-translational modification at either the  $\text{NH}_2$ -

terminal or COOH-terminal since antisera directed against the acetylated  $\text{NH}_2$ -terminal of  $\alpha$ MSH or the amidated COOH-terminal of  $\alpha$ MSH did not react. These results are not unprecedented. A non-acetylated, non-amidated form of  $\alpha$ MSH has been found in the salmon pituitary (Kawachi et al. 1982). In addition, non-acetylated  $\alpha$ MSH has been reported in the dogfish (Lowry and Scott 1975) and detectable levels of ACTH(1-13) $\text{NH}_2$  are present in mammalian pituitaries (Rudman et al. 1979; Glembotski 1982). The exact nature of the ACTH-related material in the pro-adenohypophysis is less clear. In this region a population of cells reacted with an antiserum directed against the middle region of ACTH, and accordingly these cells would appear to be the most likely source of the ACTH biological activity detected in whole pituitary extracts (Eastman and Portanova 1982). This conclusion is confounded, however, by the absence of  $\text{NH}_2$ -terminal ACTH immunoreactivity (Ann) in this region; the  $\text{NH}_2$ -terminal region of ACTH [ACTH(1-24)] is required for stimulating steroidogenic activity (Schwyzer et al. 1971). Thus the absence of immunoreactivity to the  $\text{NH}_2$ -terminal antiserum (Ann) in the pro-adenohypophysis of the lamprey probably reflects sequence modifications in the  $\text{NH}_2$ -terminal region of the putative lamprey ACTH. Such modifications are not widespread in fish as this antiserum clearly reacts with corticotropin and melanotropic cells in the pituitary of the goldfish (T. Finger, unpublished data). A recent report by Nozaki and Gorbman (1983) indicates a lack of ACTH immunoreactivity in the pituitary of a hagfish. It is possible that the failure to detect ACTH immunoreactivity in this study was due to the specificity of the ACTH antiserum which was directed against the N-terminal half of the molecule. Since the CLIP portion of ACTH is variable among species, it would be of interest to test antiserum Bertha on the pituitary of the hagfish to see if middle ACTH immunoreactivity is present.

The pattern of enkephalin-like immunoreactivity in the lamprey pituitary also differs from that in other vertebrates. In mammals, enkephalin-related immunoreactivity is dispersed throughout the neurohypophysis (Watson et al. 1977), with small amounts of immunoreactivity detected in extracts of the pars distalis (Rossier et al. 1977). In the lamprey, enkephalin-like immunoreactivity was present in the anterior neurohypophysis, but not the posterior neurohypophysis. Furthermore, the most intense enkephalin-like reactivity was detected in the meta-adenohypophysis coincident with  $\alpha$ MSH-like immunoreactivity, and in the pro-adenohypophysis coincident with middle ACTH-like immunoreactivity. The enkephalin-like immunoreactivity could be blocked by enkephalin, but not by  $\beta$ -endorphin(1-31) or dynorphin(1-13). Whether the enkephalin-related immunoreactivity in the lamprey adenohypophysis is due to an enkephalin-size molecule, or a larger form that has sequence homology with enkephalin remains unclear.

In conclusion, the present study indicates that some mammalian antisera to polypeptides of the ACTH/ $\beta$ LPH biosynthetic pathway react with material in the lamprey pituitary. The use of antisera to identify these immunoreactive forms, in conjunction with studies on the melanocyte stimulating, adrenal stimulating, and opiate biological activities of these forms will be useful towards understanding the function of the adenohypophysis of the lamprey, and towards describing the evolution of the pituitary hormones.

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