General and Comparative Endocrinology 121, 232–241 (2001)
doi:10.1006/gcen.2001.7609, available online at http://www.idealibrary.com on IDEAL®

The Distribution of Melanin-Concentrating Hormone in the Lamprey Brain

D. J. Bird,*[,1] I. C. Potter,† S. A. Sower,‡ and B. I. Baker§

*Faculty of Applied Sciences, University of the West of England, Bristol BS16 1QY, United Kingdom; †School of Environmental and Life Sciences, Murdoch University, Murdoch 6150, Western Australia; ‡Department of Biochemistry and Molecular Biology, University of New Hampshire, Rudman Building, Durham, New Hampshire 03824; and §Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom

Accepted December 14, 2000

In addition to its novel, colour-regulating hormonal role in teleosts, the melanin-concentrating hormone (MCH) serves as a neuromodulatory peptide in all vertebrate brains. In gnathostome vertebrates, it is produced in several neuronal cell groups in the hypothalamus. The present work examines the organisation of the MCH system in the brain of lampreys, which separated from gnathostome vertebrates at an early stage in evolution. In all three lamprey genera examined—*Petromyzon*, *Lampetra*, and *Geotria* spp.—MCH perikarya were found in one major anatomical site, the periventricular dorsal hypothalamic nucleus of the posterior hypothalamus. Axons from these cell bodies projected medially into the ventricular cavity, and laterally to the neuropile of the lateral hypothalamus. From here, they extended anteriorly and posteriorly to the fore- and hindbrain. Other fibres extended dorsomedially to the habenular nucleus. In *Lampetra*, but not in *Petromyzon*, MCH fibres were seen in the pituitary neurohypophysis, most prominantly above the proximal pars distalis. The hypothalamic region in which the MCH perikarya are found forms part of the paraventricular organ (PVO), which is rich in monoamines and other neuropeptides. The association of MCH neurones with the PVO, which occurs also in many other nonmammalian vertebrates, may reflect the primary location of the MCH system. These MCH neurones were present in ammocoetes, postmetamorphic juveniles, and adults. They were more heavily granulated in adults than in young lampreys but showed no marked change in secretory appearance associated with metamorphosis or experimental osmotic challenge to indicate a role in feeding or osmoregulation. In sexually maturing *Lampetra fluviatilis*, however, a second group of small MCH neurones became detectable in the telencephalon, suggesting a potential role in reproduction and/or behaviour.

Key Words: melanin-concentrating hormone (MCH); arginine vasotocin (AVT); *Lampetra*; *Petromyzon*; *Geotria*; hypothalamic neuropeptide; immunostaining.

INTRODUCTION

Melanin-concentrating hormone (MCH) is named for its role in colour regulation in teleost fish, in which it is a neurohypophysial hormone. Although it has this hormonal role only in teleosts and possibly holosteans (Sherbrooke and Hadley, 1988), the peptide is present in the hypothalamus of all vertebrates that have been examined. In most groups, the perikarya are located in two or more clusters, and fibres extend to many areas of the brain (Vallarino *et al.*, 1989; Bittencourt *et al.*, 1992; Cardot *et al.*, 1994; Francis and Baker, 1995). The peptide is assumed to exert neuromodulatory or neurotransmitter effects but the limited information about its potential central roles comes mainly from research in mammals. This work suggests that MCH may in-

[1] To whom all correspondence should be addressed.

0016-6480/01 $35.00

fluence osmoregulation, the response to stress, aspects of reproduction, and behavioural and sensory responses (Baker, 1991; Nahon, 1994).

Apart from their own intrinsic interest, lampreys are commonly studied because of their phylogenetic position as living representatives of the Agnatha, which deviated from the main line of vertebrate evolution approximately 500 million years ago. Some of their characteristics are believed to reflect the early vertebrate condition. The present work examines the distribution of MCH in the brain of three species of lamprey and compares the organisation of the MCH system with that of gnathostome vertebrates. Lampreys were examined at different developmental stages, and some were adapted to seawater from freshwater, for clues about changes in peptide secretion and hence peptide function.

MATERIALS AND METHODS

Animals

Three genera of lampreys were examined.

Petromyzon marinus. More than 100 postmetamorphic juvenile sea lampreys were captured in the river Severn during their downstream migration to the sea at the end of November (Bird *et al.*, 1994). They were transferred to freshwater aquaria and sampled either immediately after arrival or during the following weeks. Brains were removed and prepared for either wax or cryostat sections as follows: (a) They were fixed in Bouin's fixative for 1–2 days or sublimated Bouin–Hollande fixative for 5 days, before being embedded in paraffin wax; sections were cut at 7 μm. (b) They were fixed in 0.4% parabenzoquinone (Aldrich Chemical Co. Ltd) dissolved in 0.01 M phosphate-buffered saline (PBS), pH 7.2, for 5 h, then washed in 15% sucrose in PBS, and stored in this solution at 4° before sectioning on a cryostat at 14 μm. In most cases, the top and sides of the cranium and also the circumventricular membranes were removed to allow fixative to penetrate into all regions of the brain.

Prespawning, maturing female *Petromyzon*, approximately 47 cm in length and 190 g in weight, were collected in fresh water in New Hampshire, and their brains perfused in sublimated Bouin–Hollande before being embedded in paraffin wax.

Lampetra. Six ammocoetes of the nonparasitic brook lamprey, *L. planeri*, were caught by electrofishing in streams in Hampshire, United Kingdom. Twelve upstream migrating, maturing adult river lampreys, *L. fluviatilis*, and a single downstream-migrant postmetamorphic juvenile were obtained from the Severn Estuary. Dissected brains from all groups were fixed in either sublimated Bouin–Hollande fixative for wax embedding or in parabenzoquinone for cryostat sections, as described above.

Geotria australis. Ammocoetes were caught by electrofishing from streams near Pemberton, South Western Australia, and kept in a freshwater aquarium where many of them underwent metamorphosis. Six ammocoetes and twelve postmetamorphic juveniles were fixed in either Bouin's or in sublimated Bouin–Hollande. Upstream adult migrants were caught in rivers soon after their entry from the sea and their brains fixed in sublimated Bouin–Hollande fixative. All brains were wax-embedded.

Immunocytochemistry

Primary antisera against two peptides, synthetic salmonid MCH and synthetic arginine vasotocin (AVT), were used. The salmonid MCH antisera (generous gifts from Professors H. Kawauchi and A. N. Eberle), used at 2000-fold dilution, did not bind to either α-melanocytye-stimulating hormone (α-MSH) or AVT. The synthetic AVT antiserum (generous gift of Dr. P. M. Ingleton), used at 1000-fold dilution for immunostaining, showed slight ($<$0.01%) cross-reactivity with isotocin. Following overnight incubation at 20°, sections were incubated successively with anti-rabbit globulin and then peroxidase–antiperoxidase antiserum (Sigma Chemical Co., Poole, United Kingdom). Some wax-embedded sections were immunostained using an avidin/biotin peroxidase kit (Vectastain Ltd.). The enzyme substrate in all cases was 0.025% diaminobenzidene (Sigma) to which was added 0.03% H_2O_2.

Experimental

Ten postmetamorphic *P. marinus* were transferred to 50% seawater for several days and then into 100%

seawater for 35 days, before their brains were fixed for wax or cryostat sectioning. Ten recently metamorphosed *G. australis* were transferred sequentially from freshwater to 33% seawater, to 66% seawater, and then into full seawater for 3 days in each, before fixing and processing for wax sectioning.

Nuclear Area Measurement

Wax sections from both freshwater- and seawater-adapted *P. marinus* were immunostained for MCH and counterstained in haematoxylin, and the outlines of the MCH cell nuclei ($n = 25$ per brain) were drawn using a camera lucida and ×100 objective magnification. The cross-sectional area of the nuclei was determined ($\pi \times D^1/2 \times D^2/2$) after measuring their long (D^1) and short (D^2) axes. The mean values of the nuclear areas for individual animals was used to calculate the mean nuclear area (± SEM) for each condition ($n = 4$).

RESULTS

Anatomical Location of MCH Neurones

The basic organisation of the MCH neurosecretory system was similar in all species with the exception that an additional group of MCH neurones were found in sexually maturing *Lampetra.* The organisation seen in postmetamorphic *P. marinus* is described first, before comparison with the adult form and with other species.

1. Petromyzon marinus

(a) Postmetamorphic juveniles. Immunoreactive cell bodies and fibres were discernible in both wax embedded and cryostat sections. Immunoreactivity, particularly within the fibres, was better preserved in the cryostat sections but cellular morphology was less good. Neuronal perikarya were seen in only one location—the dorsal hypothalamic nucleus of the posterior hypothalamus. This nucleus borders the third ventricle, lying anterior and ventral to the posterior tubercular commissure (Fig. 1). The perikarya were sited in the ependyma and subependyma and were commonly bipolar, with one short axon projecting into the medial third ventricle (Fig. 2) and the other extending laterally into the hypothalamus. Occasional perikarya occurred deeper in the lateral hypothalamus, some distance from the ventricle. Fibres extended into many brain regions particularly in the ventral half of the brain. They were particularly abundant in the lateral hypothalamus but also projected dorsomedially towards the habenular nucleus, anteriorly to the olfactory lobes after sweeping over the optic chiasma, and posteriorly towards the spinal cord (Fig. 1a). Fibres crossed from side to side in the postoptic and posterior tubercular commissures. No immunoreactive fibres were detectable in the neurohypophysis, in either wax or cryostat sections.

(b) Adults. The pattern of MCH perikarya and fibres was similar to that of postmetamorphic juveniles but immunostaining intensity in both cell bodies and fibres was more pronounced. The cell bodies were of similar size in the two age groups but had a more rounded profile in adults (Fig. 2). Exact comparison with younger stages was difficult because of the change in brain proportions that occurs with increasing age; the adult brain appeared relatively extended dorsoventrally and the MCH perikarya were crowded more closely against the ependymal layer.

2. Lampetra spp.

Ammocoetes. Immunoreactive MCH cells and fibres were present even in the smallest ammocoete (*Lampetra planeri*), although they were most clearly visible in cryostat sections (Fig. 3a). From their length of <10 cm, these *L. planeri* ammocoetes were estimated to be about 3 years old and thus 1 or 2 years away from metamorphosis.

Postmetamorphic forms. Although staining intensity was still faint in the metamorphosed, downstream migrants of *L. fluviatilis,* it was significantly greater in upstream migrating adults, particularly in the perikarya. Fibre tracts within the brain, however, were always less striking than in *Petromyzon.*

Lampetra and *Petromyzon* appeared to differ in two respects. Firstly, in both larval and adult *Lampetra,* cryostat sections showed clear immunoreactive (ir) MCH fibres within the neurohypophysis, which en-

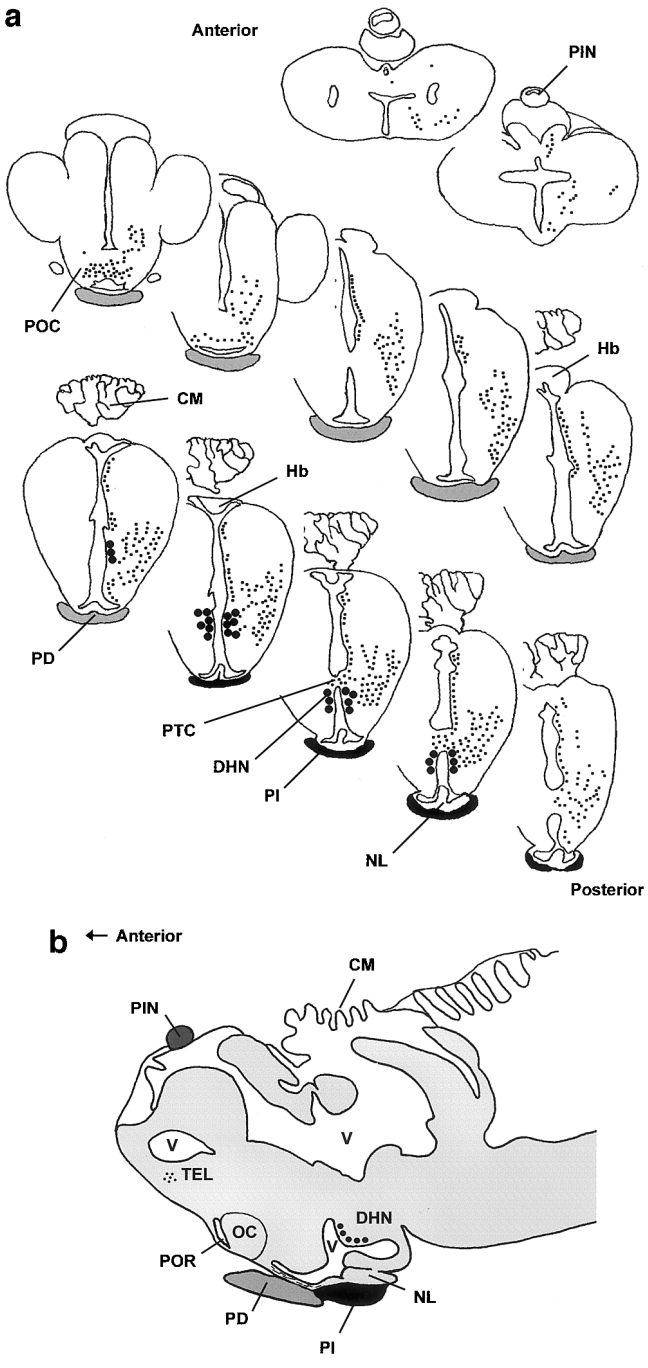


FIG. 1. (a) Transverse sections through the brain and pituitary of postmetamorphic *Petromyzon marinus,* extending from the telencephalon, anteriorly, to the posterior pituitary, and showing the position of MCH perikarya (large dots) and MCH fibre tracts (small dots). (b) Sagittal section of sexually maturing adult *Lampetra* showing position of large MCH perikarya in the dorsal hypothalamic nucleus and smaller ones in the telencephalon. Fibre tracts above the proximal pars distalis are shown as thin lines. CM, circumventricular membranes; DHN, dorsal hypothalamic nucleus; Hb, habenular nucleus; NL, neural lobe; OC, optic chiasma; PD, pars distalis; PI, pars intermedia; PIN, pineal; POC, postoptic commissure; POR, preoptic recess; PTC, posterior tubercular commissure; TEL, telencephalon; V, ventricle.

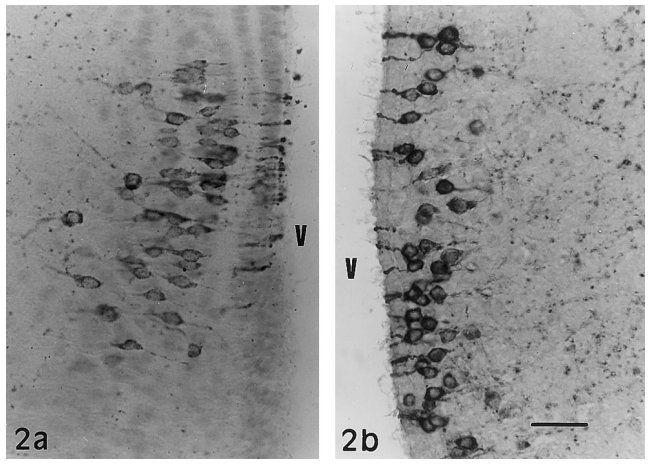
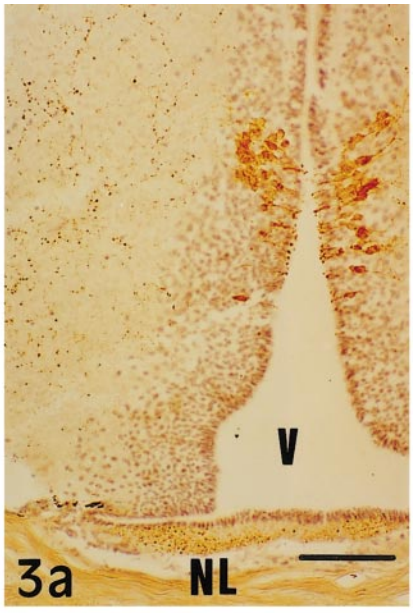
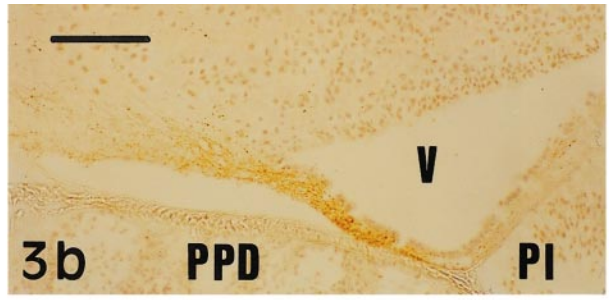


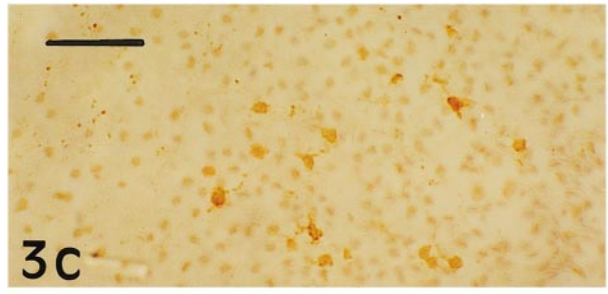
FIG. 2. Transverse sections through the dorsal hypothalamic nucleus of *Petromyzon marinus* showing immunostained MCH perikarya, with fibres projecting towards the ventricular cavity (V) in (a) postmetamorphic and (b) adult lampreys. Bar = 25 μm.

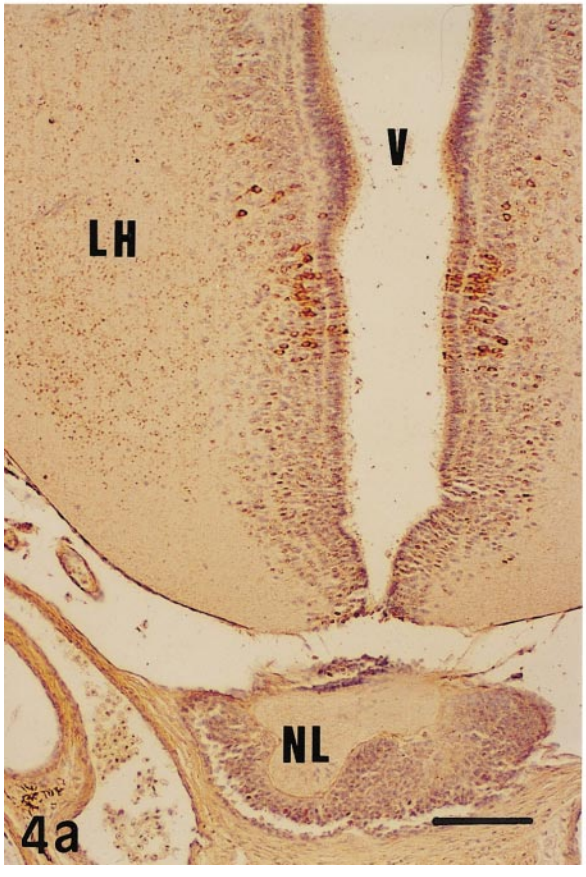
tered the pituitary anteriolaterally. In ammocoetes, such fibres were present throughout the neurohypophysis (Fig. 3a). In the sexually maturing adults, irMCH fibres were most abundant above the proximal pars distalis (Fig. 3b) but very sparse in the posterior neurohypophysis. The abundance of irMCH material in the pituitary neural lobe was relatively slight compared with sections immunostained for AVT, however (see below). MCH fibres were not detectable in the neurophysis in wax-embedded tissues.

The second difference between *Lampetra* and *Petromyzon* was the presence of another group of small, rather faintly staining irMCH neurones in the telencephalon, lying below the lateral ventricle (Figs. 1b, 3c) of sexually maturing *L. fluviatilis.* These neurones were not detectable in brains of an equivalent age processed for wax embedding and the developmental stage when they first become evident could not be determined. No such perikarya were seen in this position in cryostat sections of young postmetamorphic *Petromyzon* although irMCH fibres were readily detectable in this region.









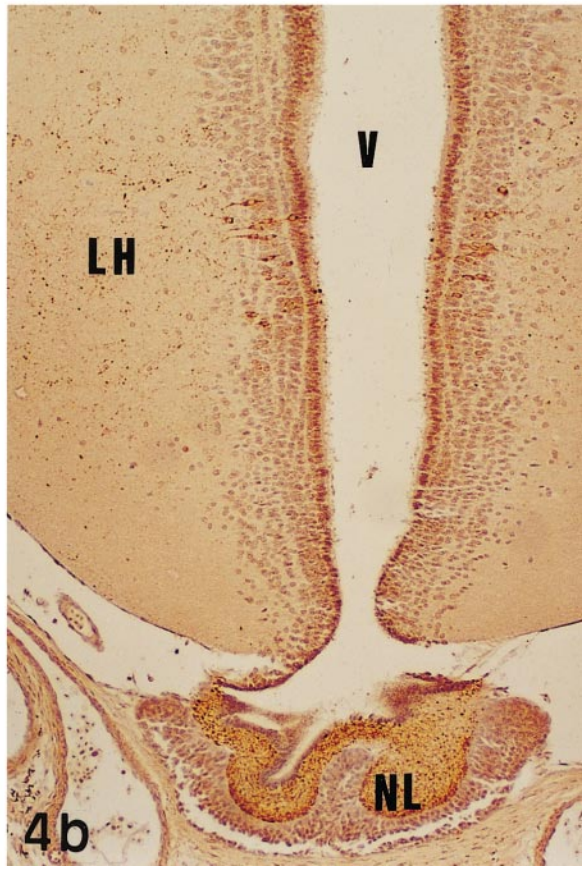


TABLE 1
Changes in Plasma Osmotic Pressure (OP) at Different Life Stages of *Geotria* and in Response to Change of Salinity

Life stage	Length (mm)	Weight (g)	Condition factor ($W/L^3 \times 10^6$)	Plasma OP (mOsm kg^{-1})	*n*
Ammocoete (Year 2/3)	85.5 ± 4.5	0.75 ± 0.12	1.00 ± 0.14	196 ± 7.5	8
Postmetamorphic					
Freshwater	101.0 ± 3.1	0.80 ± 0.06	0.76 ± 0.04	235 ± 7.7**	9
Seawater	92.0 ± 1.8	0.51 ± 0.03	0.65 ± 0.02*	321 ± 13*	8
Adult migrant (freshwater)	215.8 ± 4.9	603.90 ± 10.00	61.8 ± 4.40	296 ± 11	8

* $P < 0.05$ compared with freshwater postmetamorphic form.
** $P < 0.01$ compared with ammocoete.

3. Geotria australis

Only wax-embedded brains were examined. Immunostained fibres and cell bodies were faint in ammocoetes and early postmetamorphic juveniles. They were significantly darker in upstream migrating adults but showed no significant differences from the other two species. MCH perikarya were seen only in the posterior hypothalamus and no MCH immunoreactivity was detected in the neurohypophysis although irAVT fibres were abundant and stained densely.

Response of MCH Neurones to Increased Salinity

Transferring postmetamorphic juveniles from freshwater to seawater for several days (*Geotria*) or for a month (*Petromyzon*) did not alter the abundance of granulation in either the neuronal cell bodies or fibres, although it reduced the animals' condition factor by 13% (*Geotria*, $P < 0.05$) and 16% (*Petromyzon*, $P < 0.02$), suggesting loss of body water. Plasma osmotic pressure, measured in *Geotria* with a freezing point osmometer, increased after metamorphosis in freshwater and further increased after transfer to seawater (Table 1). Measurements of MCH neuronal nuclear area in *Petromyzon* did not reveal any alteration in size that might indicate changed synthetic activity on exposure to salt water (cell nuclear area: freshwater, 34.8 ± 2.4 μm^2; seawater, 35.8 ± 0.76 μm^2; $n = 4$, $P > 0.1$).

Position of MCH Perikarya Relative to Other Neuropeptidergic Cells

Several other peptidergic neurones, including neurones secreting AVT, have been recorded in the dorsal hypothalamic nucleus of lampreys (Nozaki *et al.*, 1984). To determine the extent of overlap between neurones secreting MCH or AVT, neighbouring sections were immunostained for one or the other peptide. Although there was some overlap in the locations of the two neuronal types, the AVT neurones extended more anteriorly in both juvenile and adult *Petromyzon* (Fig. 4b) and there was no evidence of coexistence of the two peptides.

DISCUSSION

In contrast to gnathostome vertebrates, which have several groups of hypothalamic MCH neurones

FIG. 3. Immunostained cryostat sections showing MCH neurones in *Lampetra* spp. (a) TS *L. planeri* larva, showing MCH perikarya projecting towards the ventricle (V), and fibres in the lateral hypothalamus and in the pituitary neural tissue (NL). Bar = 50 μm. (b) Sagittal section through maturing *L. fluviatilis* adult, showing fibre tracts in the pituitary neural tissue above the proximal pars distalis (PPD), with very few fibres above the pars intermedia (PI). Bar = 50 μm. (c) MCH perikarya and fibres in the telencephalon. Bar = 25 μm.
FIG. 4. Neighbouring sections through the dorsal medial hypothalamus and pituitary of postmetamorphic *Petromyzon marinus*, immunostained for (a) MCH and (b) AVT and counterstained with haematoxylin. Perikarya of the two neuronal types overlap in their locations. Both send projections towards the ventricle (V) and axons of both types (seen as brown dots) are abundant in the lateral hypothalamus (LH). Only AVT fibres are seen in the neurohypophysis (NL, derived from preoptic perikarya). Bar = 100 μm.

(Andersen *et al.,* 1986; Bittencourt *et al.,* 1992; Knollema *et al.,* 1992; Cardot *et al.,* 1994, 1999; Baker *et al.,* 1995; Groneveld *et al.,* 1995), MCH expression in the lamprey hypothalamus is restricted largely to a single cluster of perikarya in the posterior dorsal hypothalamic nucleus. It may be that this location reflects the ancestral condition. As in other vertebrates, however, axons from these MCH perikarya project extensively to many regions of the brain, suggesting that, already at this level of vertebrate organisation, MCH has a pattern of the distribution that allows widespread modulatory influence within the central nervous system.

The dorsal hypothalamic nucleus is contained within the paraventricular organ (PVO). This structure is found in all nonmammalian vertebrates and is a vascular region of the ependyma, characterised by the presence of neurones that contain serotonin and others that contain catecholamines (Baumgarten, 1972; Vigh and Vigh-Teichmann, 1973; Brodin *et al.,* 1990; Pierre *et al.,* 1992; Batten *et al.,* 1993). The aminergic neurones project into the ventricular cavity as well as towards the neuropile of the brain. Many nonmammalian vertebrates show an association between MCH neurones and the PVO (Baker and Kawauchi, 1997). In many lower vertebrates, such as lampreys, amphibia, and reptiles, this may be the major MCH grouping (Francis and Baker, 1995; Andersen *et al.,* 1986; Cardot *et al.,* 1994), but in teleosts, in which MCH is used as a colour-regulating neurohypophysial hormone, the neurones of the lateral tuberal nucleus predominate. The PVO-related MCH neurones may lie close to the ventricular surface and project into the ventricular cavity, for example, in lampreys (present paper), the holostean *Lepisosteus* (Baker and Kawauchi, 1997), the trout *Oncorhynchus* (Baker *et al.,* 1995), and the lungfish *Protopteus* (Vallarino *et al.,* 1998). In other vertebrates, MCH perikarya surround the PVO less intimately, as in amphibians (Francis and Baker, 1995) and reptiles and birds (Cardot *et al.,* 1994, 1999). In these cases, though, axonal projections from the MCH neurones are directed towards the PVO and ventricular ependyma as well as into the brain neuropile.

The functional significance of this organisation is uncertain but some intercommunication between MCH and other neurones in the PVO seems likely. Neurones producing transmitters other than monoamines and MCH are also found within the confines of the PVO. In lampreys these include AVT (present study; Nozaki *et al.,* 1984), neurotensin (Brodin *et al.,* 1990), and one of the gonadotrophin-releasing hormones (GnRH) (Nozaki *et al.,* 1984; Tobet *et al.,* 1995). In gnathostome vertebrates, GnRH (Muske *et al.,* 1994; Collin *et al.,* 1995), somatostatin (Meurling and Rodriguez, 1990), galanin (Jimenez *et al.,* 1994; Nicolini *et al.,* 1995), thyrotropin-releasing hormone (TRH) (Batten *et al.,* 1990; Zoeller and Conway, 1989), and cholecystokinin and substance P (Batten *et al.,* 1990) have all been reported within the PVO area. Synaptic contacts between unidentified neurones are abundant in the neuropile surrounding the PVO (Vigh-Teichmann and Vigh, 1989; Meurling and Rodriguez, 1990), suggesting the region may serve as an intergrative centre. The fact that this region is highly vascular and appears to lack a blood–brain barrier may be related to the finding that in the lungfish *Protopterus,* some elements in the PVO take up markers such as cobaltous lysine from the blood and transport it both into their ventricular projections and along their entire axonal length (Bartheld and Meyer, 1990).

As far as the function of the axonal projections into the ventricular cavity is concerned, a possible interpretation is that MCH is released into the CSF and thence diffuses to other regions, including the pituitary, to exert a modulatory influence. In the agnathan *Eptatretus* for instance, substances injected into the third ventricle have been shown to diffuse rapidly into the pars distalis (Nozaki *et al.,* 1975). Ultrastructural evidence for exocytosis from intraventricular terminals has never been described, however. Indeed, electron microscope studies of the PVO have led to the suggestion that these terminals have a sensory rather than a secretory role, responding to information from the CSF (Vigh and Vigh-Teichmann, 1973; Vigh-Teichmann and Vigh, 1989).

No specific function of MCH in lampreys has been demonstrated. The present study shows that the MCH system is established during larval life and that, apart from enhanced immunostaining with age, the PVO neurones showed no marked changes that could be related to a specific physiological challenge.

In teleost fish, MCH has been adopted as a skin colour-regulating hormone, causing pallor by a direct hormonal action on the chromatophores as well a by depressing the release of α-MSH, the skin-darkening hormone, from the pars intermedia. In all other verte-

brates, however, including lampreys, it has no melanin-concentrating effect on the skin. Moreover, with the exception of teleosts, it is only sparsely present in the posterior pituitary and is thus unlikely to serve as a circulating hormone (Baker, 1991).

An unexpected difference between *Petromyzon* and *Lampetra* was the presence of irMCH fibres in the neurohypophysis only of *Lampetra.* This was consistently present in the pituitary of those ammocoetes and adult *Lampetra* that had been processed for cryostat sections and confirms ultrastructural reports of MCH fibres in this region in adult *Lampetra* (Alyousuf and Mizuno, 1991). The inability to demonstrate neurohypophysial MCH in *Petromyzon* could either be a species difference or due to fixation problems, although many cryostat sections of postmetamorphic *Petromyzon* were examined that had been fixed in a manner similar to that for *Lampetra.* The functional significance of irMCH fibres in the neural lobe is still unknown. Despite its modest abundance in the pituitary, as judged by the low intensity of immunostaining, it could serve to modulate the release of some other neuropeptide by paracrine action, as appears to occur in the mammalian neurohypophysis (Parkes and Vale, 1992), or to influence the secretory activity of one or another type of pituitary cell in the proximal pars distalis following diffusion through the intervening connective tissue.

The cellular origin of these pituitary fibres is also unclear. The fact that they enter the neural lobe from an anterior tract of fibres raises the possibility that they arise from the telencephalic group of MCH neurones, again seen only in *Lampetra.* Unfortunately, lack of material makes it impossible to determine whether such telencephalic perikarya are already developed in younger animals or whether they become evident only in sexually mature lampreys. In the amphibian *Rana temporaria,* a group of irMCH perikarya become evident for the first time in gravid females close to oviposition (Francis, Ph.D. thesis, University of Bath, 1996). These neurones might be homologous/analogous to the anterior hypothalamic MCH neurones described in the rat (Knollema *et al.,* 1992) that become detectable by immunocytochemistry or *in situ* hybridization only during lactation and then disappear again immediately after weaning. Their role in reproduction is undetermined but one can speculate that they might modulate some feature, e.g., odour detection, known to be involved in migration, synchronised spawning, and parental behaviour.

The denser MCH granulation in perikarya and brain fibres of young, postmetamorphic *Petromyzon* specimens compared with the corresponding stages of other species of lampreys deserves comment. It may be associated with the fact that these *Petromyzon* were significantly larger than typical downstream migrants and had food in their guts, suggesting that they had been feeding in the Severn Estuary and had been carried upstream again before capture (Bird *et al.,* 1994). Whether their heavier MCH granulation is related to their more mature status or to the fact that they had started to feed before capture and transfer to the aquarium is not known but it may be significant that both the *Geotria* and *Lampetra* postmetamorphic juveniles would not have fed since they entered metamorphosis, several weeks previously. In mammals, MCH is thought to encourage feeding activity: MCH synthesis and presumably also its release are augmented during the early stages of starvation (Presse *et al.,* 1996; Qu *et al.,* 1996) while intracerebroventricular injections of MCH usually increase appetite (Qu *et al.,* 1996; Rossi *et al.,* 1997).

In fish, amphibians, and mammals, increased salinity affects MCH synthesis, the neurones showing either increased or decreased abundance of mRNA, depending on the cell group concerned and the duration of saline challenge (Presse and Nahon, 1993; Fellmann *et al.,* 1993; Francis *et al.,* 1997). In lampreys, however, there was no detectable response in either granulation or cell nuclear area following a brief or longer-term immersion in seawater. It is possible that the age and physiological conditions of the postmetamorphic juveniles used for these trials were inappropriate. Although a response might be detectable by using a more sensitive technique such as Northern blot or *in situ* hybridization, cell nuclear area has been successful in other studies as a measure of MCH cell synthetic activity (Bird *et al.,* 1989; Francis and Baker, 1995). It is also possible that MCH plays no part in osmoregulation in lampreys.

Although the role of MCH in lampreys remains obscure, the tentative links with feeding and reproduction imply that the subtle physiological roles demonstrated for the neuropeptide in mammals may have appeared at an early stage in vertebrate evolution.

ACKNOWLEDGMENTS

We thank the BBSRC, the Royal Society, and the Wellcome–Ramaciotti Foundation for financial support, and Dr. Glen Power for assistance in measuring plasma osmotic pressures.

REFERENCES

Alyousuf, S., and Mizuno, N. (1991). Electron-microscopic identification of axons containing melanin-concentrating hormone in the lamprey, *Lampetra fluviatilis* L. *Neurosci. Lett.* **128,** 249–252.

Andersen, A. C., Pelletier, G., Eberle, A. N., Leroux, P., Jegou, S., and Vaudry, H. (1986). Localization of melanin-concentrating hormone-like immunoreactivity in the brain and pituitary of the frog *Rana ridibunda*. *Peptides* **7,** 941–945.

Baker, B. I. (1991). Melanin-concentration hormone: A general vertebrate neuropeptide. *Int. Rev. Cytol.* **126,** 1–47.

Baker, B. I., and Kawauchi, H. (1997). MCH in non-mammalian vertebrates: Neuronal topography and functions. *In* "MCH and Seizures: Neuromolecular and Neuroendocrine Aspects" (K. Knigge, A. Prasad, S. Pretel, and J. E. Wagner, Eds.), pp. 1–30. Research Signposts, Trivandrum, India.

Baker, B. I., Levy, A., Hall, L., and Lightman, S. L. (1995). Cloning and expression of melanin-concentrating hormone genes in the rainbow trout brain. *Neuroendocrinology* **61,** 67–76.

Bartheld, C. S., and Meyer, D. L. (1990). Paraventricular organ of the lungfish *Protopterus dolloi*: Morphology and projections of CSF-contacting neurons. *J. Comp. Neurol.* **297,** 410–434.

Batten, T. F. C., Berry, P. A., Maqbool, A., Moons, L., and Vandesande, F. (1993). Immunolocalization of catecholamine enzymes, serotonin, dopamine and l-dopa in the brain of *Dicentrarchus labrax* (Teleostei). *Brain Res. Bull.* **31,** 233–252.

Batten, T. F. C., Cambre, M. L., Moons, L., and Vandesande, F. (1990). Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, *Poecilia latipinna*. *J. Comp. Neurol.* **302,** 893–919.

Baumgarten, H. (1972). Biogenic amines in the cyclostome and lower vertebrate brain. *Prog. Histochem. Cytochem.* **4,** 1–90.

Bird, D. J., Baker, B. I., and Kawauchi, H. (1989). Immunocytochemical demonstration of melanin-concentrating hormone and pro-opiomelanocortin-like products in the brain of the trout and carp. *Gen. Comp. Endocrinol.* **74,** 442–450.

Bird, D. J., Potter, I. C., Hardisty, M. W., and Baker, B. I. (1994). Morphology, body size and behaviour of recently-metamorphosed sea lampreys, *Petromyzon marinus*, from the lower river Severn, and their relevance to the onset of parasitic feeding. *J. Fish Biol.* **44,** 67–74.

Bittencourt, J. C., Presse, F., Arias, C., Peto, C., Vaughan, J., Nahon, J. L., Vale, W., and Sawchenko, P. E. (1992). The melanin-concentrating hormone system of the rat brain: An immunization and hybridization histochemical characterization. *J. Comp. Neurol.* **319,** 218–245.

Brodin, L., Theordorsson, E., Christenson, J., Cullheim, S., Hokfelt, T., Brown, J. C., Buchan, A., Panula, P., Verhofstad, A. A. J., and Goldstein, M. (1990). Neurotensin-like peptides in the CNS of lampreys: Chromatographic characterization and immunohistochemical localization with reference to aminergic markers. *Eur. J. Neurosci.* **2,** 1095–1109.

Cardot, J., Fellmann, D., and Bugnon, C. (1994). Melanin-concentrating hormone-producing neurons in reptiles. *Gen. Comp. Endocrinol.* **94,** 23–32.

Cardot, J., Griffond, B., Risold, P.-Y., Blahser, S., and Fellmann, D. (1999). Melanin-concentrating hormone-producing neurons in birds. *J. Comp. Neurol.* **411,** 239–256.

Collin, F., Chartrel, N., Fasolo, A., Conlon, J. M., Vandesande, F., and Vaudry, H. (1995). Distribution of two molecular forms of gonadotropin-releasing hormone (GnRH) in the central nervous system of the frog *Rana ridibunda*. *Brain Res.* **703,** 111–128.

Fellmann, D., Risold, P. Y., Bahjaoui, M., Compagnone, N., Bresson, J. L., Clavequin, M. C., Cardot, J., Gouget, A., Lenys, D., and Bugnon, C. (1993). Morphofunctional studies on the neurons producing melanin-concentrating hormone. *Ann. NY Acad. Sci.* **680,** 511–516.

Francis, K., and Baker, B. I. (1995). Developmental changes in melanin-concentrating hormone in *Rana temporaria*. *Gen. Comp. Endocrinol.* **98,** 157–165.

Francis, K., Suzuki, M., and Baker, B. I. (1997). Responses of melanin-concentrating hormone mRNA to salt water challenge in the rainbow trout. *Neuroendocrinology* **66,** 195–202.

Groneveld, D., Eckhardt, E. R. M., Coenen, A. J. M., Martens, G. J. M., Balm, P. H. M., and Wendelaar Bonga, S. E. (1995). Expression of tilapia prepro-melanin-concentrating hormone messenger RNA in hypothalamic and neurohypophyseal cells. *J. Mol. Endocrinol.* **14,** 199–207.

Jimenez, A. J., Mancera, J. M., Perez Figares, J. M., and Fernandez Llebrez, P. (1994). Distribution of galanin-like immunoreactivity in the brain of the turtle *Mauremys caspica*. *J. Comp. Neurol.* **349,** 73–84.

Knollema, S., Brown, E. R., Vale, W., and Sawchenko, P. E. (1992). Novel hypothalamic and preoptic sites of prepro-melanin-concentrating hormone messenger ribonucleic acid and peptide expression in lactating rats. *J. Neuroendocrinol.* **4,** 709–717.

Meurling, P., and Rodriguez, E. M. (1990). The paraventricular and posterior recess organs of elasmobranchs: A system of cerebrospinal fluid-contacting neurons containing immunoreactive serotonin and somatostatin. *Cell Tissue Res.* **259,** 463–473.

Muske, L. E., King, J. A., Moore, F. L., and Millar, R. P. (1994). Gonadotropin-releasing hormones in microdissected brain-regions of an amphibian: Concentration and anatomical distribution of immunoreactive mammalian GnRH and chicken GnRH-II. *Regul. Peptides* **54,** 373–384.

Nahon, J.-L. (1994). The melanin-concentrating hormone: From the peptide to the gene. *Crit. Rev. Neurobiol.* **8,** 211–262.

Nicolini, V. G., Orezzoli, A. A., Achi, M. V., Villar, M. J., and Tramezzani, J. H. (1995). Galanin-like immunoreactive expression in the central nervous system of the toad. *Biocell* **19,** 95–111.

Nozaki, M., Fernholm, B., and Kobayashi, H. (1975). Ependymal absorption of peroxidase into the third ventricle of the hagfish, *Eptatretus burgeri* (Girard). *Acta Zool. Stockholm* **56,** 265–269.

Nozaki, M., Tsukahara, T., and Kobayashi, H. (1984). An immunocytochemical study on the distribution of neuropeptides in the brain of certain species of fish. *Biomed. Res.* **4** (Suppl.), 135–143.

Parkes, D., and Vale, W. (1992). Secretion of melanin-concentrating hormone and neuropeptide-EI from cultured rat hypothalamic cells. *Endocrinology* **131,** 1826–1831.

Pierre, J., Reperant, J., Ward, R., Vesselkin, N. P., Rio, J.-P., Miceli, D., and Kratskin, I. (1992). The serotoninergic system of the brain of lamprey, *Lampetra fluviatilis*: An evolutionary perspective. *J. Chem. Neuroanat.* **5,** 195–219.

Presse, F., and Nahon, J.-L. (1993). Differential regulation of melanin-concentrating hormone gene expression in distinct hypothalamic areas under osmotic stimulation in rat. *Neuroscience* **55,** 709–720.

Presse, F., Sorokovsky, I., Max, J.-P., Nicolaidis, S., and Nahon, J.-L. (1996). Melanin-concentrating hormone is a potent anorectic peptide regulated by food-deprivation and glucopenia in the rat. *Neuroscience* **71,** 735–745.

Qu, D. Q., Ludwig, D. S., Gammeltoft, S., Piper, M., Pelleymounter, M. A., Cullen, M. J., Mathes, W. F., Przypek, J., Kanarek, R., and Maratos-Flier, E. (1996). A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* **380,** 243–247.

Rossi, M., Choi, S. J., O'Shea, D., Miyoshi, T., Ghatei, M. M., and Bloom, S. R. (1997). Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. *Endocrinology* **138,** 351–355.

Sherbrooke, W. C., and Hadley, M. E. (1988). Exploring the evolutionary history of melanin-concentrating and melanin-stimulating hormone receptors on melanophores: Neopterygian (holostean) and chondrostean fishes. *Pigm. Cell Res.* **1,** 344–349.

Tobet, S. A., Nozaki, M., Youson, J. H., and Sower, S. A. (1995). Distribution of lamprey gonadotropin-releasing hormone-III (GnRH-III) in brains of larval lampreys (*Petromyzon marinus*). *Cell Tissue Res.* **279,** 261–270.

Vallarino, M., Andersen, A. C., Delbende, C., Ottonello, I., Eberle, A. N., and Vaudry, H. (1989). Melanin-concentrating hormone (MCH) immunoreactivity in the brain and pituitary of the dogfish *Scyliorhinus canicula*: Colocalization with alpha-melanocyte-stimulating hormone (α-MSH) in hypothalamic neurons. *Peptides* **10,** 375–382.

Vallarino, M., Trabucchi, M., Chartrel, N., Jäggin, V., Eberle, A. N., and Vaudry, H. (1998). Melanin-concentrating hormone system in the brain of the lungfish *Protopterus annectens*. *J. Comp. Neurol.* **390,** 41–51.

Vigh, B., and Vigh-Teichmann, I. (1973). Comparative ultrastructure of the cerebrospinal fluid-contacting neurons. *Int. Rev. Cytol.* **35,** 189–251.

Vigh-Teichmann, I., and Vigh, B. (1989). The cerebrospinal fluid-contacting neuron: a peculiar cell type of the central nervous system: Immunocytochemical aspects. *Arch. Histol. Cytol.* **52,** 195–207.

Zoeller, R. T., and Conway, K. M. (1989). Neurons expressing thyrotropin-releasing hormone-like messenger ribonucleic acid are widely distributed in *Xenopus laevis* brain. *Gen. Comp. Endocrinol.* **76,** 139–146.