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Melanin-Concentrating Hormone-Producing Neurons in Reptiles

J. CARDOT, D. FELLMANN, AND C. BUGNON

Laboratoire d'Histologie-Embryologie-Cytogénétique, Unité de Recherche Associée 0561 du Centre National de la Recherche Scientifique, Faculté de Médecine, 25030 Besançon Cedex, France

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Melanin-concentrating hormone (MCH)-like producing neurons were mapped in the brains of several reptiles using antisera (AS) prepared against salmon MCH (sMCH) and peptides derived from the rat MCH precursor (rMCH, NGE, NEI) or cross-reacting with these peptides (anti-GRF37 and anti-α-MSH). MCH neurons were detected in the periventricular and lateral hypothalamic nuclei. The coexpression of MCH-, GRF37- and NEI-like immunoreactivities suggests that the reptile precursor presents large sequence homologies with the rat/human precursor. MCH neurons project to many brain areas, but fibers are very scarce in the median eminence, and the neurohypophysis is devoid of immunoreactive processes. Thus the MCH produced by these neurons would not be a neurohormone as in fish. The great quantity of processes observed in the optic lobes and in the olfactive encephalic areas (particularly in the septum) is most probably related to behavioral and adaptive regulations controlled by the hypothalamus.

In mammals a discrete neuron population whose perikarya are exclusively located in lateral and dorsal areas of the hypothalamus synthesizes a neuropeptide closely related to the teleost fish melanin-concentrating hormone (MCH) (Skofitsch *et al.*, 1985; Naito *et al.*, 1986, 1988; Bresson *et al.*, 1989; Risold *et al.*, 1989; Vaughan *et al.*, 1989) (for reviews see Baker, 1988, 1991; Eberlé, 1988).

This peptide was first isolated and sequenced from the salmon brain (Kawauchi *et al.*, 1983), and the structure of its precursor molecule has been elucidated by cDNA cloning in rat (Nahon *et al.*, 1989a,b; Breton *et al.*, 1989; Fellmann *et al.*, 1989a,b) and human (Presse *et al.*, 1990). In salmonids, two slightly different precursors yield the same 17-residue MCH peptide (Ono *et al.*, 1988; Minth *et al.*, 1989). Rat and salmon MCH are closely related.

In rat and in human, two additional peptides might be released by proteolytic cleavage of the MCH precursor: a 13-residue amidated peptide (neuropeptide glutamic acid-isoleucine amide, NEI) and a 19-residue peptide (neuropeptide glycine-glutamic acid, NGE) (Nahon *et al.*, 1989a,b). It has been shown that an epitope generated by the amidated C-terminus of the NEI can be recognized by antisera to α-melanotropin (α-MSH) and rat corticoliberin (Nahon *et al.*, 1989a). The C-terminal epitope of NEI would thus explain the immunostaining of the neuron population expressing the MCH gene by antisera to α-MSH (Watson and Akil, 1979; Fellmann *et al.*, 1986; Naito *et al.*, 1986; Risold *et al.*, 1989), adrenorphin (Merchenthaler *et al.*, 1986), and rat corticoliberin (Daikoku *et al.*, 1985; Nahon *et al.*, 1989a). Moreover, antisera to the 1-37 fragment of the human somatocrinin (GRF 37), which strongly stained the same neuron population (Fellmann *et al.*, 1985, 1986; Risold *et al.*, 1989), recognized a synthetic NGE molecule (Nahon *et al.*, 1989a; Breton *et al.*, 1989; Risold *et al.*, 1992). In the salmon precursor, the NEI sequence would be replaced by a NEV (neuropeptide glutamic acid-valine) (Nahon *et al.*, 1989a,b).

Immunocytochemical investigations performed for several years in our laboratory enabled us to analyze the phylogenic evo-

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lution of neurons producing MCH-like immunoreactivity in various vertebrates, including fishes, amphibians, reptiles, and birds. We report here the findings obtained in reptile brains.

MATERIALS AND METHODS

The present work was performed mainly on the brains of young water turtles (*Chrysemis scripta elegans*) (Figs. 1–15)[1] measuring 6 to 8 cm (commercial origin), but also on lizards (*Podarcis muralis*) and snakes (*Natrix natrix* and *Natrix maura*) taken in spring or summer.

After several preliminary tests, brain sections were prepared as follows: Brains were quickly dissected after decapitation and fixed for 5 hr in formaldehyde–lysine solution with or without added sodium periodate, as described by McLean and Nakane (1974). They were soaked in a 15% (w/v) sucrose solution in sodium phosphate buffer (0.1 *M*, pH 7.4) for 2 to 8 days at 4°, frozen over liquid nitrogen (vapors), and cut into serial 10-μm-thick sections which were collected on gelatin-subbed slides and stored at −80° until use.

Several antisera (AS) prepared against salmon MCH (sMCH) and peptides derived from the rat MCH precursor (rMCH, NGE, NEI), or cross-reacting with these peptides (anti-GRF37 and anti-α-MSH), have been previously prepared in our laboratory. Their specificities have been thoroughly tested by liquid-phase and dot blot experiments (Risold *et al.*, 1992). Sections were incubated for 16 hr at 4° with AS diluted 1/200 to 1/400 in phosphate-buffered saline (PBS) added with 0.3% (v/v) Triton X-100, 1% (w/v) bovine albumin, and 10% (w/v) defatted milk. Antigen–antibody complexes were detected by peroxidase-anti-peroxidase (PAP) complexes or by fluorescein (FITC) or rhodamine-labeled anti-rabbit antibodies (TRITC). The results obtained with these sera were compared on adjacent sections. We also performed double-staining experiments either by successively staining the first antigen with the PAP technique and the second with immunofluorescence method or by performing two consecutive immunofluorescence detections on a single section, the first with a fluorescein conjugate and the second with a rhodamine conjugate. The potassium permanganate method was occasionally tried to elute the first antigen–antibody complexes before the second staining (Tramu *et al.*, 1978). However, this drastic method is not easy to use because cryostat sections are very brittle. Fluorescent nuclear counterstain with ethidium bromide was sometimes used as described (Schmued *et al.*, 1982). The nomenclature of the reptile brain structures has been taken from Reiner *et al.* (1984) and Weindl *et al.* (1984).

RESULTS

In the turtle, lizard, and snake brains, sMCH AS and rMCH AS stained perikarya exclusively located in the hypothalamus. They were mainly distributed in the periventricular nucleus, which is ventral to the paraventricular organ (PVO) and to the lateral hypothalamic sulcus (Figs. 9, 10, and 16–18). Scarce perikarya were also

[1] Abbreviations used in the figures: A, alveus; AC, nucleus centralis amygdalae; AP, area pretectalis; AV, area vestibularis; BA, nucleus basalis amygdalae; BN, bed nuclei; BO, bulbus olfactorius; BOR, basal optic root; C, cerebellum; c, area c (Riss, Halpern, Scalia); CA, commissura anterioris; CH, commissura pallii anterioris (Hippocampi); CO, chiasma opticum; CP, commissura posterioris; cd, cortex dorsalis; cdm, cortex dorsomedialis; cm, cortex medialis; cp, cortex piriformis; d, area d (Riss, Halpern, Scalia); DB, fasciculus diagonalis Brocae; DLA, nucleus dorsolateralis anterior; DMA, nucleus dorsomedialis anterior; d IV, decussatio nervi trochlearis; DVR, dorsal ventricular ridge of the telencephalon; EM, eminentia mediana; FL, funiculus lateralis; FLM, fasciculus longitudinalis medialis; GP, globus pallidus; H, Primordium hippocampi; Hb, habenula; HT, hypothalamus; Hy, hypophysis; IP, nucleus interpeduncularis; LG, lamina glomerularis; LGr, lamina granularis; LT, nucleus laminaris of the torus semicircularis; MC, mitral cell layer; MO, medulla oblongata; MV, nucleus mesencephalicus nervi trigemini; nI, nuclei isthmi; NP, neuroglia periventricularis (subependymal layer); nLH, nucleus lateralis hypothalami; nPH, nucleus periventricularis hypothalami; nSL, nucleus septalis lateralis; nSM, nucleus septalis medialis; nTS, nucleus tracti solitarii; OSC, organum subcommissurale; OT, tractus opticus; PA, paleostriatum augmentatum; PC, pedunculus cerebellaris; PD, pedunculus dorsalis fasciculi prosencephali lateralis; PT, pallial thickening; PV, pedunculus ventralis fasciculi prosencephali lateralis; PVO, organum paraventricularis; R, nucleus rotundus; Rai, nucleus raphe inferior; Re, nucleus reuniens; Ri, nucleus reticularis inferior; Ris, nucleus reticularis isthmi; Rm, nucleus reticularis medius; S, septum; SM, stria medullaris; SGD, substantia grisea dorsalis; SGIL, substantia grisea intermediolateralis; SVD, substantia grisea ventralis; T, tuber; TE, telencephalon; TO, tectum opticum; TOL, tuberculum olfactorium; II, nervus opticus; III, nervus oculomotorius; IV, nervus trochlearis; V, nervus trigemini; VIII, nervus octavus (and VI, nervus abducens; VII, nervus facialis).

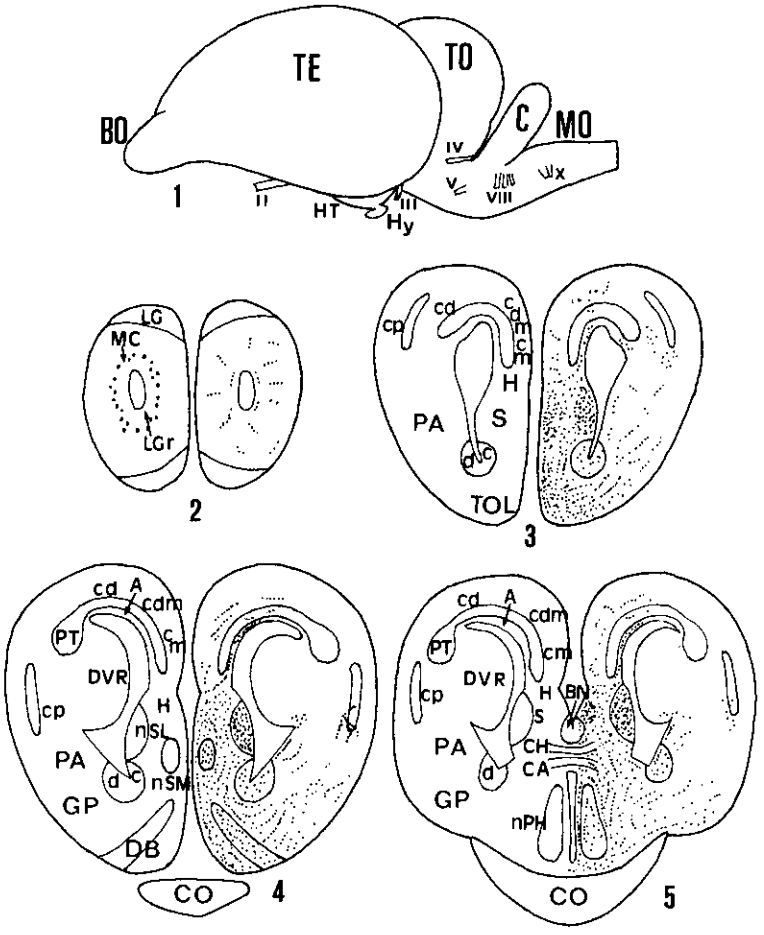


FIG. 1. Line drawing illustrating a lateral view of the brain of a turtle (*Chrysemis scripta elegans*). The major subdivisions are indicated, except for the diencephalon, which is hidden from view by the overlying telencephalon.

FIG. 2. Line drawing of transverse section through turtle olfactory bulb, illustrating the distribution of MCH-containing fibers.

FIG. 3. Transverse section through the rostral telencephalon.

FIG. 4. Section through more caudal telencephalic level than shown in Fig. 3.

FIG. 5. Section through the telencephalon and junction between the telencephalon and the diencephalon.

stained in the lateral hypothalamic nucleus (Figs. 9, 10, 19, and 20).

In periventricular nucleus, MCH perikarya were abundant, elongated, and bipolar. They emitted a thin process toward the periphery of the third ventricle. The opposite fibers were more prominent. They projected in the lateral hypothalamic regions (Figs. 9, 10, and 18) where they were very conspicuous (Figs. 9 and 10). However, fibers were scarce in the ventral tuberal area (Figs. 9 and 10). They were very few in the median eminence and they did not reach the neurohypophysis. Abundant processes ran dorsally toward the thalamus. They innervated the major part of this structure, the exceptions being the nucleus rotundus and the nucleus reuniens. They were always very varicose, often long and unramified (Fig. 23). Some thick fibers as-

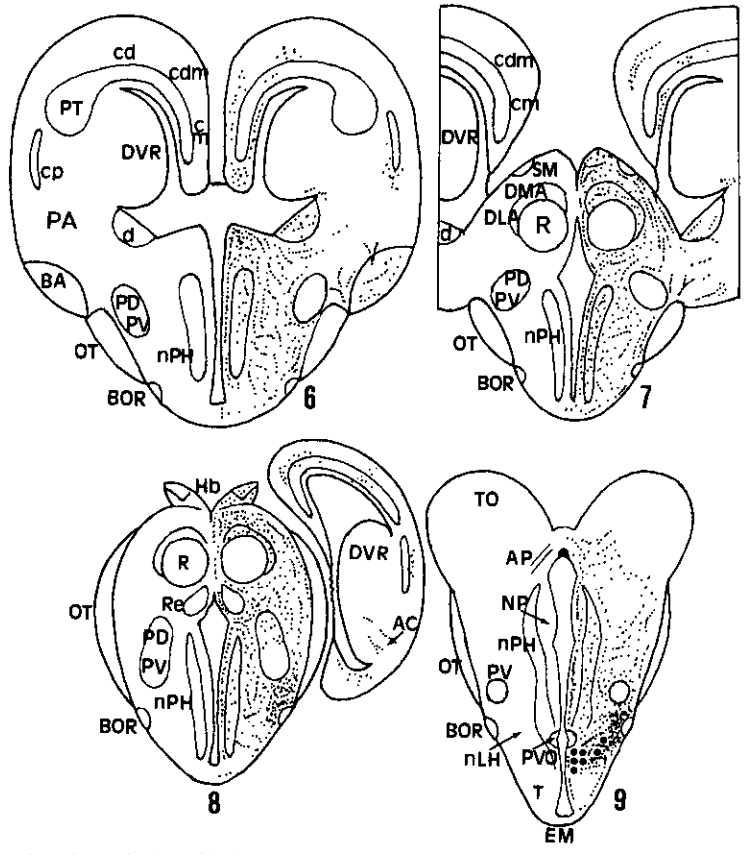


FIG. 6. Section through the midtelencephalon and the rostral diencephalon.

FIG. 7. Section through the caudal telencephalon and middiencephalon. The distribution of MCH-containing fibers in the telencephalon is identical to that in Fig. 6.

FIG. 8. Section through the middiencephalon at level of habenula.

FIG. 9. Section through the diencephalon at level of PVO and rostral mesencephalon (tectum opticum) showing the localization of MCH-perikarya (large dots).

cended through the subependymal neuroglial layer (Figs. 6–10).

The preoptic area, the junction between the telencephalon and the diencephalon, the diagonal band of Broca, the septum (especially the lateral and the medial nuclei), the olfactory tubercle, and the *c* and *d* areas of Riss, Halpern, and Scalia were densely innervated by MCH fibers (Figs. 3–6 and 30). MCH processes were less abundant in the primordium hippocampi (and alveus), in the paleostriatum augmentatum, in the globus pallidus, in the amygdala, and in the medial, dorsal, and dorsomedial cortex, just as in the piriformis cortex (Figs. 3–8). In the olfactory bulb, MCH fibers, often very thin, were present in all layers but the glomerular layer (Fig. 2). In the posterior brain structures, processes spread in the pretectal areas, in most layers of the optic lobes (Figs. 9–12 and 22), and in all parts of the brain stem. They were abundant in the isthmus area (except in the isthmus ganglia) and particularly in the roof of the third ventricle under the decussation of the trochlear nerve (Fig. 12). In the medulla oblongata, they were located mainly in the reticular matter (Figs. 13 and 14). They were few in number and very thin in the intermediolateralis gray, lateral funiculi, and sometimes

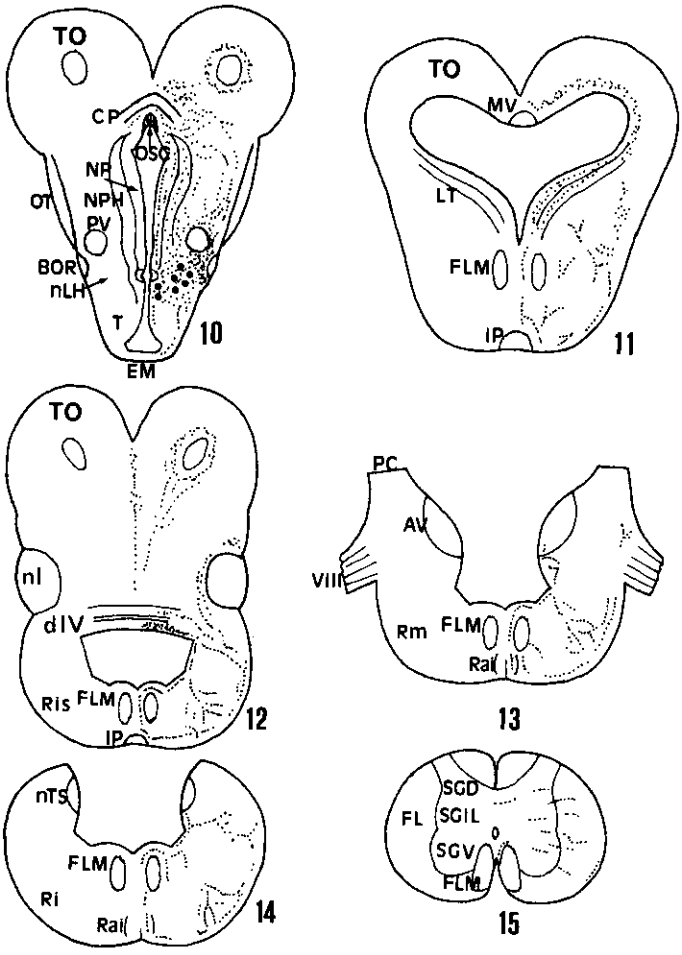


FIG. 10. Section through the optic lobes and post-diencephalon (MCH-perikarya: large dots).

FIG. 11. Section through the caudal mesencephalon (tectum opticum) and the mesencephalic tegmentum.

FIG. 12. Section at the level of the isthmus area and tegmentum.

FIG. 13. Section through the rostral rhombencephalon. The cerebellum, which does not contain MCH-fibers, is not represented.

FIG. 14. Section through the midrhombencephalon (medulla oblongata).

FIG. 15. Section through the cervical cord.

in the ventral horn of the cervical medulla (Fig. 15). They were not observed in the more distal parts of the medulla.

Close contacts suggesting the existence of synapses between MCH processes and/or terminals with perikarya were frequently observed, but these contacts were most striking in the septal nuclei (Figs. 31–33).

On serial sections AS to sMCH, rMCH, GRF37, and NEI stained the same perikarya. The stainings obtained with GRF37 and NEI sera were less intense and more granular than those observed with MCH AS (Figs. 24–29). The brain areas containing many MCH processes were also rich in GRF37 and NEI fibers. Moreover, double-staining experiments also confirmed the coexistence of MCH with NEI or GRF37 immunoreactivities. This coexistence was also evident in the septal areas, in

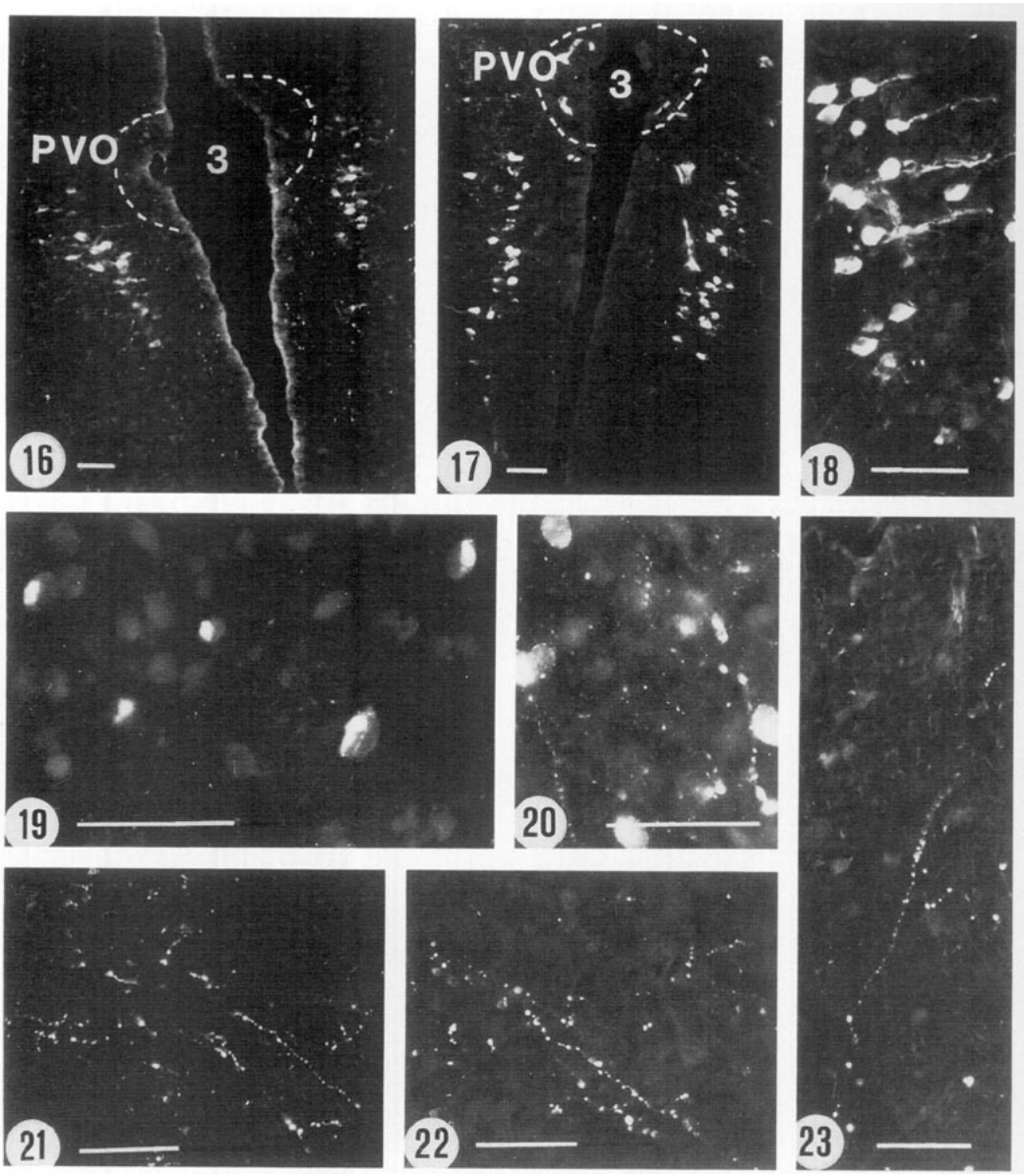


FIG. 16. Perikarya in the periventricular nucleus of the turtle hypothalamus, stained by MCH-AS (PVO): paraventricular organ; 3: Third Ventricle). Frontal section.

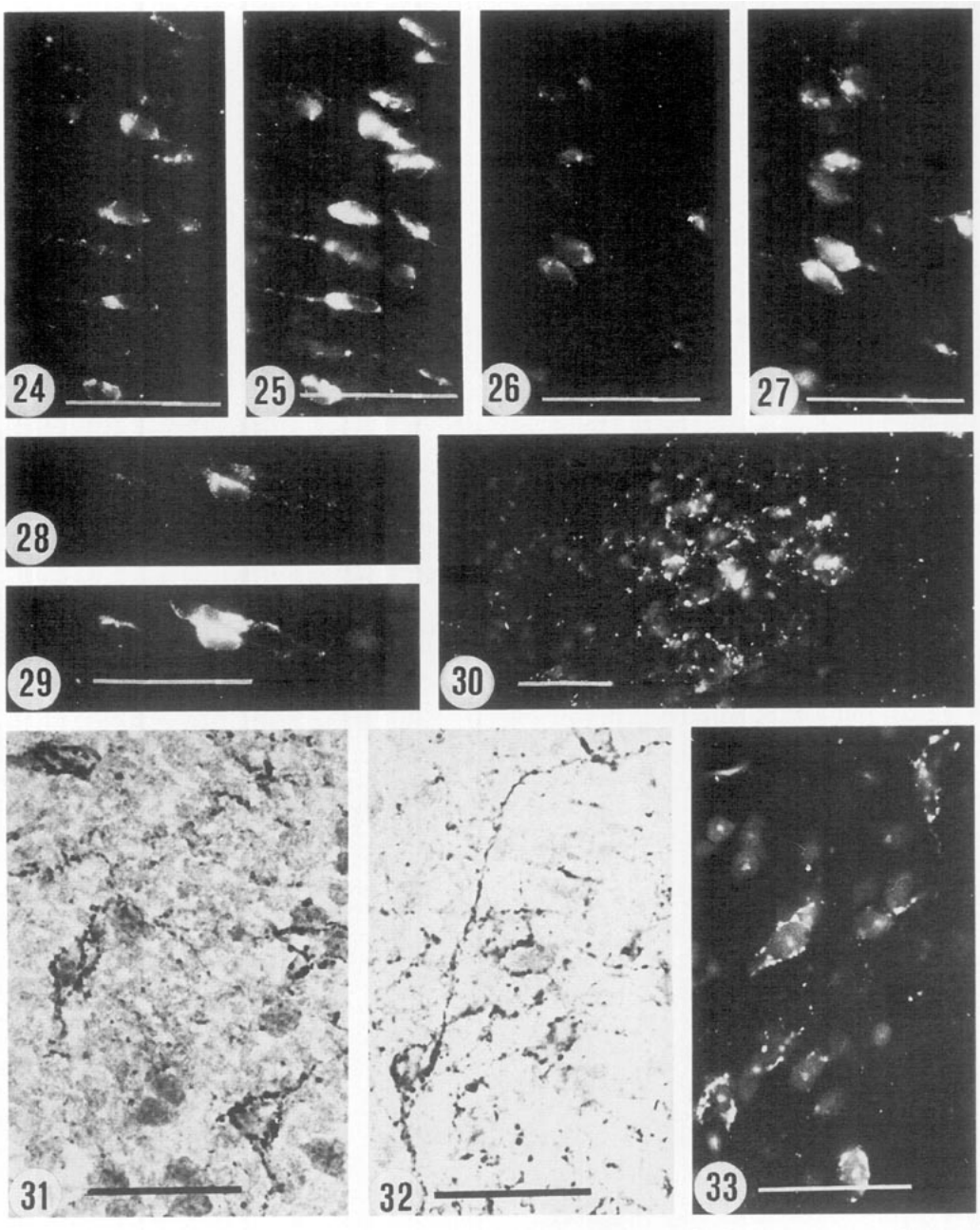
FIG. 17. MCH perikarya in periventricular nucleus of the lizard hypothalamus. Frontal section.

FIG. 18. MCH perikarya and lateral immunoreactive processes in the turtle hypothalamus. Frontal section.

FIGS. 19 AND 20. MCH perikarya in the lateral hypothalamus of the snakes *Natrix natrix* (Fig. 19) and *Natrix maura* (Fig. 20).

FIGS. 21 AND 22. MCH processes in the lateral hypothalamus and in the optic lobes (Turtle brain).

FIG. 23. Long MCH processes extending toward the thalamus (Turtle brain, frontal section).



FIGS. 24 AND 25. The same perikarya of the turtle periventricular hypothalamic nucleus are stained by GRF 37 (Fig. 24) and then by sMCH antisera (Fig. 25) (FITC).

FIGS. 26 AND 27. Successive NEI (Fig. 26) and sMCH (Fig. 27) stainings of the same perikarya in the turtle hypothalamus (FITC and TRITC).

FIGS. 28 AND 29. Successive GRF37 (Fig. 28; FITC) and sMCH (Fig. 29; TRITC) immunolocalization show distinct intracellular patterns of staining.

FIG. 30. Abundant sMCH innervation of the turtle septum (frontal section).

FIGS. 31–33. Pericellular basket in the turtle septum as seen with sMCH, GRF37 (immunoperoxidase) and NEI-AS (immunofluorescence; FITC). Scale bar: 5 μm.

which MCH processes formed heavy basket-like terminals around unstained perikarya (Figs. 31–33). We never observed α-MSH, NGE, and MCH immunoreactivities in the same neurons.

The results obtained in the turtle brain were also observed in the lizard (Fig. 17). In the investigated animals, MCH neurons of the periventricular nucleus were strongly immunoreactive. Conversely, lateral hypothalamic neurons were more intensely stained in the examined snakes (Figs. 19 and 20).

DISCUSSION

The present results show that, in the reptile brain, the pattern of hypothalamic perikarya producing a peptide immunologically related to fish MCH is similar to that described in mammals. They also suggest that putative peptides derived from the MCH precursor would mostly act as neuroregulators but not as hormone-like MCH in fish since (i) there is no significant MCH innervation in the median eminence and in pituitary neural lobe and (ii) extra-hypophyseal processes and terminals are very abundant.

Intracellular patterns of GRF37 and NEI-immunoreactivities differ from those obtained with MCH antibodies; however, we could not assign these stainings to a distinct intracellular compartment corresponding to maturational stages of the precursors as in mammals (Risold *et al.*, 1989). Although our results provide no information about the extent of post-translational cleavage or the number of cleavage products that are released, this precursor presents important immunological homologies with the rat/human pre-pro-MCH (Breton *et al.*, 1989; Nahon *et al.*, 1989a,b; Parkes and Vale, 1992).

The dramatic extension of the immunoreactive processes in the optic lobes and especially in the olfactory telencephalon (which corresponds to the primitive telencephalon) is similar to that observed in cyclostomes (Baker, 1991), elasmobranches (personal results), teleost fishes (Naito *et al.*, 1985; Batten and Baker, 1988, and personal observations), amphibia: urodeles and anurans (Baker, 1991, and personal results), as well as in birds (in preparation) and mammals. In all these cases, these relationships are probably involved in the sensorimotor modulation of the arousal behavior and in different adaptation conditions such as stress as already stated in fishes (see Baker, 1991) but also in the rat (Jezovā *et al.*, 1992).

REFERENCES

Baker, B. I. (1988). Melanin concentrating hormone. *In* "The Melanotropic Peptides" (M. E. Hadley, Ed.), Vol. 2, 159–174. CRC Press, Boca Raton, FL.

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

Batten, T. F. C., and Baker, B. I. (1988). Melanin concentrating hormone (MCH)-immunoreactive hypophysial neurosecretory system in the teleost *Poecilia latipinna*, light and electron microscopic study. *Gen. Comp. Endocrinol.* **70**, 193–205.

Bresson, J. L., Clavequin, M. C., Fellmann, D., and Bugnon, C. (1989). Human hypothalamic neuronal system revealed with a salmon melanin-concentrating hormone (MCH) antiserum. *Neurosci. Lett.* **102**, 39–43.

Breton, C., Fellmann, D., and Bugnon, C. (1989). Clonage et séquençage d'ADNc du précurseur de trois neuropeptides hypothalamiques immunologiquement apparentés à la somatocrinine humaine 1-37, à l'α-mélanotropine et à l'hormone de mélano-concentration du saumon. *C. R. Acad. Sci. Paris Ser. 3* **309**, 749–754.

Daikoku, S., Okamura, Y., Kowano, H., Tsuruo, Y., Maegawa, M., and Shibasaki, Y. (1985). CRF-containing neurons of the rat hypothalamus. *Cell Tissue Res.* **240**, 575–584.

Eberlé, A. N. (1988). Melanin concentrating hormone. *In* "The Melanotropins" (A. N. Eberlé, Ed.), pp. 321–332. Karger, Basel.

Fellmann, D., Bresson, J. L., Breton, C., Bahjaoui, M., Rouillon, A., Gouget, A., and Bugnon, C. (1989a). Cloning of cDNAs encoding a rat neuropeptide immunologically related to salmon melanin-concentrating hormone. *Neurosci. Lett.* **106**, 23–28.

Fellmann, D., Breton, C., Bahjaoui, M., and Bugnon, C. (1989b). Hybridation spécifique d'un oligonucléotide de synthèse dans les neurones hypothalamiques du rat immunoréactifs à un immunsérum dirigé contre l'hormone de mélanoconcentration du saumon. *C. R. Acad. Sci. Paris Ser. 3* **309**, 631–636.

Fellmann, D., Bugnon, C., and Lavry, G. N. (1985). Immunohistochemical demonstration of a new neuron system in rat brain using antibodies against human growth hormone-releasing factor (1-37). *Neurosci. Lett.* **58**, 91–96.

Fellmann, D., Bugnon, C., Verstegen, J., and Lavry, G. N. (1986). Coexpression of human growth hormone-releasing factor 1-37-like and α-melanotropin-like immunoreactivities in neurones of the rat lateral dorsal hypothalamus. *Neurosci. Lett.* **68**, 122–126.

Ježová D., Bartanusz, V., Westergren, I., Johansson, B. B., Rivier, J., Vale, W., and Rivier, C. (1992). Rat melanin-concentrating hormone stimulates adrenocorticotropin secretion: Evidence for a site of action in brain regions protected by the blood-barrier. *Endocrinology* **130**, 1024–1029.

Kawauchi, H., Kawazoe, I., Tsubokawa, M., Kishida, M., and Baker, B. I. (1983). Characterization of melanin-concentrating hormone in chum salmon pituitaries. *Nature* **305**, 324–323.

Merchenthaler, I., Maderdrut, J. L., Altschuler, R. A., and Petrusz, P. (1986). Immunocytochemical localization of proenkephalin-derived peptides in the central nervous system of the rat. *Neuroscience* **17**, 325–348.

Minth, C. D., Qiu, H., Akil, H., Watson, S. J., and Dixon, J. E. (1989). Two precursors of melanin-concentrating hormone: DNA sequence analysis and *in situ* and immunochemical localization. *Proc. Natl. Acad. Sci. USA* **86**, 4292–4296.

McLean, I. W., and Nakane, P. K. (1974). Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.* **22**, 1077–1083.

Nahon, J. L., Presse, F., Bittencourt, J. C., Sawchenko, P. E., and Vale, W. (1989a). The rat melanin-concentrating hormone messenger ribonucleic acid encodes multiple putative neuropeptides coexpressed in the dorsolateral hypothalamus. *Endocrinology* **125**, 2056–2065.

Nahon, J. L., Presse, F., Vaughan, J., Fischer, W., Bittencourt, J., Hoeger, C., Schoepfer, R., Rivier, J., Sawchenko, P., and Vale, W. (1989b). Characterization of mammalian melanin-concentrating hormones and their precursors. *In* "Recent Advances in Basic and Clinical Neuroendocrinology" (F. F. Casanueva and C. Dieguez, Eds.), Vol. 864, pp. 15–23. Elsevier, New York.

Naito, N., Kawazoe, I., Nakai, Y., and Kawauchi, H. (1988). Melanin-concentrating hormone-like immunoreactive material in the rat hypothalamus, characterization and subcellular localization. *Cell Tissue Res.* **253**, 291–295.

Naito, N., Kawazoe, I., Nakai, Y., Kawauchi, H., and Hirano, T. (1986). Coexistence of immunoreactivity for melanin-concentrating hormone and α-melanocyte-stimulating hormone in the hypothalamus of the rat. *Neurosci. Lett.* **70**, 81–85.

Naito, N., Nakai, Y., Kawauchi, H., and Hayashi, Y. (1985). Immunocytochemical identification of melanin-concentrating hormone in the brain and pituitary gland of the teleost fishes *Oncorhynchus keta* and *Salmo gairdneri*. *Cell Tissue Res.* **242**, 41–48.

Ono, M., Wada, C., Oikawa, I., Kawazoe, I., and Kawauchi, H. (1988). Structures of two kinds of mRNA encoding the chum salmon melanin-concentrating hormone. *Gene* **71**, 433–438.

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

Presse, F., Nahon, J. L., Fisher, W. H., and Vale, W. (1990). Structure of the human melanin concentrating hormone mRNA. *Mol. Endocrinol.* **4**, 632–637.

Reiner, A., Krause, J. E., Keyser, K. T., Eldred, W. D., and McKelvy, J. F. (1984). The distribution of substance P in turtle nervous system. A radioimmunoassay and immunohistochemical study. *J. Comp. Neurol.* **226**, 50–75.

Risold, P. Y., Fellmann, D., Lenys, D., and Bugnon, C. (1989). Coexistence of acetylcholinesterase, human growth hormone-releasing factor (1-37), α-melanotropin and melanin-concentrating hormone-like immunoreactivities in neurons of the rat hypothalamus: A light and electron microscope study. *Neurosci. Lett.* **100**, 23–28.

Risold, P. Y., Fellmann, D., Rivier, J., Vale, W., and Bugnon, C. (1992). Immunoreactivities for antisera to three putative neuropeptide of the rat melanin-concentrating hormone precursor are coexpressed in neurons of the rat lateral dorsal hypothalamus. *Neurosci. Lett.* **136**, 145–149.

Schmued, L. C., Swanson, L. W., and Sawchenko, P. E. (1982). Some fluorescent counterstains for neuroanatomical studies. *J. Histochem. Cytochem.* **30**, 123–128.

Skofitsch, G., Jacobowitz, D. M., and Zamir, N. (1985). Immunohistochemical localization of a melanin-concentrating hormone-like peptide in the rat brain. *Brain Res. Bull.* **15**, 635–649.

Tramu, G., Pillez, A., and Leonardelli, J. (1978). An efficient method of antibody elution for the successive or simultaneous localization of two antigens by immunocytochemistry. *J. Histochem. Cytochem.* **26**, 322–324.

Watson, S. J., and Akil, H. (1979). The presence of two α-MSH positive cell groups in the rat hypothalamus. *Eur. J. Pharmacol.* **48**, 101–105.

Vaughan, J. M., Fischer, W. H., Hoeger, C., Rivier, J., and Vale, W. (1989). Characterization of melanin-concentrating hormone from rat hypothalamus. *Endocrinology* **125**, 1660–1665.

Weindl, A., Triepel, J., and Kuchling, G. (1984). Somatostatin in the brain of the turtle *Testudo hermani* Gmelin. An immunohistochemical mapping study. *Peptides* **5**, 91–100.