Mapping of α-Melanocyte-Stimulating Hormone-Like Immunoreactivity in the Cat Diencephalon

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COVEÑAS, R., M. DE LEÓN, J. A. NARVÁEZ, G. TRAMU, J. A. AGUIRRE AND S. GONZÁLEZ-BARÓN. Mapping of α-melanocyte-stimulating hormone-like immunoreactivity in the cat diencephalon. PEPTIDES 17(5) 845-852, 1996.—Using an indirect immunoperoxidase technique, we studied the location of α-melanocyte-stimulating hormone-like fibers and cell bodies in the cat diencephalon. In the thalamus, almost all the immunoreactive fibers were found in the midline region, whereas in the hypothalamus immunoreactive fibers were observed in the whole structure. The hypothalamus showed a higher density of both immunoreactive fibers and cell bodies; no immunoreactive neurons were found in the thalamus. The densest network of immunoreactive fibers was observed in the epithalamus (nucleus periventricularis anterior) and in the hypothalamic nuclei filiformis, hypothalami ventromedialis, arcuatus, periventricularis hypothalami, area hypothalami ventromedialis, and hypothalamus posterior. A high density of immunoreactive neurons was found in the nucleus arcuatus, in the hypothalamus lateralis, and in the area hypothalami ventromedialis and in the hypothalamus dorsomedialis. By comparison with the studies of previous researchers, these data showed a more widespread distribution of α-melanocyte-stimulating hormone-like immunoreactive fibers and perikarya in the feline hypothalamus. Moreover, our findings indicate that the peptide is widely distributed in the cat diencephalon, suggesting that α-melanocyte-stimulating hormone might be involved in several physiological functions.

α-Melanocyte-stimulating hormone  Mapping  Immunocytochemistry  Diencephalon  Cat

It is known that pro-opiomelanocortin is the precursor of opiate (α-, β-, and γ-endorphin) and nonopiate (adrenocorticotropin hormone, β-lipotropin, and α-melanocyte-stimulating hormone) peptides (20). Only scarce data are available on the distribution of immunoreactive structures containing such peptides in the cat CNS. In the feline, only the distribution of β-endorphin and α-melanocyte-stimulating hormone (α-MSH) in the hypothalamus (25) and the presence of β-lipotropin in the brain stem (15) have been described. In addition, the distribution of α-melatonin in several regions of the cat brain has been described using radioimmunoassay techniques (27). Further, α-MSH has been reported to be involved in several physiological roles, such as hyperalgesia, cardiovascular and neuroendocrine regulation, thermoregulation, stress, learning, memory, sexual behavior, grooming, and in attentive, aggressive, and defensive behavior (21). However, no previous information appears to be available in the literature concerning the distribution of immunoreactive structures containing α-MSH in the cat thalamus. The aims of this work are: 1) to study the distribution of fibers and cell bodies containing such peptide in the cat thalamus; 2) to reexamine the distribution of fibers and perikarya containing α-MSH in the cat hypothalamus; and 3) to compare our findings both with previous studies reported on the presence of α-MSH in the diencephalon of other species (e.g., rat) and with the distribution of several peptides previously described in the cat diencephalon.

METHOD

Five male adult cats (2-3 kg body weight), obtained from commercial sources, were used in this study. Three animals, under deep ketamine (40-50 mg/kg, IP) anesthesia, received unilateral IV (lateral ventricle) injections of colchicine (300 μg of

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FIG. 1. Distribution of α-MSH-IR fibers and cell bodies in frontal planes of the diencephalon of the cat corresponding to the posteroanterior stereotaxic plane levels A 7.5 to A 13.5 of the Jasper and Ajmone-Marsan (18) stereotaxic atlas. The anteriority (A) (in mm) with respect to the zero stereotaxic point of each section is indicated at the lower left. The distribution of α-MSH-IR structures presented in this figure is based on the results obtained from both control and colchicine-treated cats. Cell bodies containing α-MSH have been indicated by dots (1 dot = 9 immunoreactive neurons). The density of such immunoreactive neurons has been considered as high (>20 cell bodies/section), middle (10–20 cell bodies/section), and low (<10 cell bodies/section), whereas α-MSH-IR fibers are represented by continuous lines. Examples of moderate and low density of immunoreactive fibers are observed, respectively, in Fig. 3(C) and 3(E). AD: nucleus anterior dorsalis, AHD: area hypothalamiæ dorsalis, AM: nucleus anterior medialis, ARC: nucleus arcuatus, AV: nucleus anterior ventralis, CL: nucleus centralis lateralis, CM: nucleus centrum medianum, FIL: nucleus filiformis, FX: fornix, GL: corpus geniculatum laterale, GLV: pars ventralis of the corpus geniculatum laterale, GM: corpus geniculatum mediale, HA: hypothalamus anterior, HBL: nucleus habenularis lateralis, HBM: nucleus habenularis medialis, HDM: hypothala-
the drug diluted in 5 μl of saline solution). Two days after the administration of the drug, both the treated and untreated (control) animals were deeply anesthetized with ketamine, heparinized, and perfused transcardially with 500 ml of cold 0.9% NaCl. This perfusion was followed immediately by the fixative, 3 l of cold 4% paraformaldehyde, in 0.15 M phosphate-buffered saline (PBS) (pH 7.2). The brains were removed and the diencephalons dissected out and placed in the same fixative for 12 h at 4°C. After this post-fixation, diencephalons were cryoprotected by immersion in increasing sucrose baths (10–30%) until they sank. With the use of a cryostat, 80-μm frontal frozen sections were cut, collected into PBS, and kept at 4°C. Six of seven free-floating sections were processed for immunostaining, in common with previously described studies (2,5,8,9,34), and the remaining section was stained for Nissl.

The antiserum was raised in rabbits against immunogens prepared by coupling the peptide, synthetic human α-MSH, to a carrier protein (human serum albumin) with glutaraldehyde. The specificity of the immunostaining was controlled as follows: a) the preabsorption of the first antiserum with synthetic α-MSH (100 μg per ml of diluted antiserum at 1:1000): b) omitting the α-MSH antiserum in the first incubation bath (in both cases, no residual immunoreactivity was found); c) no significant reduction in the immunolabeling was found when α-MSH antiserum was preabsorbed with an excess (10^-7 M) of synthetic α-, β-, and γ-endorphin, β-lipotropin, α-melanocyte-stimulating hormone, β- and γ-MSH, methionine- and leucine-enkephalin, dynorphin A, α-neo-endorphin, or dynorphin B; d) possible interference by endogenous peroxidases was ruled out by staining some sections with the preabsorption of the first antiserum with synthetic α-MSH 100 pg per ml of diluted antiserum at 1:1000 (10-20 μm). By contrast, in the area hypothalami dorsalis and in the hypothalami lateralis immunoreactive cell bodies were large (20-35 μm) and round or fusiform, and showed long processes.

Diencephalic–Mesencephalic Junction

This junction is located from anteriority (A) 3.0 to A 7.0 (not shown in Fig. 1). A moderate density of immunoreactive fibers was observed in the colliculus superior and in the griseum centrale. A low density in the substantia reticularis mesencephalica, praetectum, nuclei interpeduncularis, and commissurae posteriores, along the midline and surrounding the nucleus ruber. Single fibers were seen in the nuclei of Edinger Westphal, of Darkschewitsch, and of ruber. At this level no α-MSH-IR cell bodies were observed.

Thalamus

The epithalamus (from A 6.0 to A 13.5) showed the highest immunoreactivity. A moderate density of α-MSH-IR fibers was observed in the nucleus periventricularis anterior (its caudal part) [Figs. 1(A–D), 2(A)]. A low density was observed in the nuclei habenulares lateralis [Fig. 1(A, B)], in the habenulares medialis [Fig. 1(A, B)], and in the most rostral part of the nucleus periventricularis anterior [Fig. 1(E, F)]. In the midline or central group (from A 8.0 to A 12.5), we observed a low density of immunoreactive processes in all the nuclei included in this group (rhomboidensi, reuniens, centralis medialis, interventricularis, and interanteromedialis) [Figs. 1(B–E), 2(B, C)]. In the medial group (from A 6.5 to A 13.0), a low density of immunoreactive fibers was found in the nuclei medialis dorsalis [Figs. 1(A–D); 2(D)] and single fibers in the nucleus parataenialis [Figs. 1(C–E), 2(E)]. In the intralaminar group (from A 6.5 to A 11.0), a low density of fibers containing α-MSH was observed in the nuclei parafascicularis, subparafascicularis [Fig. 1(A)], and centralis medius [Fig. 1(A)]. No immunoreactive fibers were found in the nuclei paracentralis [Fig. 1(B, C)] and centralis lateralis [Fig. 1(B, C)]. Further, no immunoreactive fibers were found in either the ventral group (from A 7.0 to A 12.5).

Hypothalamus

In the zona incerta (from A 7.5 to A 13.5), we observed a moderate density of immunoreactive fibers was found in the zona incerta dorsalis [Figs. 1(A, C), 2(F)]. Single fibers were observed in the nuclei anterior dorsalis [Fig. 1(D, E)] and anterior ventralis [Fig. 1(D, E)]. The nucleus anterior medialis [Fig. 1(D, E)] was devoid of immunoreactivity. Regarding the ventral thalamus (from A 7.5 to A 13.5), a low density of fibers containing α-MSH was found in the zona incerta [Fig. 1(A–C)], whereas no immunoreactive fiber was observed in the nucleus reticularis [Fig. 1(A–F)]. Finally, no α-MSH-IR cell bodies were observed in the cat thalamus.

Hypothalamus

In the mamillary region (from A 8.0 to A 9.5), a low density of immunoreactive fibers was observed in the corpus mamillare [Fig. 1(B, C)] and in the nucleus mamillaris lateralis [Fig. 1(B, C)]. In the periventricular region (from A 11.5 to A 13.5), the nucleus arcuatus [Figs. 1(D, E), 3(A)] and the region located near the ventricle [Fig. 1(D–F)] showed a moderate density of α-MSH IR fibers. In the medial region (from A 8.5 to A 14.5), a moderate density of immunoreactive fibers was observed in the area hypothalami dorsalis [Fig. 1(D)], in the hypothalami posterior (its rostral part) [Fig. 1(C)], and in the nucleus filamentum [Fig. 1(D, E)], hypothalani ventromedialis [Figs. 1(D), 3(A)], and periventricularis hypothalami [Figs. 1(E, F), 3(C)].

By contrast, a low density was found in the nucleus suprachiasmaticus [Figs. 1(F), 3(D)]. Hypothalami dorsomedialis [Figs. 1(E, F), 3(B)], hypothalami posterius (its caudal part) [Fig. 1(B)], and hypothalami anterior [Fig. 1(F)]. No immunoreactive fibers were observed in the nucleus supraopticus [Fig. 1(D–F)]. In the lateral hypothalamus (from A 9.0 to A 13.0) and in the praeoptica area (from A 14.0 to A 15.0), a low density of immunoreactive fibers was found in the hypothalami lateralis [Fig. 1(C–E)] and in the regio praeoptica [Fig. 3(E)]. Moreover, α-MSH-IR fibers were observed in the fornix [Fig. 1(D–F)] and in the median forebrain bundle [Fig. 1(C–E)]. Finally, a high density of α-MSH IR cell bodies was observed in the hypothalami lateralis (from A 10.0 to A 13.0) [Fig. 1(D, E)]. This high density extended ventromedially from this region to the nucleus arcuatus (from A 10.0 to A 13.0). In the most ventral part of the area hypothalami dorsalis (from A 10.0 to A 11.5) [Fig. 1(D)], in the nucleus arcuatus (from A 11.5 to A 13.0) [Figs. 1(D, E), 3(A)], and around the fornix (from A 10.5 to A 13.0) [Fig. 3(F)]. In contrast, a low density of α-MSH-IR fibers was found in the most dorsal part of the hypothalami dorsomedialis (A 12.5) [Figs. 1(E), 3(B)] and in the ventromedial part of the nucleus hypothalami ventromedialis (from A 11.0 to A 12.0) [Figs. 1(D), 3(A)].

DISCUSSION

Comparison of the Distribution of α-MSH in the Mammalian Diencephalon

We have described in detail, for the first time, the distribution of α-MSH-IR fibers in the cat hypothalamus. In comparison with a previous study carried out by Micevych and Elde (25) on the distribution of α-MSH-IR fibers and cell bodies in the cat hypothalamus, the distribution of α-MSH-IR structures observed in our study is in general more widespread. Thus, we observed immunoreactive fibers in the hypothalamus posterior, area hypothalamic dorsalis, nucleus filiformis, nucleus periventricularis hypothalami, hypothalami anterior, nucleus suprachiasmaticus, and the regio praeoptica, in none of which did Micevych and Elde (25) observe α-MSH-IR fibers. These same authors (25) found α-MSH-IR cell bodies in the hypothalami lateralis, area hypothalami dorsalis, and the nucleus arcuatus, as we have observed in our own study. The same authors (25) demonstrated that in the cat β-endorphin and α-MSH coexist in neurons located in the nucleus arcuatus and that the neuronal population containing α-MSH located in the dorsolateral hypothalamus is devoid of other components of the pro-opiomelanocortin precursor. We have described a more widespread distribution of α-MSH-IR perikarya in the cat hypothalamus: we found α-MSH-IR cell bodies in the hypothalami dorsomedialis, around the fornix, and in the nucleus hypothalami ventromedialis, where Micevych and Elde (25) did not find perikarya containing α-MSH. These immunoreactive cell bodies only showed α-MSH immunoreactivity, being devoid of β-endorphin or adrenocorticotropic hormone. The discrepancies in the distribution of α-MSH-IR fibers and cell bodies between our study and those carried out by Micevych and Elde (25) could be due to technical considerations, particularly to the different antisera used: except for the antiserum, the methodology used in both studies was quite similar.

Our findings in the cat hypothalamus are generally consistent with the microdissection radioimmunoassay study reported by O’Donohue et al. (27) in the cat brain. However, in the thalamus there are great differences, because these authors (27) only described α-MSH in the thalamic nuclei anterior ventralis, periventricularis anterior, and anterior medialis. Except in the latter, we have similarly described α-MSH-IR fibers in such nuclei, as well as in other thalamic nuclei (e.g., pulvinar, lateralis posterior, lateralis dorsalis, centralis medialis). In sum, our study has shown a more widespread distribution of α-MSH in the cat thalamus than that described by O’Donohue et al. (27).

Finally, our results are generally in agreement with the findings described by several authors on the distribution of α-MSH-IR structures in the rat diencephalon (3,12,17,21,24,28,30,31).

Anatomical Relationship of α-MSH With Other Neuropeptides in the Cat Diencephalon

The anatomical distribution of several peptides (vasoactive intestinal polyepitope, substance P, neurokinin A, neuropeptide Y, methionine-enkephalin, cholecystokinin, neotensin, and somatostatin) has been studied in the cat diencephalon (2,4–9,22,26,34,36). In general, cell bodies containing the above-mentioned neuropeptides were more broadly distributed in the cat diencephalon than were those containing α-MSH. By contrast, on comparing the distribution of α-MSH-IR fibers with those diencephalic regions containing the above-mentioned eight peptides (except the vasoactive intestinal polyepitope), it appears that the distribution is quite similar. Thus, for example, α-MSH and all the mentioned peptides (except the vasoactive intestinal polyepitope) have been observed in fibers in several diencephalic nuclei of the cat (e.g., habenularis lateralis, periventricularis anterior, centralis medialis, hypothalami ventromedialis, hypothalami posterior). These data suggest possible interactions among some of the eight cited peptides in the diencephalon of the cat. For example, an interaction between α-MSH and neuropeptide Y could be possible, because in addition to the
anatomical relationship of both peptides shown in the cat dien-cephalon, it has been pointed out that neuropeptide Y may act as an MSH release-inhibiting factor (35). Also, the regulation of pro-opiomelanocortin gene expression by neuropeptide Y has been described in the nucleus arcuatus (10).

Finally, the coexistence of neuropeptide Y and α-MSH can be suggested in cell bodies located in the nucleus arcuatus, because the morphological characteristics of perikarya containing α-MSH were similar to the neuronal population containing neuropeptide Y (8).

Possible α-MSH-Containing Pathways in the Cat

In the cat hypothalamus, we still do not know whether the α-MSH-IR neurons are intrinsic or extrinsic. However, according
FIG. 3. α-MSH-IR cell bodies and fibers in the cat hypothalamus. (A) Anteriority 11.5. Immunoreactive cell bodies (arrows) and fibers (arrowheads) in the nucleus hypothalami ventromedialis (NHVM) and in the nucleus arcuatus (ARC). V: ventricle. (B) Anteriority 12.5. Immunoreactive perikarya (arrows) and fibers (arrowheads) in the hypothalamus dorsomedialis. D: dorsal; M: medial. (C) Anteriority 12.0. Fibers containing α-MSH (arrowheads) in the nucleus periventricularis hypothalami. V: ventricle. (D) Anteriority 14.5. Immunoreactive fibers (arrowheads) in the nucleus suprachiasmaticus. CH: chiasma opticum. (E) Anteriority 14.0. α-MSH-IR fibers (arrowheads) in the regio praeoptica. V: ventricle. (F) Anteriority 10.5. Immunoreactive cell bodies (arrows) around the fornix (FX). Scale bar: 100 μm.

to the morphological data observed in the feline, it appears that, as with the rat (21,23), the α-MSH-IR neurons located in the nucleus arcuatus have projections throughout the epithalamus, thalamic medial group, hypothalamus anterior, and preoptic area. In the feline, a high density of perikarya containing α-MSH was found in the nucleus arcuatus, and the diencephalic regions mentioned above only showed α-MSH-IR fibers; they were devoid of immunoreactive cell bodies. Moreover, it is also known that the rat griseum centrale receives α-MSH afferents arising from cell bodies located in the hypothalamus lateralis (3). This could
also occur in the cat, inasmuch as we observed a high and low
density, respectively, of immunoreactive cell bodies and fibers
in the hypothalamus lateralis and a moderate density of fibers in
the griseum centrale. This mesencephalic region was devoid of
α-MSH-IR neurons. In addition, α-MSH-IR cell bodies located in
the hypothalamus lateralis might send fibers to the parabrachial
nuclei, locus coeruleus, nucleus of the solitary tract, nucleus me-
dialis dorsalis, and nucleus habenularis lateralis. In these brain
stem and thalamic nuclei we observed fibers containing α-MSH
but no immunoreactive cell bodies. This observation is in agree-
ment with previous works carried out in the cat, in which, using
horseradish and autoradiographic techniques, researchers de-
scribed pathways from the hypothalamus lateralis to the five
brain areas mentioned above (16,29).

Possible Physiological Functions of α-MSH in the Cat
Diencephalon

The presence of α-MSH at multiple sites in the cat thalamus
and hypothalamus implies that the peptide serves different func-
tions at these sites. Moreover, the location of α-MSH in fibers
(data suggesting that the peptide is released from the hypothal-
amus) (23) and the behavioral effects produced by α-MSH when
it is administered (19) indicate that α-MSH plays a role as neu-
rotransmitter and/or neuromodulator in the central nervous sys-
tem. The presence of α-MSH-IR fibers in the mediodorsal thal-
amus, dorsomedial hypothalamus, and the nuclei hypothalam-
ventromedialis, suprahypothalamus, and periventricular hypoth-
alamus indicates that α-MSH might be involved in vigilance and
attentive behavior, regulation of thermogenesis, control of cir-
cadian rhythms, feeding and affective defense behavior, visual
processes, stress, and cardiovascular mechanisms (1,11,
13,14,32,33).

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