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Immunocytochemical Study of Enkephalin-Like Cell Bodies in the Thalamus of the Rat

R. COVEÑAS,*[1] J. R. ALONSO* AND M. CONRATH†

**Citología e Histología, Facultad de Biología, Salamanca, Spain*
†Département de Cytologie, Institut des Neuroscience, Université P. et M. Curie, URA 09, Paris, France

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COVEÑAS, R., J. R. ALONSO AND M. CONRATH. *Immunocytochemical study of enkephalin-like cell bodies in the thalamus of the rat.* BRAIN RES BULL **23**(4/5) 277–281, 1989.—The distribution of enkephalin-like cell bodies in the thalamus of the rat was studied by means of intratissular injections of colchicine and using an indirect immunoperoxidase technique. The densest clusters of immunoreactive perikarya were observed in the nuclei geniculatum lateralis ventralis, medialis dorsalis, centralis lateralis, centralis medialis and anterior ventralis. Whereas the nuclei praetectalis lateralis, lateralis posterior, habenularis lateralis, parataenialis (its caudal part), parafascicularis, centrum medianum, reuniens and ventralis medialis had the lowest density. In other thalamic nuclei geniculatum mediale, paraventricularis and parataenialis (its rostral part) the density of enkephalin-like cell bodies was intermediate. These results suggest that the intratissular injection of colchicine is the better way of administration of the drug in order to study the distribution of peptidergic cell bodies in the mammalian CNS. The similarities and differences found in the distribution of enkephalinergic cell populations in the thalamus of different mammals are discussed.

Enkephalin Immunocytochemistry Rat Thalamus Cell bodies

SEVERAL immunocytochemical studies have been carried out on the distribution of enkephalins in the CNS of mammals (7, 9, 12–20). In order to facilitate the visualization of enkephalinergic perikarya, animals were pretreated with colchicine, administrated subcutaneously (7,18), intracisternally (12) or intraventricularly (7, 9, 13, 15–17, 19). Thus, enkephalin immunoreactive cell bodies have been observed in the rat and monkey thalamus in nuclei localized near the ventricles such as reuniens, centrum medianum, parafascicularis, habenularis lateralis and medialis, paraventricularis anterior, parataenialis and pretectum. In nuclei located far from the ventricles, e.g., geniculatum laterale and geniculatum mediale, enkephalin-like perikarya were also observed. In general, in almost all these thalamic nuclei, particularly in those located far from the ventricles the density of immunoreactive perikarya was rather low.

We have recently developed the use of systematic intratissular injections of colchicine to reveal enkephalin-like cell bodies of the cat thalamus and hypothalamus (3,4). This route of administration of the drug led to the demonstration of a greater density of enkephalin-like cell bodies and to a larger distribution in the different thalamic nuclei, compared to the results in rat and monkey thalamus obtained with intraventricular or subcutaneous injections of colchicine (see the references above).

However, few studies of the literature concerned the cat thalamus; it was thus questionable to know whether the way of administration of the drug was responsible for the differences observed between our study (4) and previous data or might be attributed to species variations.

In order to elucidate this question, a reexamination of the distribution of enkephalin-like cell bodies in the rat thalamus was carried out after intratissular injections of colchicine in different nuclei of the thalamus and neighbouring regions. A cartography of enkephalin-like cell bodies was thus established and compared to those of other mammalians.

METHOD

Twenty-seven adult male Sprague-Dawley rats (200–250 g body weight) were pretreated with colchicine under deep ketamine anaesthesia (50 mg/kg). The drug was injected intraparenchymally (80–150 μg in 1.5–3 μl of distilled water) or intraventricularly (300 μg in 5 μl of distilled water) according to the stereotaxic atlas of Albe-Fessard *et al.* (1) in the following nuclei: praetectalis lateralis (2 rats), geniculatum mediale (1 rat), praetectalis medialis (1 rat), lateralis posterior (2 rats), geniculatum lateralis dorsalis (1 rat), centrum medianum (2 rats), ventralis posterior (1 rat), reuniens (1 rat), medialis dorsalis (1 rat), centralis medialis (1 rat), centralis lateralis (1 rat), lateralis anterior (2 rats), anterior

[1]Requests for reprints should be addressed to Dr. R. Coveñas, Facultad de Biología, Dpto. Citología, Plaza de La Merced s/n, 37008-Salamanca, Spain.

ventralis (1 rat), ventralis anterior (1 rat), anterior medialis (1 rat), reticularis (2 rats), parataenialis (1 rat), colliculus superior (1 rat), globus pallidus (2 rats) and lateral ventricle (2 rats).

Two or three days after the injection of colchicine rats were reanaesthetized with ketamine and perfused through the ascending aorta with 250 ml of 0.9% saline followed by 1 l of 4% paraformaldehyde in 0.15 M phosphate buffer (pH 7.2). The brains were removed, the thalamic region dissected out and postfixed in the same solution for twelve hours and then put in solutions of increasing sucrose concentrations (10–30%) until they sank. With a freezing microtome, 50 μm sections were cut and processed for immunostaining.

The free-floating sections were preincubated 30 min in phosphate buffer containing 1% normal sheep serum and 0.3% Triton X-100 in order to enhance the penetration of antibodies. Then, the sections were incubated overnight in the same phosphate buffer containing the antienkephalin antibody at the dilution of 1/1000 (this antibody was kindly provided by Professor M. Hamon and F. Cesselin, INSERM U 288, Paris). After a 30 min wash with phosphate buffer, the sections were incubated for 2 hr with sheep anti-rabbit IgG coupled to horseradish peroxidase as second antibody, diluted 1/250 in phosphate buffer. Finally, the sections were washed in the phosphate buffer and the peroxidase activity was revealed by the 3,3′diaminobenzidine method.

It is important to note that the antibody used here recognizes both methionine and leucine-enkephalins, since when the preabsorption of the enkephalin antibody was carried out with synthetic leucine-enkephalin or methionine-enkephalin (100 μg per ml diluted antibody), a lack of immunoreactivity was observed in both cases. On the contrary, no significant reduction of the immunolabeling was found when the enkephalin antibody was preabsorbed with synthetic dynorphin A_{1-8}, dynorphin B, Met^5-enk-Arg^6-Gly^7-Leu^8, β-neoendorphin, β-endorphin and peptide E (100 μg per ml diluted antibody). In addition, the specificity of the immunostaining was controlled by omission of the antienkephalin antibody in the first incubation bath. In this last case, no residual immunoreactivity was observed. Moreover, possible interference by endogenous peroxidases was ruled out by staining some sections beginning with the diaminobenzidine step. No reaction was visualized.

The mapping was carried out according to the stereotaxic atlas of Albe-Fessard *et al.* (1). The same atlas was used for the terminology of the thalamic nuclei. The term "enkephalin-like immunoreactivity" (ELI) was used to describe staining in our material.

RESULTS

Enkephalin fibers and cell bodies are widely distributed throughout the thalamus of the rat (Fig. 1). Thus, all thalamic nuclei possess enkephalin immunoreactivity, except the nuclei geniculatum lateralis dorsalis, ventralis posterior, ventralis lateralis and reticularis. ELI perikarya of the thalamus were generally small (10–15 μm) and round, however, some fusiform cells were also observed.

In posterior regions (A 2.6) (Fig. 1A), ELI cell bodies were grouped in the most medial region of the nucleus geniculatum mediale, below this nucleus, and between the nucleus geniculatum mediale and the nucleus praetectalis lateralis. The density of ELI cell bodies was moderate except in the group localized below the nucleus geniculatum mediale where they were scarcely distributed.

At A 3.5 (Fig. 1B), immunoreactive neurons were separated in two groups, the first being localized dorsally and the second ventrolaterally. In the dorso-caudal aspect of the thalamus, ELI

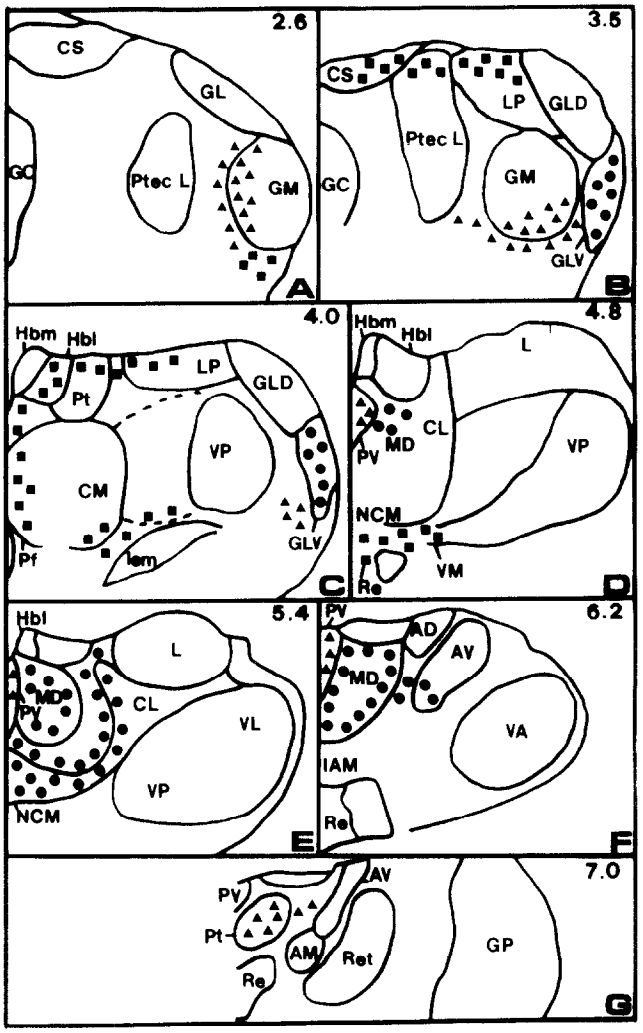

FIG. 1. Distribution of ELI cell bodies in the thalamus of the rat. ELI perikarya are indicated by closed circles, triangles and squares, their shape being related to the density of cell bodies (●, high density: >10 cell bodies; ▲, middle density: 5–10 cell bodies; ■, lower density: 1–5 cell bodies). The anteriority of each section is indicated in the upper right. AD: N. anterior dorsalis; AM: N. anterior medialis; AV: N. anterior ventralis; CL: N. centralis lateralis; CM: N. centrum medianum; CS: Colliculus superior; GC: Griseum centrale; GL: N. geniculatum lateralis; GLD: N. geniculatum lateralis dorsalis; GLV: N. geniculatum lateralis ventralis; GM: N. geniculatum mediale; GP: Globus pallidus; Hbl: N. habenularis lateralis; Hbm: N. habenularis medialis; IAM: N. interanteromedialis; L: N. lateralis; lem: Lemniscus medialis; LP: N. lateralis posterior; MD: N. medialis dorsalis; NCM: N. centralis medialis; Pf: N. parafascicularis; Pt: N. parataenialis; PtecL: N. praetectalis lateralis; PV: N. paraventricularis; Re: N. reuniens; VA: N. ventralis anterior; VL: N. ventralis lateralis; VM: N. ventralis medialis; VP: N. ventralis posterior.

cell bodies were observed in the dorsal part of the nuclei praetectalis lateralis and lateralis posterior. In addition, the dorsal population extends medially to the colliculus superior. In the three nuclei, a low number of perikarya was observed. In the ventrolateral cluster a high density of ELI cell bodies was found in the nucleus geniculatum lateralis ventralis, whereas in the nucleus geniculatum mediale and ventrally to this latter nucleus, a moderate number of immunoreactive cell bodies was visualized.

More rostrally (A 4.0) (Fig. 1C), the dorso-caudal and ventrolateral immunoreactive populations remain, but a new cluster

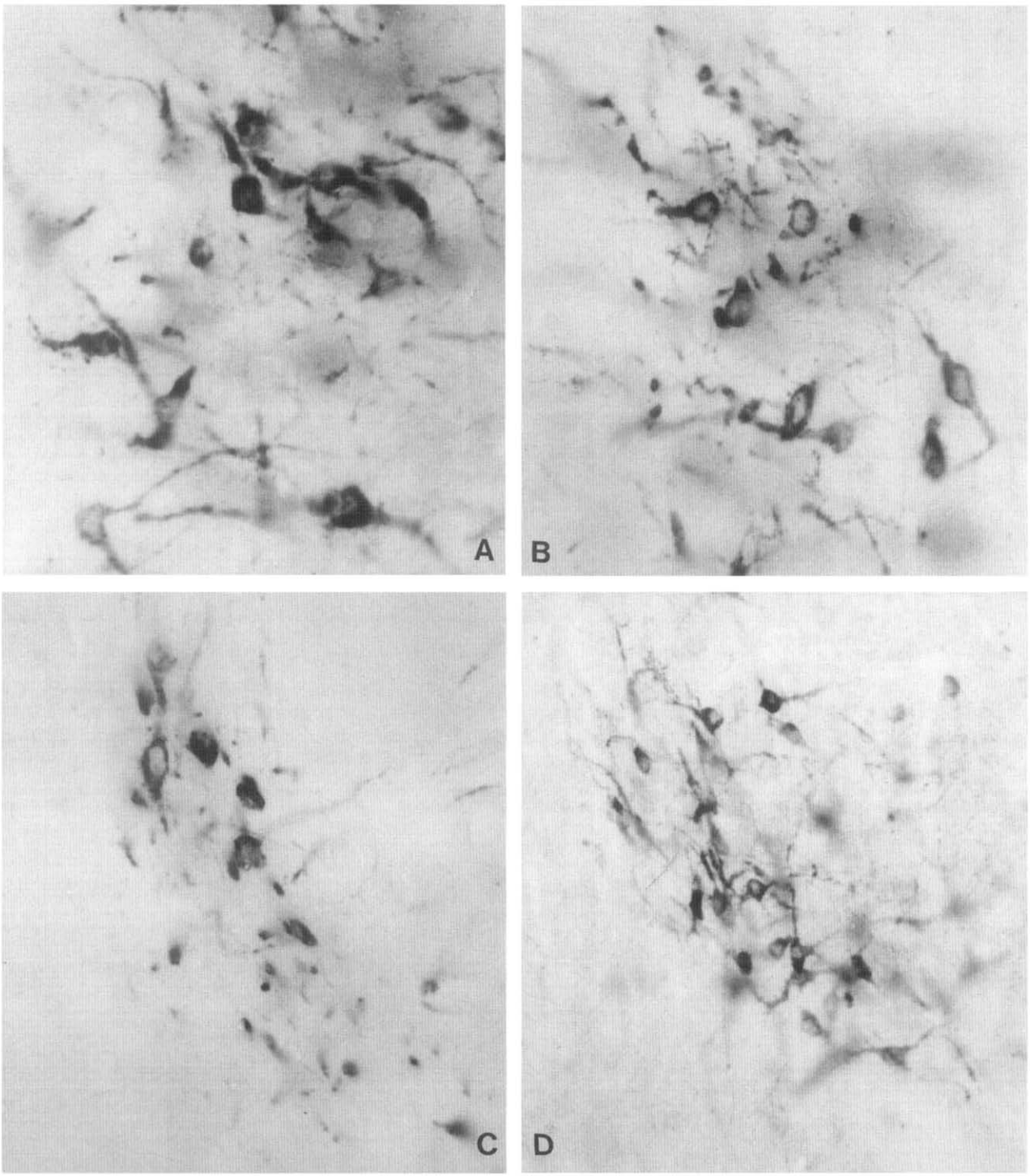

FIG. 2. ELI cell bodies in the thalamus of the rat. (A) Immunoreactive perikarya in the nucleus centralis medialis (×400). (B) ELI cell bodies in the nucleus geniculatum mediale (×400). (C) Cluster of immunoreactive neurons in the nucleus anterior ventralis (×400). (D) Group of immunoreactive perikarya in the nucleus ventralis medialis (×400).

appears in the midline area. Scarce ELI cell bodies were found in the nucleus habenularis lateralis, as well as in the dorsalmost aspect of the nuclei parataenialis and lateralis posterior. In the ventro-lateral cluster, numerous ELI neurons were distributed in the whole nucleus geniculatum lateralis ventralis as well as between this nucleus and the nucleus ventralis posterior, in a moderate number. In the midline area a low number of ELI perikarya was observed in the most medial part of the nucleus parafascicularis and in the ventro-lateral area of the nucleus centrum medianum, and in its lateral extension to the lemniscus

medialis.

At A 4.8 (Fig. 1D), the dorso-caudal and ventro-lateral neuronal clusters have disappeared. However, in the midline area two well defined clusters of immunoreactive neurons can be observed, one placed dorsally and the other ventrally. Dorsally, ELI cell bodies were localized moderate in the nucleus paraventricularis. This group extended to the dorsal part of the nucleus medialis dorsalis, where a high density of perikarya was observed. In the ventral cluster, scarce ELI cell bodies were found in the nucleus reuniens, which extend laterally to the nucleus ventralis medialis.

More rostrally (A 5.4) (Fig. 1E) a large number of ELI perikarya was visualized in the nuclei medialis dorsalis, centralis medialis and centralis lateralis, whereas a moderate density was found in the nucleus paraventricularis.

At A 6.2 (Fig. 1F), a rich cluster of ELI perikarya was visualized in the nuclei medialis dorsalis and anterior ventralis (its ventral part) and between both nuclei. The nucleus paraventricularis showed a moderate number of ELI neurons.

Rostrally, at A 7.0 (Fig. 1G), a moderate number of ELI perikarya was found in the nucleus parataenialis, as well as between this nucleus and the nucleus anterior ventralis.

Clusters of ELI cell bodies in different thalamic nuclei of the rat are shown in Fig. 2.

DISCUSSION

In comparison with previous studies carried out on the distribution of ELI cell bodies in the CNS of rat (7, 12, 15, 17–20), monkey (9,13) and man (2), it seems that immunoreactive perikarya in the thalamus are more numerous and widely distributed in our study.

Previous data on both rat and monkey have shown ELI cell bodies in relatively few thalamic nuclei including centrum medianum, habenularis lateralis and medialis, parafascicularis, paraventricularis, parataenialis, reuniens, and geniculatum lateralis (7, 9, 15, 17, 19). In human thalamus, no ELI cell body was observed (2). By contrast, in the present study we have found ELI neurons in thalamic nuclei reported previously in rat and monkey (except in the nucleus habenularis medialis), but in addition in other nuclei such as praetectalis lateralis, lateralis posterior, ventralis medialis, centralis lateralis, anterior ventralis, geniculatum mediale, medialis dorsalis and centralis medialis. In the same time, it has to be noticed that our observations on the distribution of ELI fibers in the thalamus of the rat (not described here) is in agreement with previous reports (7, 15, 17–19). The lonely discrepancy observed concerns the distribution and number of ELI perikarya.

When we have injected colchicine intraventricularly, most ELI perikarya were observed in nuclei localized near the ventricles (paraventricularis, parafascicularis, parataenialis and habenularis lateralis). These results fit well with those obtained in the rat and monkey (7, 8, 13, 15, 17, 19) which have received also intraventricular colchicine. After intratissular injections of the drug, in addition to immunoreactive cell bodies next to the ventricles, we have also observed ELI neurons located far from the ventricles in nuclei centralis lateralis, ventralis medialis, geniculatum mediale and anterior ventralis. Thus, the intratissular injections of colchicine performed in the present work could be responsible for the most lateral extension of the labeling found in the thalamus of the rat. In addition, comparing intraventricular and intratissular injections, we have observed that the number of immunoreactive perikarya is greatly enhanced in the second case. We have made the same observation in the cat thalamus and hypothalamus (3,4).

In comparison with studies on the cat (4), ELI cell bodies in the rat thalamus showed more or less a similar distribution. In both species, ELI perikarya were found in dorso-caudal, midline and lateral thalamic regions, when both animals received intratissular injections of colchicine. Thus, in the cat and rat thalamus, immunoreactive neurons were found for example in the following nuclei: lateralis posterior, habenularis lateralis, parafascicularis, centrum medianum, medialis dorsalis and centralis medialis. However, some species differences can be observed in the ventrobasal complex and in the nuclei ventralis lateralis and reuniens, where ELI neurons were found in the cat, but not in the rat. On the contrary, in the rodent, we have observed cell bodies containing ELI in the nuclei anterior ventralis and centralis lateralis, whereas in the cat immunoreactive perikarya were absent.

So, we may conclude first that the way of administration of colchicine may be responsible for the differences observed between the present study and previous cartography of ELI cell bodies of the rat thalamus. Secondly, our comparison between rat and cat (4), using the same technique, has shown nevertheless that some species variation occurs. A complete comparison between species (with monkey in particular) would require the systematic injection of colchicine in the thalamus.

Our results are partly in agreement with in situ hybridization of preproenkephalin mRNA in the rat (11). They have found neurons containing preproenkephalin mRNA in the following thalamic nuclei: lateralis posterior, parafascicularis and geniculatum lateralis, whereas we have observed ELI perikarya in the same nuclei of the rat. In this way, it is known that both methionine-enkephalin and leucine-enkephalin are synthesized from the preproenkephalin. However, by in situ hybridization, Harlan *et al.* (11) have not seen neurons containing preproenkephalin mRNA in other thalamic nuclei, e.g., paraventricularis, parataenialis, habenularis lateralis, reuniens, in which we and other authors (7, 9, 15, 19) have observed ELI immunoreactive cell bodies. Thus, in the thalamus, our study has revealed considerably more immunopositive neurons than those labeled by in situ hybridization. This discrepancy could be explained, since the localization of mRNA coding for the peptide does not necessarily indicate the presence of a translation product (11). Alternatively, according to the same authors (11), other possibilities such as alteration of gene expression by colchicine cannot be excluded. In fact, it seems that, locally, there is a mismatch between localization of a given peptide by immunocytochemistry and of its gene by in situ hybridization. This may perhaps depend on physiological states of activation or inhibition of the neurons.

Hitherto, we have no data indicating whether enkephalin perikarya observed in the rat thalamus are local or projecting neurons. In a few nuclei such as centralis lateralis, centralis medialis, geniculatum lateralis ventralis and anterior ventralis, both dense enkephalin terminals and cell bodies were observed, suggesting that these cells may be interneurons. Alternatively, such nuclei could send distant enkephalinergic projections and receive enkephalinergic afferences [e.g., the nucleus anterior ventralis receives enkephalinergic projections from the medial mammillary nucleus (8)]. Numerous ELI neurons and scarce immunoreactive fibers were found in the nucleus medialis dorsalis indicating that they could be projecting neurons.

Enkephalin cell bodies are concentrated in the thalamus of the rat not only in regions concerned by a motivational or affective aspect of the sensory transmission, but also in regions involved in discriminative functions such as the specific thalamic nuclei, ventralis medialis, geniculatum lateralis and geniculatum mediale. Thus, such data could indicate a possible role of the enkephalins in auditive and visual mechanisms. In addition, it is known that the medial thalamic nuclei are involved in pain perception (5, 6, 10, 21). We have observed enkephalin in such nuclei, suggesting a role of this substance in the analgesic function.

REFERENCES

1. Albe-Fessard, D.; Stutinsky, F.; Libouban, S. Atlas stéréotaxique du diencéphale du rat blanc. Paris: C.N.R.S.; 1966.
2. Bouras, C.; Taban, C. H.; Constantinidis, J. Mapping of enkephalins in human brain. An immunofluorescence study on brains from patients with senile and presenile dementia. Neuroscience 12:179–190; 1984.
3. Coveñas, R.; Burgos, C.; Conrath, M. Immunocytochemical study of Met-enkephalin-like cell bodies in the cat hypothalamus. Neurosci. Res. 5:353–360; 1988.
4. Coveñas, R.; Romo, R.; Cheramy, A.; Cesselin, F.; Conrath, M. Immunocytochemical study of enkephalin-like cell bodies in the thalamus of the cat. Brain Res. 377:355–361; 1986.
5. Duggan, A. W.; Hall, J. G. Morphine, naloxone and the responses of medial thalamic neurons of the cat. Brain Res. 122:49–57; 1977.
6. Emmers, R. Thalamic mechanisms that process a temporal pulse code for pain. Brain Res. 103:425–441; 1976.
7. Finley, J. C. W.; Maderdrut, J. L.; Petrusz, P. The immunocytochemical localization of enkephalin in the central nervous system of the rat. J. Comp. Neurol. 198:541–565; 1981.
8. Fujii, S.; Senba, E.; Kiyama, H.; Ueda, Y.; Tohyama, M. Mammillothalamic enkephalinergic pathway in the rat: an immunocytochemical analysis. Brain Res. 401:1–8; 1987.
9. Haber, S.; Elde, R. The distribution of enkephalin immunoreactive neuronal cell bodies in the monkey brain: preliminary observations. Neurosci. Lett. 32:247–251; 1982.
10. Halpern, B. P.; Halverson, J. D. Modification of escape from noxious stimuli after bulbar reticular formation lesions. Behav. Biol. 11: 215–229; 1974.
11. Harlan, R. E.; Shivers, B. D.; Romano, G. J.; Howells, R. D.; Pfaff, D. W. Localization of preproenkephalin mRNA in the rat brain and spinal cord by in situ hybridization. J. Comp. Neurol. 258:159–184; 1987.
12. Hökfelt, T.; Elde, R.; Johansson, O.; Terenius, L.; Stein, L. The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neurosci. Lett. 5:25–31; 1977.
13. Inagaki, S.; Parent, A. Distribution of enkephalin-immunoreactive neurons in the forebrain and upper brainstem of the squirrel monkey. Brain Res. 359:267–280; 1985.
14. Johansson, O.; Hökfelt, T.; Elde, R. P.; Schultzberg, M.; Terenius, L. Immunohistochemical distribution of enkephalin neurons. In: Costa, E.; Trabucchi, M., eds. Advances in biochemical psychopharmacology, vol. 18. New York: Raven Press; 1978:51–70.
15. Khachaturian, H.; Lewis, M. E.; Watson, S. J. Enkephalin systems in diencephalon and brainstem of the rat. J. Comp. Neurol. 220: 310–320; 1983.
16. Lewis, M. E.; Khachaturian, H.; Watson, S. J. Combined autoradiographic-immunocytochemical analysis of opioid receptors and opioid peptide neuronal systems in brain. Peptides 6:37–47; 1985.
17. Merchenthaler, I.; Maderdrut, J. L.; Altschuler, R. A.; Petrusz, P. Immunocytochemical localization of proenkephalin-derived peptides in the central nervous system of the rat. Neuroscience 17:325–348; 1986.
18. Sar, M.; Stumpf, W. E.; Miller, R. J.; Chang, K-J.; Cuatrecasas, P. Immunohistochemical localization of enkephalin in rat brain and spinal cord. J. Comp. Neurol. 182:17–38; 1978.
19. Wamsley, J. K.; Young, W. S.; Kuhar, M. J. Immunohistochemical localization of enkephalin in rat forebrain. Brain Res. 190:153–174; 1980.
20. Watson, S. J.; Akil, H.; Sullivan, S.; Barchas, J. D. Immunocytochemical localization of methionine enkephalin: preliminary observations. Life Sci. 21:733–738; 1977.
21. Zimmerman, M. Neurophysiology of nociception. Int. Rev. Physiol. 10:179–221; 1976.