Distribution of Neuropeptide Y-Like Immunoreactive Fibers in the Cat Thalamus

R. COVENAS,* J. A. AGUIRRE, † J. R. ALONSO,* M. DIOS,* J. LARA* AND J. AIJON*

*Departamento de Biologia Celular y Patologia, Facultad de Biologia
Plaza de la Merced s/n, 37008 Salamanca, Spain
†Departamento de Fisiologia, Facultad de Medicina, Malaga, Spain

Received 26 June 1989

COVENAS, R., J. A. AGUIRRE, J. R. ALONSO, M. DIOS, J. LARA AND J. AIJON. Distribution of neuropeptide Y-like immunoreactive fibers in the cat thalamus PEPTIDES 11(1) 45–50, 1990 — Neuropeptide Y-like immunoreactivity was studied in the thalamus of the cat using an indirect immunoperoxidase method. The densest network of immunoreactive fibers was observed in the nucleus (n) paraventricularis anterior. In the anterior, intralaminar and midline thalamic nuclei, as well as in the n. geniculatum mediales, n. geniculatum lateralis, n. habenularis lateralis, n. medialis dorsalis, n. lateralis posterior and n. pulvinar a low density of neuropeptide Y-like immunoreactive fibers was observed. Neuropeptide Y-like fibers were totally absent in the n. ventralis lateralis, n. ventralis medialis, n. ventralis posteromedialis and n. ventralis postero-lateralis. In addition, neuropeptide Y-like perikarya were found in the n. parafascicularis, n. suprageniculatus, n. geniculatum lateralis ventralis, n. medialis dorsalis and n. lateralis posterior.

Neuropeptide Y Immunocytochemistry Thalamus Cat

SINCE neuropeptide Y was isolated and sequenced by Tatemoto et al. (40), this substance has been reported to be involved in several physiological roles such as the regulation of the cardiovascular system (7, 17, 19), ingestive and sexual behaviors (20), the secretion of the melanoocyte-stimulating and luteinizing-hormone releasing hormones (12, 42), alterations of the circadian rhythms (2) and respiratory mechanisms (16, 19).

The distribution of neuropeptide Y in the mammalian CNS has been characterized in rats (3, 9, 15, 18, 28, 31, 33, 37, 46), golden hamsters (36), rabbits (5, 6), monkeys (4, 24, 38) and humans (1, 8, 14, 21, 43) using immunocytochemistry and radioimmunoassay techniques. Comparing both techniques, in general a similar distribution of neuropeptide Y can be observed in the CNS of mammals. In sum, all these studies point to a widespread distribution of neuropeptide Y in the brain, this substance being the most abundant peptide found in the rat and human brain (1, 3), with the telencephalon and the hypothalamus showing the highest concentrations. In addition, neuropeptide Y is the only member of a family of structurally related peptides (avian pancreatic polypeptide, bovine pancreatic polypeptide, peptide YY) which is widely distributed in the CNS of mammals, since it is now accepted that neuropeptide Y is the peptide recognized by antisera to avian pancreatic polypeptide and bovine pancreatic polypeptide (26, 32) in the central and peripheral nervous system and that little, if any, peptide YY has been found in the CNS (15, 16, 18, 27, 36). The presence of neuropeptide Y in the brain of the frog (13) and fish (34) suggests that the structure of the peptide has been preserved across vertebrate evolution.

In the cat, few data are available on the distribution of neuropeptide Y in the CNS. The only regions in which such a distribution has been studied in detail are the cortex (44, 45), hypothalamus (22, 25) and the thalamic nucleus geniculatum lateralis ventralis (41). Thus, in this study our aim was to localize neuropeptide Y-like immunoreactive (NPY-ir) structures in the thalamus of the cat, and to compare the findings with those previously reported in other species.

METHOD

Eight male adult cats (2–3 kg) were used. Under deep ketamine anesthesia (40–50 mg/kg) the animals were perfused through the ascending aorta with 500 ml of 0.9% NaCl and 1% sodium nitrite, followed by 3 l of 1% paraformaldehyde in 0.15 M phosphate buffer (PB), pH 7.2. The brains were postfixed in the same solution overnight and kept in increasing sucrose baths (10–30%) until they sank. Frontal sections of 60 μm of the thalamic region were obtained with a freezing microtome and processed for immunostaining. The sections were incubated in PB containing 1% normal sheep serum and 0.3% Triton X-100 for 30 min. The sections were then placed overnight in the same PB solution containing neuropeptide Y antiserum (this antibody was kindly provided by Prof. J. Polak, Royal Postgraduate Medical School, London), at a dilution of 1/1000. After a 30-min wash with PB, the sections were incubated for 60 min with sheep antirabbit IgG coupled to horseradish peroxidase as the second antibody, diluted 1/250 in PB. Finally, the sections were washed in PB and the peroxidase was revealed by 3,3′ diaminobenzidine method.

The specificity of the immunostaining was controlled by the preabsorption of the primary antiserum with synthetic neuropeptide Y (100 μg per ml diluted antiserum) and by omitting the
neuropeptide Y antibody in the first incubation bath. In both cases, no residual immunoreactivity was found. In addition, immunohistochemical cross-reactivities were performed by incubating neuropeptide Y antiserum absorbed with an excess (10^{-7} M) of peptide YY, bovine pancreatic polypeptide, avian pancreatic polypeptide, FMRFamide, metenkephalin, leucine-enkephalin, substance P, angiotensin II, somatostatin, neurotensin and cholecystokinin-8. In all cases, no significant reduction in immunoreactivity was observed. Finally, cartography was carried out following the stereotaxic atlas of Jasper and Ajmone-Marsan (23).

RESULTS

As shown in Fig. 1, NPY-ir fibers are widely distributed throughout the thalamus of the cat. Thus, for example, immunoreactivity was found in the n. paraventricularis anterior, n. centralis lateralis, n. pulvinar, n. geniculatum medialis, n. geniculatum lateralis, n. centralis medialis, n. rhomboidens, n. centrum medianum, n. habenularis lateralis, n. reuniens, n. anterior ventrals and n. interanteromedialis. However, in other thalamic regions, e.g., n. reticularis, n. habenularis medialis, n. ventralis anterior, n. ventralis medialis, n. ventralis postero-lateralis and n. ventralis postero-medialis, no NPY-ir fibers were observed. Furthermore, NPY-ir perikarya (Fig. 1A, B, C) were located in the n. suprageniculatus, n. lateralis posterior, n. geniculatum lateralis ventralis, n. medialis dorsalis (Fig. 2A) and n. parafascicularis. In all the thalamic nuclei in which immunoreactivity was observed, the density of the NPY-ir fibers was low, except in the n. paraventricularis where a moderate density of immunoreactive fibers was visualized.

In posterior thalamic regions (A 5.0) (Fig. 1A), NPY-ir fibers were found in the most dorsal aspect of the n. praetectum and n. lateralis posterior, whereas in the n. pulvinar immunoreactive fibers were observed throughout the nucleus. Finally, NPY-ir fibers were found in the dorsolateral region of the n. geniculatum medialis (Fig. 2B). At the same level of anteriority (A 5.0), a cluster of NPY-ir perikarya (5-10 cell bodies) was located in the medial regions of both the n. suprageniculatus and the n. lateralis posterior.

At A 7.0 (Fig. 1B), NPY-ir fibers were found in the midline and dorsolateral regions of the thalamus. This immunoreactivity was found in regions located near the midline such as the n. habenularis lateralis, n. parafascicularis, n. medialis dorsalis (Fig. 2C) and n. centrum medianum. In the dorsolateral region NPY-ir fibers were found in the n. lateralis dorsalis, n. pulvinar, n. lateralis posterior (Fig. 2D), n. geniculatum lateralis ventralis and n. geniculatum lateralis. In the n. lateralis posterior immunoreactivity was observed in the dorsal region, whereas in the geniculatum lateralis fibers were only found in the ventral region. Finally, a moderate density of NPY-ir perikarya was found in the most ventral region of the n. parafascicularis and in the n. geniculatum lateralis ventralis.

In comparison with the previous anteriority (A 7.0), a similar pattern of NPY-ir fibers was observed more rostrally (A 8.0) (Fig. 1C). Thus, in the midline region NPY-ir fibers were located in the n. medialis dorsalis, n. habenularis lateralis, n. centralis medialis and n. centrum medianum. Moreover, immunoreactive fibers were located dorsolaterally in the n. lateralis dorsalis, n. lateralis posterior, n. pulvinar and n. geniculatum lateralis.

FIG. 1 Distribution of NPY-ir fibers and cell bodies in frontal planes of the thalamus of the cat corresponding to the posteroanterior stereotaxic plane levels A 5.0 to A 11.5 of the Jasper and Ajmone-Marsan stereotaxic atlas (23). Immunoreactive fibers are represented by continuous lines, whereas cell bodies are represented by closed circles. The anteriority (A), in mm with respect to the zero stereotaxic point of each section, is indicated at the lower right AD nucleus anterior dorsalis, AM nucleus anterior medialis, AV nucleus anterior ventralis, CL nucleus centralis lateralis, CM nucleus centralis medialis, GL nucleus geniculatum lateralis, GLv nucleus geniculatum lateralis ventralis, GM nucleus geniculatum medialis, Hbl nucleus habenularis lateralis, IAM nucleus interanteromedialis, LD nucleus lateralis dorsalis, LP nucleus lateralis posterior, MD nucleus medialis dorsalis, NCM nucleus centrum medialis, P nucleus posterior, Pr nucleus paracentralis, Pf nucleus parafascicularis, Pt praetectum, Pn nucleus parataenialis, Pul nucleus pulvinar, PVA nucleus paraventricularis anterior, R nucleus reticularis, RE nucleus reuniens, Rh nucleus rhomboidalis, SG nucleus suprageniculatus, VA nucleus ventralis anterior, VL nucleus ventralis lateralis, VM nucleus ventralis medialis, VPL nucleus ventralis postero-lateralis, VPM nucleus ventralis postero-medialis.

At A 9.0 (Fig. 1D), NPY-ir fibers were located in all the thalamic nuclei present at this level, except in the n. reticularis, n.
ventrals postero-laterals and n. ventralis postero-medialis. Immunoreactivity was observed in the n. paraventricularis anterior, n. centralis medialis (Fig. 2E), n. medialis dorsalis, n. paracentralis, n. centralis lateralis (Fig. 2F), n. lateralis posterior, n. pulvinar, n. lateralis dorsalis and n. geniculatum lateralis.

At A 9.5 (Fig. 1E), NPY-ir structures remained in the dorsalmost aspect of the n. lateralis posterior. NPY-ir fibers were also observed in the n. lateralis dorsalis and n. centralis lateralis, as well as in nuclei located near or in the midline such as the n. parataenialis, n. medialis dorsalis, n. paracentralis, n. paraventricularis anterior, n. rhomboidens (Fig. 2G), n. centralis medialis and n. reuniens. Rostrally, at A 11.5 (Fig. 1F), NPY-ir processes were found in the n. paraventricularis anterior, n. parataenialis, n. anterior dorsalis, n. anterior ventralis (dorsal part), n. anterior medialis, n. rhomboidens, n. interanteromedialis and n. reuniens.

**DISCUSSION**

The results found in the thalamus of the cat are generally consistent with the findings observed in the rat and monkey CNS, since in all three species NPY-ir fibers were visualized in certain nuclei of the thalamus such as the n. geniculatum lateralis ventrals, n. centralis lateralis, n. centralis medialis, n. habenularis lateralis, n. lateralis posterior, n. medialis dorsalis, n. paracentralis, n. pulvinar, n. reuniens, n. rhomboidens and n. paraventricularis anterior (9, 31, 38). In addition, as has been previously described in the rat and monkey (9, 31, 38), in the cat we found no NPY-ir structures in the n. habenularis medialis, n. ventralis postero-laterals and n. ventralis postero-medialis. Our results are also in agreement with previous studies carried out in the cat on the localization of NPY-ir structures in the n. geniculatum lateralis ventrals (41) and in the n. parafascicularis (45), since we also observed NPY-ir fibers and cell bodies in the former nucleus and a moderate density of NPY-ir perikarya in the latter. That the density of NPY-ir fibers is low in all the thalamic nuclei in which we observed immunoreactive processes, except in the n. paraventricularis anterior, where a moderate density was found. These observations are also in agreement with previous studies, since the thalamus is one of the weakest NPY-ir regions of the monkey and rat brains (9, 31, 38). Moreover, in both the latter species the n. paraventricularis anterior showed the highest density of NPY-ir fibers. These immunocytochemical data correlate well with radioimmunoassay studies which have shown a low concentration of NPY in the thalamus of the rat, monkey and humans (1, 3, 4).

However, comparing the distribution of NPY-ir structures in the thalamus of the cat, rat and monkey some differences can be observed. In this sense, in the cat we did not find NPY-ir structures in the n. ventralis anterior and n. ventralis lateralis, in which NPY-ir fibers were found in the monkey (38) On the other hand, in the present work, we visualized NPY-ir fibers in the n. anterior dorsalis, n. anterior medialis, n. geniculatum medialis, n. interanteromedialis, n. lateralis dorsalis, n. parafascicularis and n. parataenialis, these have never been reported in previous studies on the mammalian thalamus. Thus, using an indirect immunoperoxidase technique, the present report demonstrates a more complete distribution of NPY-ir fibers in the mammalian thalamus.

We also observed in the cat NPY-ir perikarya in the n. lateralis posterior, n. suprageniculatus, n. medialis dorsalis and n. parafascicularis. In these thalamic nuclei no immunoreactivity has been observed in cell bodies either in the monkey or the rat (9, 31, 38). In the monkey, after intraventricular injections of colchicine, NPY-ir perikarya have been observed in the n. paraventricularis and n. habenularis lateralis, both nuclei placed near the ventricle (38). However, in rats, when intraventricular injections of the drug were carried out, no immunoreactive cell bodies were observed in the thalamus except in the n. geniculatum lateralis ventrals (9, 31). In this latter nucleus, we found NPY-ir cell bodies, as has been previously reported in the cat (41), but in the monkey no NPY-ir cell bodies have been described in this nucleus (38). In sum, at present there are differences regarding the distribution of NPY-ir cell bodies and fibers in the mammalian thalamus. These discrepancies could be due to species variations and/or technical considerations (e.g., antisera used, injections of colchicine). In this sense, there are data suggesting the first possibility since species differences on the (125)I Bolton-Hunter NPY binding sites in the thalamus of mammals have been described (29). Thus, for example, high densities of NPY binding sites were present in various rat and hamster thalamic nuclei such as the n. lateralis posterior and n. ventralis posterior, whereas only low densities were found in the corresponding nuclei in the guinea pig. Accordingly, in order to know the differences in the immunoreactivity found in the mammalian thalamus, additional experiments should be carried out.

In the cat thalamus the distribution of fibers and cell bodies containing methionine-enkephalin has been described (10, 11). In this diencephalic region a clear anatomical relationship between methionine-enkephalin and NPY can be suggested. Thus, in all the thalamic nuclei in which methionine-enkephalin fibers have been observed (e.g., n. centrum medianum, n. parafascicularis, n. medialis dorsalis, n. centralis medialis, n. rhomboidens), we have also observed NPY-ir processes NPY-ir processes have also been found in the same anatomical locations in which methionine-enkephalin cell bodies are present in the cat thalamus. Thus, dorsally (at A 5.0), NPY-ir fibers and methionine-enkephalin perikarya were observed in the dorsalmost aspect of the n. praetectum and n. lateralis posterior, as well as being distributed throughout the n. pulvnr. More rostrally (A 9.5), both NPY-ir fibers and methionine-enkephalin cell bodies were found in nuclei of the midline region such as the n. rhomboidens, n. centralis medialis, n. reuniens and in the intralaminar n. paracentralis.

The origin of the NPY-ir fibers observed in the thalamus of the cat is at present unknown. However, according to morphological data reported in the cat, it appears that NPY-ir cell bodies found in the n. geniculatum lateralis ventrals might send projections to the n. lateralis posterior and to the n. pulvinar, since in the cat horseradish peroxidase-labelled cell bodies have been visualized after injections of the enzyme into the n. lateralis posterior and n. pulvinar, in the ventral region of the n. geniculatum lateralis ventrals (35), the same place where we observed NPY-ir cell bodies. In the rat a neuropetide Y pathway has been demonstrated from the n. geniculatum lateralis ventrals to the hypothalamic n. suprachiasmaticus (30), but in the cat such a pathway is not probable since Leger et al. (25) have reported in the feline that the n. suprachiasmaticus is almost devoid of NPY-ir fibers. It also appears that the neurons observed in the medial regions of both the n. suprageniculatus and n. lateralis posterior might be projecting neurons since in both nuclei a moderate density of NPY-ir cell bodies was visualized, but no NPY-ir fibers. Moreover, in the cat it has been demonstrated that neurons located in the same places where we found NPY-ir cell bodies in the n. suprageniculatus and in the n. lateralis posterior send afferents to the putamen (39), in which numerous NPY-ir fibers have been observed (45). However, another possibility might be that the NPY-ir fibers found in the putamen have their origin in the same nucleus, since numerous NPY-ir cell bodies have been also described (45). In addition, cell bodies located in the n. parafascicularis in the same region where we observed NPY-ir neurons project to the putamen (39). Thus, these NPY cell bodies might send afferents to the putamen. Alternatively, the NPY-ir neurons located in the n. parafascicularis might be interneurons, since in this nucleus we also visualized NPY-ir fibers.

Finally, the widespread distribution of NPY-ir structures in the
cat thalamus suggests that neuropeptide Y may be involved in several functions.

ACKNOWLEDGEMENTS

We are very grateful to Professor J M Polak, Royal Postgraduate Medical School, London, for kindly providing the NPY antisera. This work was supported by the Caja de Ahorros and the University of Salamanca.


40. Tatemoto, K. Neuropeptide Y complete amino acid sequence of the brain peptide Proc Natl Acad Sci USA 79:5485–5489, 1982


