Distribution of Neuropeptide Y-Like Immunoreactive Cell Bodies and Fibers in the Brain Stem of the Cat

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COVENAS, R., J. A. AGUIRRE, M. DE LEÓN, J. R. ALONSO, J. A. NARVÁEZ, R. AREVALO AND S. GONZALEZ-BARON. Distribution of neuropeptide Y-like immunoreactive cell bodies and fibers in the brain stem of the cat. BRAIN RES BULL 25(5), 675-683, 1990.—By using intratissue injections of colchicine and an indirect immunoperoxidase technique, we studied the distribution of cell bodies and fibers containing neuropeptide Y-like immunoreactivity in the brain stem of the cat. The densest clusters of immunoreactive perikarya were observed in the following nuclei: anteroventral cochlear, lateral reticular (internal and external divisions), dorsal tegmental, inferior colliculus and dorsal nucleus of the lateral lemniscus. By contrast, the nuclei abducens, the nucleus of the trapezoid body, preolivary, interpeduncularis, infratrigeminal, gigantocellular tegmental field, coeruleus and dorsal motor nucleus of the vagus had the lowest density. Finally, a moderate density of neuropeptide Y-like immunoreactive cell bodies was found in the nuclei: lateral tegmental field, laminar spinal trigeminal, praepositus hypoglossi, superior colliculus, lateral vestibular and motor trigeminal. In addition, a mapping of the neuropeptide Y-like immunoreactive fibers was carried out. Thus, the densest network of immunoreactive fibers was observed in the laminar spinal trigeminal nucleus. The nuclei periaqueductal gray, inferior central, praepositus hypoglossi, postpyramidal raphe, dorsal raphe, incertus and medial vestibular contained a moderate density of immunoreactive fibers, whereas the nuclei interpeduncularis, inferior colliculus, superior central, gracile, retrorubral, Kölliker-Fuse, dorsal tegmental, ambiguous and alaminar spinal trigeminal had the lowest density of neuropeptide Y-like immunoreactive fibers. The anatomical location of neuropeptide Y-like immunoreactivity suggests that the peptide could play an important role in several physiological functions, e.g., those involved in cardiovascular, auditory, motor, visual, nociceptive and somatosensory mechanisms.

Neuropeptide Y Brain stem Immunocytochemistry Cat

SINCE neuropeptide Y was isolated and sequenced by Tatemoto et al. (37), the distribution of neuropeptide Y has been studied in brain of rat (5, 11, 12, 20, 22, 31-33, 35, 43), golden hamster (34), rabbit (8,9), monkey (6, 27, 36) and man (1, 11, 25, 40). It has been shown that neuropeptide Y has a widespread distribution in the CNS of mammals. The peptide has been proposed to have several roles such as in the regulation of the cardiovascular system (10, 21, 23), in neuroendocrine and respiratory functions (3, 18, 39), the alteration of circadian rhythms (4) and ingestive and sexual behaviors (24).

The distribution of neuropeptide Y in the cat remains poorly studied (3, 26, 29, 38, 41, 42). Neuropeptide Y immunoreactive structures have been described in the cortex (41,42), hypothalamus (26, 29, 38), brain stem respiratory nuclei (3) and in the thalamic geniculatum lateralis nucleus (38) of the cat. However, no data are available on the distribution of neuropeptide Y in the brain stem of the cat. In order to facilitate the visualization of peptidergic cell bodies, we have demonstrated that intratissue injections of colchicine are more potent than intraventricular injections, because the number and extent of immunoreactive perikarya is greatly enhanced in the first case (2, 3, 14-17). The aim in the present study was to locate neuropeptide Y immunoreactive structures in the cat brain stem using intratissue injections of colchicine.

METHOD

Under deep ketamine anesthesia (40-50 mg/kg body weight), eleven male adult cats (2-3 kg) were treated with colchicine. Two animals received unilateral intraventricular injections into the fourth ventricle (350 µg in 5 µl of distilled water) following the stereotaxic atlas of Berman (7). Nine other cats received unilateral intratissue injections of the drug (100 µg in 1 µl of distilled water) according to the same atlas in the paralemniscal tegmental field (1 cat), lateral tegmental field (2 cats), nucleus of the trapezoid body (1 cat), lateral nucleus of the superior olive (1 cat), gigantocellular tegmental field (1 cat), inferior central nucleus (1 cat), near the retrofacial nucleus (1 cat) and into the internal division of the lateral reticular nucleus (1 cat). In addition, four cats...
were used as controls (without colchicine).

Two days after the injection the pretreated animals, as well as the controls, were anesthetized, heparinized and perfused with physiological saline followed by 4 litres of 4% paraformaldehyde in 0.15 M phosphate buffer (pH 7.2); the brains were postfixed in the same solution overnight and kept in sucrose baths. Coronal sections of 90 µm of the brain stem were cut on a vibratome and processed for immunostaining as previously described (3). Briefly, the sections were preincubated for 90 min in 1% normal sheep serum in 0.15 M Sörensen buffer, with 0.3% Triton X-100 and then incubated overnight in the same buffer containing neuroneptide Y antiserum (kindly provided by Professor J. Polak, Royal Postgraduate Medical School), diluted 1/1500. The sections were then washed and incubated for 60 min with sheep anti-rabbit immunoglobulin coupled to horseradish peroxidase as the second antiserum, diluted 1/250. Finally, the peroxidase was developed by the 3,3'-diaminobenzidine method. Mapping was carried out according to the stereotaxic atlas of Berman (7). To test the specificity of the immunostaining, the primary antiserum was preabsorbed with synthetic neuropeptide Y (100 µg per ml of diluted antiserum). Neuropeptide Y antiserum was omitted in the first incubation bath. No immunoreactivity was found in either case. In addition, no significant reduction in immunoreactivity was observed incubating neuropeptide Y antiserum absorbed with an excess (10^{-7} M) of peptide YY, bovine pancreatic polypeptide, FMRF-amide, methionine-enkephalin, leucine-enkephalin, substance P, angiotensin II, somatostatin, neurotensin, cholecystokinin-8 and α-melanocyte-stimulating hormone. Finally, the term neuropeptide Y-like immunoreactive (NPY-ir) was used to describe staining in our material.

**RESULTS**

NPY-ir cell bodies and fibers were widely distributed throughout the brain stem of the cat (Fig. 1). The densest clusters of NPY-ir perikarya were observed in the anteroventral cochlear nucleus, in the inferior colliculus, in the dorsal tegmental nucleus, in the dorsal nucleus of the lateral lemniscus and in the external and internal divisions of the lateral reticular nucleus, whereas the densest cluster of immunoreactive fibers was found in the laminar spinal trigeminal nucleus.

**Distribution of NPY-ir Cell Bodies**

At rostral level (A 1.6) (Fig. 1A), a low density of immunoreactive cell bodies was observed in the nucleus interpeduncularis, whereas in the superficial division of the superior colliculus a moderate number of NPY-ir perikarya was observed. At P 0.9 (Fig. 1B) a high density of NPY-ir cell bodies was observed in the inferior colliculus and in the dorsal nucleus of the lateral lemniscus (Fig. 2A). More caudally (P 4.0) (Fig. 1C), immunoreactive neurons were located in two groups, the first located dorsally and the second ventrally. Thus, dorsally, immunoreactive perikarya were observed in the nucleus coeruleus and dorsal tegmental nucleus to the motor trigeminal nucleus. Kölliker-Fuse area and lateral tegmental field (Fig. 2B). In the three latter regions a moderate number of NPY-ir cell bodies was visualized, whereas in the dorsal tegmental nucleus, a high number of NPY-ir cell bodies (Fig. 2C) and in the nucleus coeruleus a low number of NPY-ir perikarya was observed. Ventrally, in both the preolivary nucleus (Fig. 2D) and the nucleus of the trapezoid body a few immunoreactive cell bodies were found. At posterior level (P 6.0) (Fig. 1D), NPY-ir cell bodies were observed dorsally in the abducens nucleus and in the gigantocellular tegmental field (low density), ventrally in the nucleus of the trapezoid body (low density) and laterally in the nucleus anteroventral cochlear (high density) (Fig. 2E). At P 8.5 (Fig. 1E), a moderate number of NPY-ir cell bodies was found in both the nucleus praepositus hypoglossi (Fig. 2F) and lateral vestibular nucleus (Fig. 2G). More caudally, at P 10.8 (Fig. 1F) immunoreactive cell bodies were visualized, whereas in the other two nuclei a moderate density of immunoreactive perikarya was observed. Moreover, NPY-ir cell bodies were observed between the retrofacial nucleus and the inferior olive. At P 13.5 (Fig. 1G), numerous NPY-ir cell bodies were located in the internal and external divisions of the lateral reticular nucleus (Fig. 3A). In the nucleus ambiguus, lateral nucleus of the solitary tract and in the dorsal motor nucleus of the vagus, a few NPY-ir cell bodies were also found. Finally, at the caudal-most level P 16.0 (Fig. 1H), a moderate number of NPY-ir perikarya was visualized in the laminar spinal trigeminal nucleus.

**Distribution of NPY-ir Fibers**

At A 1.6 (Fig. 1A) a moderate density of NPY-ir fibers was found in the periaqueductal gray, and few fibers were visualized in the nuclei interpeduncularis, retrolateral and in the superficial division of the superior colliculus. In addition, a few NPY-ir fibers were found on the midline from the periaqueductal gray to the nucleus interpeduncularis, as well as distributed ventrolaterally from the periaqueductal gray into the central tegmental field. More caudally, at P 0.9 (Fig. 1B) the periaqueductal gray, the dorsal raphe nucleus (medial division) and the nucleus incertus showed a moderate density of NPY-ir fibers, whereas a low den-
sity was visualized in the nuclei superior central, coeruleus, inferior colliculus (its most dorsolateral part), paramestrical tegmental field and in the commissure of the inferior colliculus. Finally, a few NPY-ir fibers were distributed ventrolaterally from the nucleus coeruleus into the central tegmental field. At P 4.0 (Fig. 1C), a moderate number of NPY-ir fibers was located in the midline (inferior central nucleus), whereas in the most medial zone of the medial nucleus of the superior olive a low density of NPY-ir fibers was observed. Dorsally, a moderate number of NPY-ir fibers was found in the nucleus incertus. Other nuclei, the dorsal tegmental, coeruleus, marginal nucleus of the brachium conjunctivum. Kölliker-Fuse and lateral tegmental field showed a low density of NPY-ir fibers. More caudally at P 6.0 (Fig. 1D), NPY-ir fibers were distributed from dorsal to ventral regions. A moderate number of immunoreactive fibers was found in the nucleus praepositus hypoglossi, medial vestibular, inferior central, as well as in the magnocellular tegmental field, whereas a few fibers were visualized in the abducens nucleus and nerve, lateral tegmental field, gigantocellular tegmental field and lateral nucleus of the superior olive (its most dorsolateral part). At P 8.5 (Fig. 1E), NPY-ir fibers were located mainly in the ventral region of the section throughout the postpyramidal nucleus of the raphe (Fig. 3B), the magnocellular tegmental field and the medial division of the facial nucleus. In addition, NPY-ir fibers were also found in the midline in the inferior central nucleus and dorsally in the nucleus praepositus hypoglossi and in the lateral tegmental field. In all the nuclei mentioned at this anteriority a moderate number of NPY-ir fibers was observed, except in the lateral tegmental field in which a few fibers were visualized. At P 10.8 (Fig. 1F), NPY-ir fibers were found in the midline in the inferior central nucleus and in the postpyramidal nucleus of the raphe, dorsally in the nucleus praepositus hypoglossi, in the medial vestibular nucleus and in the gigantocellular and lateral tegmental fields, ventrally in the retrofacial nucleus and laterally in the paraventricular division of the alamian spinal trigeminal nucleus. In the midline and dorsal regions (nucleus praepositus hypoglossi, medial vestibular nucleus) a moderate number of NPY-ir fibers was observed, whereas in dorsal (gigantocellular and lateral tegmental fields), ventral and lateral regions a low density of immunoreactive fibers was visualized. Like the previous posterior region (P 10.8), at P 13.5 a moderate density of NPY-ir fibers was observed in the midline (inferior central nucleus) and dorsal (dorsal motor nucleus of the vagus and lateral nucleus of the solitary tract) regions and a few NPY-ir fibers in the ventral and lateral regions [nucleus ambiguus, paraventricular division of the alamian spinal trigeminal nucleus, internal and external divisions of the lateral reticular nucleus and in the medial accessory inferior olive (Fig. 3C)]. Finally, rostrally, at P 16.0 (Fig. 1H), a high number of NPY-ir fibers was observed in the laminar spinal trigeminal nucleus (Fig. 3D). In other regions, the rostral division of the gracile nucleus, the lateral tegmental field and the decussation of the medial lemniscus, a few NPY-ir fibers were observed.

DISCUSSION

In the present study we have demonstrated that NPY-ir cell bodies and fibers are widely distributed in the cat brain stem. Thus, in the midbrain NPY-ir structures have been observed, for example, in the periaqueductal gray, the dorsal raphe nucleus and inferior colliculus, in the pons, in the nuclei coeruleus, dorsal tegmental, superior olive and motor trigeminal, whereas in the medulla oblongata NPY-ir fibers or cell bodies were observed in the nuclei anteroventral cochlear, lateral vestibular, medial vestibular, lateral reticular, lamina spinal trigeminal and praepositus hypoglossi. However, several nuclei and tracts in the brain stem of the cat showed no NPY-ir structures, e.g., dorsal and posteroventral cochlear, cuneate, superior and inferior vestibular, cuneiform, area postrema, pontine gray, trapezoid body, pyramidal and spinal trigeminal tracts.

In comparison with previous studies on the distribution of NPY-ir fibers in the rat and cat brain stem (12,43), it seems that in general the distribution found in the brain stem of the cat is quite similar. Thus, immunoreactive fibers were found in both the rat and cat in the abducens, facial, intermediomedialis, medial vestibular, dorsal tegmental, coeruleus, dorsal motor nucleus of the vagus, internal and external divisions of the lateral reticular nucleus, superior colliculus, gigantocellular tegmental field and periaqueductal gray. However, some differences can be observed in the medial accessory inferior olive and in the nucleus praepositus hypoglossi. We found NPY-ir fibers in the cat, but no immunoreactive fibers have been found in any of these nuclei in the rat (12,43). By contrast, in the rat, NPY-ir fibers have been observed in the anteroventral cochlear and lateral vestibular nuclei, where we did not visualize immunoreactive fibers in the cat. In addition, our results are in agreement with the findings reported for monkey (36), since in both cat and monkey NPY-ir fibers have been described in the nuclei coeruleus, intermediomedialis and periaqueductal gray.

Previous data from both rats and humans (12,25,43) have shown NPY-ir cell bodies in the nuclei coeruleus, dorsal motor nucleus of the vagus, lateral tegmental field, inferior colliculus, intermediomedialis, lateral reticular, praepositus hypoglossi, dorsal tegmental, lamina spinal trigeminal and gigantocellular tegmental field. Our results are in agreement with these findings, since in the cat we have found NPY-ir perikarya in all the above-mentioned nuclei. However, we also visualized other brain stem nuclei of the cat containing NPY-ir cell bodies, not found in rats or humans (12,25,43), e.g., anteroventral cochlear, prepyramidal, superior colliculus, nucleus of the trapezoid body, lateral vestibular, motor trigeminal and abducens. By contrast, in rats and humans NPY-ir perikarya have been found in the periaqueductal gray, gracile, medial vestibular and facial nuclei, but not in the cat.

In summary, the distributions of NPY-ir fibers appear to be quite similar in the rat and cat. However, NPY-ir cell bodies seem to be more widely distributed in the brain stem of the cat as compared with the rat, the monkey and humans (12,25,43). This discrepancy could be due to technical considerations (injections of colchicine, antisera used) and/or species differences. In fact, the results of the present study suggest that these differences could be due to the intratissue injections of colchicine. In the control cats, without pretreatment with the drug, we found NPY-ir cell bodies in brain stem nuclei intermediomedialis and in-
fibers and superior colliculi. Moreover, when intraventricular injections of colchicine were carried out we also observed NPY-ir perikarya in nuclei located near the ventricles, e.g., the praepositus hypoglossi, lateral nucleus of the solitary tract, coeruleus, dorsal tegmental and dorsal motor nucleus of the vagus. These are the same nuclei in the rat in which NPY-ir neurons were visualized after intraventricular injections of colchicine. However, in nuclei located distant from the ventricles, e.g., the dorsal nucleus of the lateral lemniscus, the preolivary nucleus, the nucleus of the trapezoid body, the anteroventral cochlear nucleus, the infratrigeminal nucleus, the retrofacial nucleus, and the nucleus ambiguus, NPY-ir perikarya were only observed in the cat when intratissue injections of colchicine were made. In these nuclei, no NPY-ir cell body was found in the control or intraventricular-treated rats. It therefore seems that intratissue injections of the drug are needed to reveal immunoreactive cell bodies located distant from the ventricles in the cat. suggesting that the way of administration of the colchicine might be responsible for the differences found in the distribution of labeled neurons. In conclusion, it appears that a complete comparison between species (the rat and monkey, in particular) would require a reexamination of the distribution of NPY-ir cell bodies in the brain stem of mammals using systematic intratissue injections of colchicine. However, as pointed out above, species variations could also be responsible for the differences found in the distribution of NPY-ir cell bodies in the mammalian brain stem, as we have observed between the cat and rat thalamus using the same technique of intratissue colchicine injections (14).

In the present work, we have found a widespread distribution of NPY-ir structures in the brain stem of the cat using intratissue injections of colchicine. Several data (e.g., the immunocytochemical controls, the brain stem regions mapped) suggest that the immunoreactivity found is specific for NPY, since, for example, all the findings presented here were observed in regions of the brain stem located far from the injection sites of colchicine, in order to avoid a possible nonspecific staining as a consequence of tissue damage produced by colchicine. In addition, sections stained with Nissl’s procedure showed that the morphology of the tissue was preserved in those regions of the cat brain stem in which we have observed NPY immunoreactivity. Moreover, the morphology of NPY-ir cell bodies and fibers visualized was also preserved.

Hitherto, we have no data indicating whether NPY-ir perikarya observed in the brain stem of the cat are local or projecting neurons. However, according to morphological data obtained in the cat, it appears that the NPY-ir neurons found in the nucleus praepositus hypoglossi could be interneurons, since this nucleus showed a moderate density of both NPY-ir fibers and perikarya. Alternatively, neurons located in the nucleus praepositus hypoglossi may send distant NPY projections, whereas NPY-ir fibers may be NPY afferents. It is known, for example, that in the cat the neurons located in the nucleus praepositus hypoglossi send projections to the vestibular nuclei, medial accessory olive and superior colliculus (30). In the latter case an NPY pathway is unlikely since we found NPY-ir fibers in the superficial division of the superior colliculus, a region quite different from that observed by McCrea and Baker (30), into which the neurons of the nucleus praepositus hypoglossi project. However, NPY pathways from the nucleus praepositus hypoglossi to the medial vestibular nucleus and medial accessory olive are possible since in these latter two nuclei NPY-ir fibers have been observed, but no NPY-ir neurons. Moreover, NPY-ir processes found in the nucleus praepositus hypoglossi could be NPY afferents. Thus, it is known in the cat that the nucleus praepositus hypoglossi receives afferents from its contralateral homologous and from the vestibular nuclei (30). Thus, it seems that NPY-ir neurons visualized in the lateral vestibular nucleus could send NPY-ir projections to the nucleus praepositus hypoglossi, since in this vestibular nucleus we have observed a moderate density of NPY-ir cell bodies and no NPY-ir fiber.

It also appears that the nuclei inferior central, facial, the dorsal postpyramidal nucleus of the raphe, the periaqueductal gray and the dorsal nucleus of the raphe could receive NPY-ir afferents since in all these nuclei a moderate density of NPY-ir fibers was observed but no NPY-ir perikarya.

The results of the present study reveal that NPY-ir structures are widely distributed in the brain stem of the cat, suggesting that neuropeptide Y might be involved in several physiological functions. Thus, for example, the presence of immunoreactive structures located in the nucleus of the solitary tract and in the dorsal motor nucleus of the vagus could be involved in cardiovascular mechanisms. The location of neuropeptide Y in the inferior colliculus indicates that neuropeptide Y might also be involved in auditory mechanisms, whereas the presence of NPY-ir structures in the nucleus praepositus hypoglossi suggests that the peptide could be involved in the control of eye and head movements. Moreover, neuropeptide Y could also be involved in motor mechanisms, since NPY-ir structures have been observed in the lateral vestibular nucleus and in the nucleus interpeduncularis. In addition, the presence of NPY-ir fibers or cell bodies in the superior colliculus, periaqueductal gray, dorsal nucleus of the raphe, alaminar and laminar spinal trigeminal nuclei, gracile nucleus and lateral reticular nuclei suggests the involvement of neuropeptide Y in visual, nociceptive and somatosensory behaviors. Finally, it seems that an anatomical relationship between methionine-enkephalin and neuropeptide Y can be suggested. Thus, in almost all the cat brain stem nuclei in which Conrath et al. (13) observed methionine-enkephalin fibers or cell bodies, e.g., praepositus hypoglossi, medial vestibular, interpeduncularis, periaqueductal gray, lateral reticular and facial, we have observed NPY-ir processes. On the other hand, according to the results found by Kahama et al. (28) on the distribution of catecholaminergic neurons in the cat medulla oblongata, it seems that in general the distribution of both catecholaminergic and NPY-ir neurons in the cat medulla oblongata is quite different. Thus, for example, in the nuclei laminar spinal trigeminal, ambiguous, praepositus hypoglossi and lateral nucleus of the solitary tract we have found NPY-ir neurons; however, no catecholaminergic neurons have been visualized in these nuclei.
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REFERENCES


