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IMMUNOCYTOCHEMICAL STUDIES WITH ANTISERA AGAINST LEU-ENKEPHALIN AND AN ENKEPHALIN-PRECURSOR FRAGMENT (BAM-22P) IN THE RAT BRAIN

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Summary

Rats given high doses of colchicine (300-400 ug, i.c.v.) were used to investigate the comparative distribution of Leu-enkephalin and bovine adrenal medullary enkephalin-precursor fragment (BAM-22P) in the brain. Leu-enkephalin immunoreactive neurons have a widespread distribution throughout the neuraxis. In most areas adjacent section analysis showed BAM-22P and Leu-enkephalin immunoreactive perikarya to be similarly localized. Brain enkephalin biosynthetic mechanisms might therefore be similar to those regulating adrenal medullary enkephalin biosynthesis.

The enkephalins (1) have been shown to occur in neuronal systems in many brain sites. The highest radioimmunoassayable levels occur in striatum, with lower amounts found in diencephalon, brainstem, and cerebral cortex (2). With the use of immunohistochemical techniques, many investigators have been able to show widespread immunoreactivity in brain and spinal cord (3-10). In many regions, such as cerebral cortex and several limbic areas, enkephalins occur in very low concentrations, and are thus not easily detectable by immunocytochemistry. To overcome this problem we have used high doses of colchicine administered i.c.v. to enhance cell body visualization and have been able to detect widespread enkephalin immunoreactivity in several cortical-limbic structures (Khachaturian et al., submitted).

The enkephalins also occur outside of the brain. In particular, the adrenal medullary cells synthesize enkephalins via a precursor containing 6 copies of Met-enkephalin and 1 copy of Leu-enkephalin (11-13). In the present study, we have used antisera to both Leu-enkephalin and an enkephalin precursor fragment from bovine adrenal medulla (BAM-22P; 14) to demonstrate a close anatomical correlation between the two immunoreactivities in the rat central nervous system.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were injected i.c.v. with high doses of colchicine (300-400 ug in 10 ul 0.9% saline). After 24-48 hours, the animals were anesthetized with sodium pentobarbital and then perfused through the aorta with ice-cold 4% neutral-buffered formaldehyde for 30 minutes. The brains were removed promptly and refrigerated overnight in 15% sucrose. The tissues were frozen in -40°C isopentane and processed for PAP immunocytochemistry (15) using antisera generated against Leu-enkephalin and the non-enkephalin portion of BAM-22P. The tissues were incubated with primary

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rabbit antiserum to each peptide for 1 hour at 37°C and overnight at 4°C. These were then washed in phosphate-buffered saline (PBS), incubated with goat-anti-rabbit serum (Sternberger-Meyer) for 30 minutes at 37°C and overnight at 4°C. The tissues were again washed in PBS, incubated with horseradish peroxidase (HRP) antiserum for 40 minutes, and subsequently with HRP enzyme (Sigma, Type VI) for 30 minutes. The tissues were then washed in PBS and immersed in a solution of diaminobenzidine (Sigma) and 0.03% hydrogen peroxide for 15 minutes. They were then washed in distilled water, osmicated (2%), washed, and dehydrated.

RESULTS

Leu-enkephalin immunoreactivity was observed to be widely distributed throughout the neuraxis (Table I). Most of these areas also contained BAM-22P immunoreactivity (Table I). Serial section analysis with antisera against Leu-enkephalin and BAM-22P showed these two immunoreactivities to be distributed similarly (Figure 1). Leu-enkephalin immunoreactivity occurred

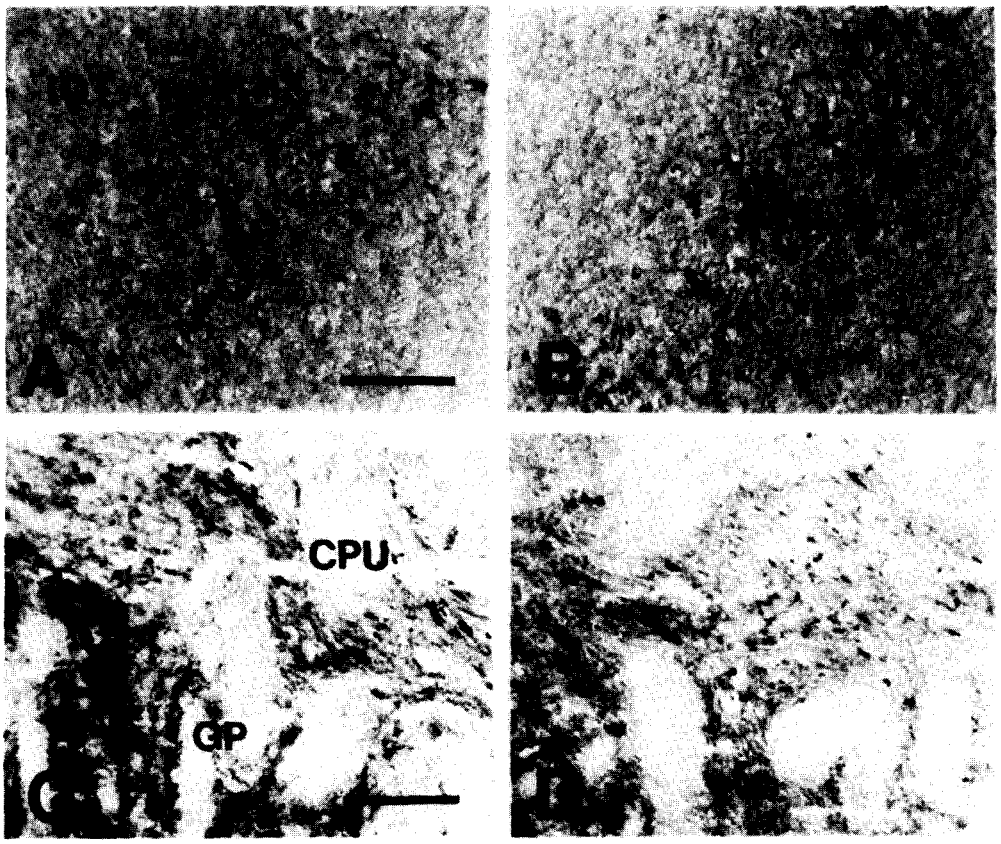

FIG. 1

Comparative immunocytochemistry of Leu-enkephalin (A,C) and BAM-22P (B,D) in adjacent tissue sections through the anterior olfactory nucleus (A,B) and caudate-putamen (CPU)/globus pallidus (GP) (C,D). Note the similar anatomical distribution of perikarya, fibers, and terminals in each respective region. Bar = 100 um.

TABLE I

Comparative distribution of Leu-enkephalin (ENK) and BAM-22P (BAM) immunoreactivities in selected rat brain sites

Region	Perikarya		Fibers/Terminals	
	ENK	BAM	ENK	BAM
Olfactory bulb	+		+	
Anterior Olfactory n.	+	+	+	+
Neocortex	+		+	
Paleocortex: cingulate	+	+		
piriform	+	+	+	+
entorhinal	+	+	+	+
Amygdala	+	+	+	
Hippocampal formation: perforant path			+	+
dentate gyrus	+		+	
hippocampus	+		+	+
N. accumbens	+	+	+	+
Caudate-putamen	+	+	+	+
Globus pallidus			+	+
Septum	+	+	+	+
Hypothalamus: supraoptic n.			+	
paraventricular n. (parvo)	+	+	+	+
ventromedial n.	+	+	+	+
arcuate n.	+	+	+	+
lateral hypothalamic area	+	+	+	+
mammillary bodies	+	+	+	+
Thalamus: anterior n.			+	+
periventricular n.	+	+	+	+
Habenula			+	+
Interpeduncular n.	+	+	+	+
Substantia nigra			+	+
Inferior colliculus	+	+	+	+
Periaqueductal gray	+	+	+	+
N. raphe dorsalis	+	+	+	+
N. locus coeruleus			+	+
Parabrachial n.	+	+	+	+
Trigeminal sensory nn.			+	+
N. raphe magnus	+	+	+	+
N. reticularis paragigantocellularis	+	+	+	+
Vestibular nn.	+	+	+	+
N. tractus solitarius	+	+	+	+
Spinal cord	+	+	+	+

in perikarya, processes and terminals of neurons, whereas the immunoreactive signal of BAM-22P was for the most part confined to neuronal perikarya. Furthermore, the immunoreactive signal obtained by the anti- Leu-enkephalin serum was completely blocked by the addition of excess Leu-enkephalin but not BAM-22P. Likewise, while BAM-22P blocked the immunoreactive signal of anti-BAM-22P serum, neither Leu- nor Met-enkephalin demonstrated an effective blockade of that signal.

DISCUSSION

Colchicine-pretreated rats have been used to investigate the comparative distribution of Leu-enkephalin and BAM-22P in the central nervous system.

Careful cross-blocking studies confirmed the specificity of each antiserum for its particular peptide. Treatment with large doses of colchicine allowed the visualization of immunoreactivity in neuronal perikarya otherwise undetectable in rats treated with lower doses or without colchicine (10). Leu-enkephalin immunoreactivity was also enhanced in processes and terminals of neurons. In contrast, BAM-22P immunoreactivity was mainly confined to perikarya, although faint terminal-like or diffuse immunoreactivity was also noted. This observation might be due to the fact that BAM-22P is a part of the enkephalin precursor molecule and might, therefore, be more concentrated within neuronal perikarya. When compared through serial section analysis, Leu-enkephalin and BAM-22P immunoreactive perikarya, fibers and terminals appeared to be similarly distributed in many brain sites. This observation is suggestive of similarity between the adrenal medullary enkephalin precursor (11-13) and the enkephalin precursor in brain. We are presently investigating whether these two immunoreactivities are co-localized within the same neurons. The present results indicate that at least some brain enkephalin biosynthesis might occur via a precursor similar or identical to the enkephalin precursor found in the adrenal medulla.

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