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DYNORPHIN IS LOCATED THROUGHOUT THE CNS AND IS OFTEN CO-LOCALIZED WITH ALPHA-NEO-ENDORPHIN

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Summary

The opioid peptide dynorphin has been described as widely distributed in CNS when measured by RIA. Our previous immunohistochemical studies have only demonstrated dynorphin cells as those containing AVP. We now report the specific localization of dynorphin throughout the neuraxis. Further, dynorphin and alpha-neo-endorphin have been co-localized to the same magnocellular neurosecretory cells in hypothalamus. We report agreement with the findings of others and extend them to include a cell group in dorsomedial hypothalmus, further strengthening the association between dynorphin and alpha-neo-endorphin.

The potent opioid peptide dynorphin has been the subject of intense anatomical, physiological and biochemical study. Its distribution and connectivity in CNS has been very difficult to precisely determine. Data from RIA studies have shown that dynorphin can be found throughout the neuraxis (1,2,3). Yet anatomical studies have reported a much more limited distribution (4,5,6,7). We previously demonstrated that dynorphin was located in vasopressin (AVP) containing cells of the supraoptic (SON) and paraventricular (PVN) nuclei. Recently with the purification and sequencing of dynorphin through residue number seventeen (8,9), it has been possible to develop antisera against the COOH-terminus of dynorphin-17. The problem of potential cross-reactivity between dynorphin antisera and the several opioid peptides has been solved by developing an antiserum against a non-enkephalin fragment of dynorphin (i.e., residues 7-17).

We employed several antisera (against dynorphin-17 and dynorphin-(1-13) from Dr. Avram Goldstein, and dynorphin-(7-17)) in these studies. All animals were Sprague-Dawley rats either pretreated with colchicine (400 ug, icv, 48 hrs prior to sacrifice) or untreated. Immunocytochemistry was carried out as reported elsewhere (4).

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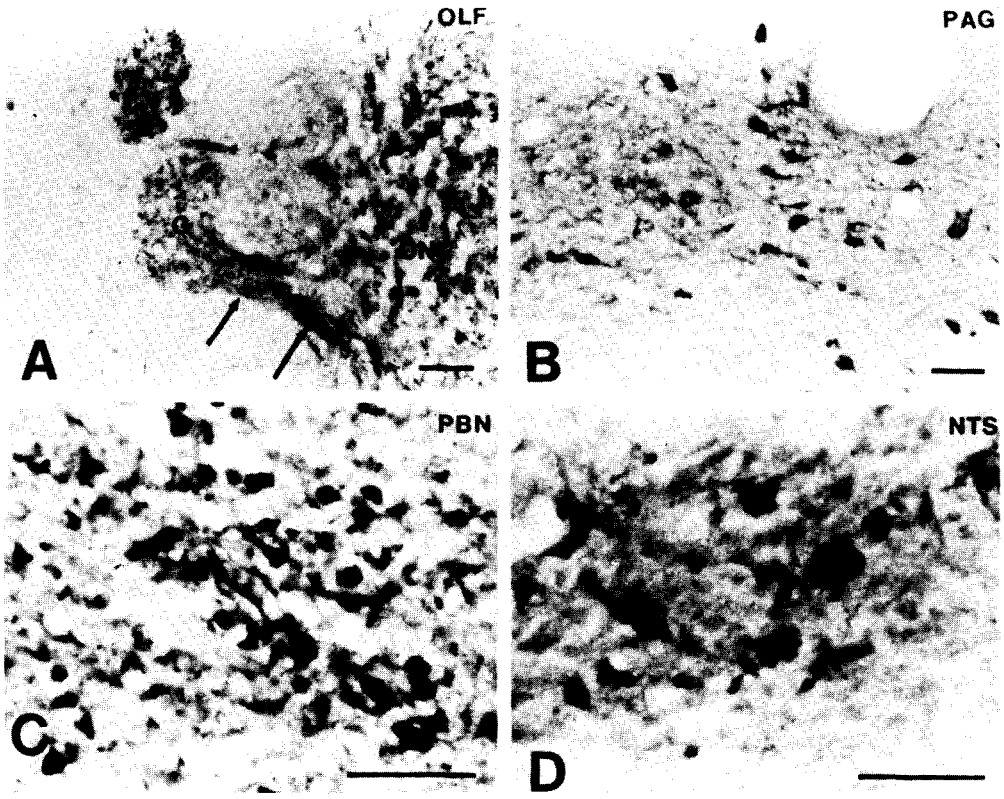

FIG. 1

Dynorphin is distributed widely throughout the neuraxis. For example, immunoreactive dynorphin terminals are localized to some olfactory bulb (OLF) glomeruli (A), while dynorphin-positive perikarya and fibers are seen in the periaqueductal gray (PAG) (B), lateral parabrachial nucleus (PBN) (C), and nucleus tractus solitarius (NTS) (D). Arrows in (A) point to the connection between the olfactory nerve layer (ONL) and a glomerulus (G). Bar is 50 um.

The results of the use of all three types of dynorphin antisera are highly consistent. They demonstrate a broad set of dynorphin positive cells and fibers (see Fig. 1) at every level of the neuraxis (10). For example, some of the glomeruli of the olfactory bulb are stained for dynorphin, where as others are not. Fibers are seen in the globus pallidus; cells and fibers are seen in the central nucleus of the amygdala with a heavy set of fibers in the stria terminals. Within the hippocampal formation, dynorphin-positive fibers are seen in the dentate gyrus and mossy fiber projection to areas CA3 and CA2. The hypothalamus has a distribution of cells and fibers, reported previously by us, to be magnocellular in origin (4,5). Further, we have been able to see a large dynorphin-positive cell group

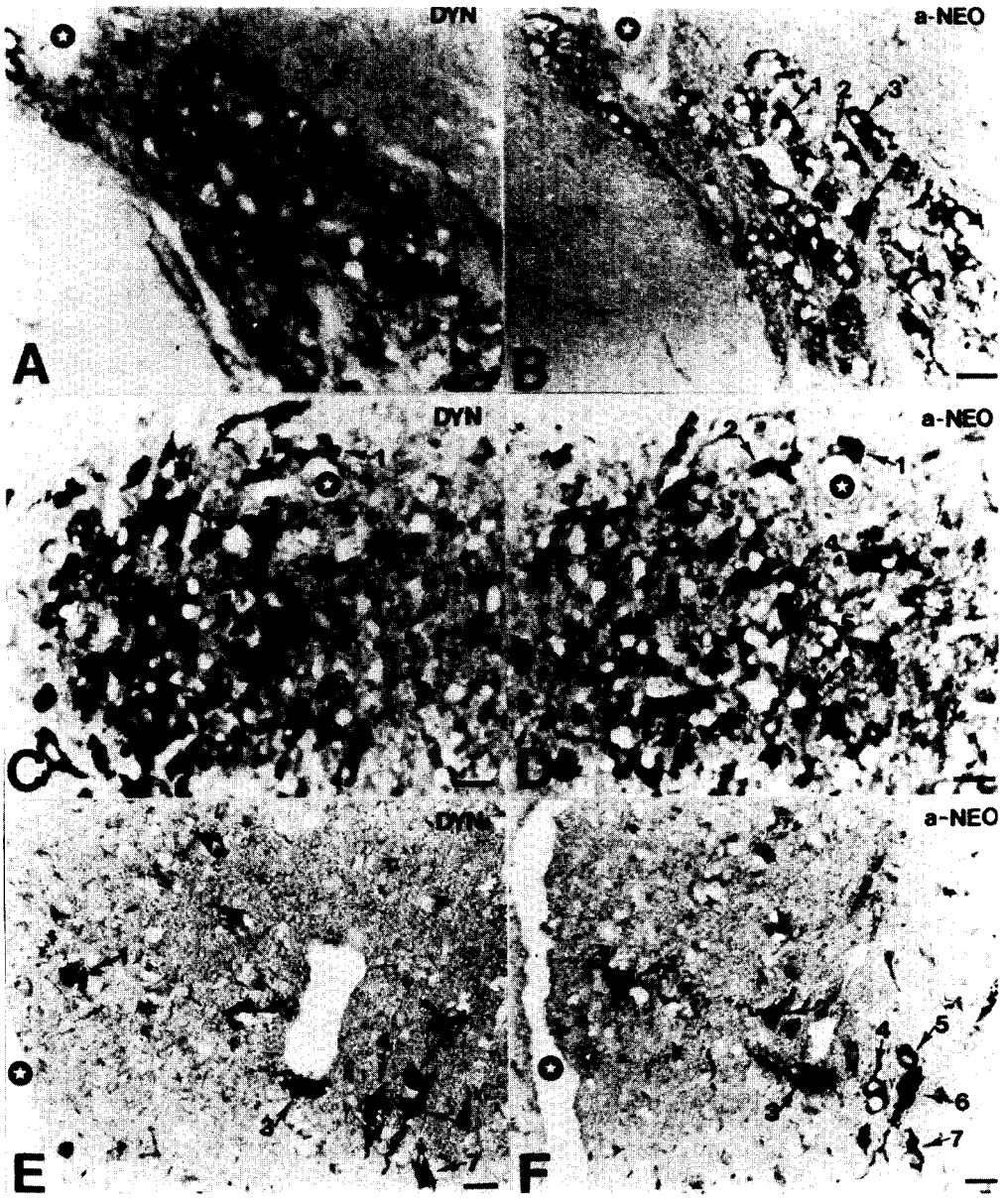

FIG. 2

Dynorphin (A,C,E) and alpha-neo-endorphin (B,D,F) are co-localized in the same cells of the supraoptic nucleus (SON) (A,B), paraventricular nucleus (PVN) (C,D) and the cells in the dorsomedial/lateral hypothalmic areas (E,F). The same numbered cells can be seen stained with both antisera in A and B; C and D; and E and F. Stars are in common vessels for each area. Bar is 20 um.

in the posterior hypothalamus beginning in the dorsomedial nucleus extending laterally past the fornix into the lateral hypothalamic area. Fibers were also visible in the ventromedial nucleus, with cells and fibers appearing in the arcuate nucleus. Progressing caudally, one can see heavy fiber staining in the zona reticulata of the substantia nigra and cells in the ventral aspect of the PAG. At the level of the fourth ventricle, it is possible to stain cells in the n. parabrachialis. Other brain stem and spinal areas included cells in the nucleus tractus solitarius, n. cuneatus, and dorsal horn. All demonstrations were blockable with dynorphin-17 and not with any other opioid peptide tested.

The other aspect of this paper involves the anatomy of alpha-neo-endorphin structures. Immunocytochemical studies of alpha-neo-endorphin place it in the posterior pituitary, SON and PVN with AVP and dynorphin (6,7). While it has a very similar distribution to that of dynorphin in the magnocellular nuclei, the biosynthesis of this peptide is not as yet clearly linked to dynorphin. Many peptides are co-localized in the same neurons but not synthesized from the same precursor. However, co-localization of peptides in several different cell groups may provide stronger evidence for synthesis from the precursor. Accordingly, we have studied alpha-neo-endorphin and dynorphin in both the magnocellular nuclei as well as the dorsal medial-lateral cell groups. As shown in Fig. 2, both peptides can be localized to both cell types. These data provide increased support for the hypothesis of a common biosynthetic route for dynorphin and alpha-neo-endorphin.

Acknowledgement

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