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DISTRIBUTION OF IMMUNOREACTIVE DYNORPHIN IN DISCRETE BRAIN NUCLEI; COMPARISON WITH VASOPRESSIN

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The distribution of the opioid peptide, dynorphin, has been studied in discrete, microdissected hypothalamic nuclei, and compared with the distribution of vasopressin. Both peptides were found in relatively high concentration in the supraoptic and paraventricular nuclei. However, dynorphin-like immunoreactivity (DYN-LI) was much more widely distributed in the hypothalamus than vasopressin-like immunoreactivity, with highest concentrations in the anterior hypothalamic and ventromedial nuclei. DYN-LI was also observed in some extra-hypothalamic structures; concentrations which were comparable to the highest levels in the hypothalamus were found in the tractus diagonalis and the nucleus interstitialis stria terminalis. Among the three brainstem nuclei examined, DYN-LI levels were highest in the sensory nucleus of the trigeminal nerve.

The potent opioid peptide, dynorphin, was initially isolated from porcine pituitary [7, 8]. It is present in highest concentration in the pars nervosa of pituitary [6], and recent immunocytochemical studies have shown that it is localized in the vasopressin-containing cells of the supraoptic and paraventricular nuclei of hypothalamus [19]. However, a more detailed quantitative analysis of its distribution in both hypothalamic and extra-hypothalamic structures is not presently available. Interest in the possible physiological functions of this peptide is heightened by the demonstration that it is a selective ligand of the *kappa* subclass of opioid receptors [2]. We have therefore measured the dynorphin content of microdissected rat brain nuclei using a specific radioimmunoassay. Because of the association of dynorphin with vasopressin in the hypothalamo-hypophysial tracts we have also measured the concentration of vasopressin in each sample. Nuclei from the hypothalamus and selected extra-hypothalamic sites have been examined. Emphasis has been placed on structures implicated in cardiovascular regulation, because of recent interest in the roles of opioid peptides in this function [10, 16].

Male Sprague–Dawley rats, 14–16 weeks old, were killed by decapitation between 09.00 and 10.00 h. The brain was quickly removed and immediately frozen on dry ice. The brains were mounted on specimen plates, and 300 μm coronal sections were

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cut on a cryostat at −8°C. Specific areas from the sections were then microdissected by the technique of Palkovits [13]. Forebrain nuclei were punched with a 500 μm cannula (internal diameter), except for the amygdala nuclei, nucleus caudatus-putamen and the hippocampus, which were punched with a 1 mm cannula. The number of punches per rat has been described previously [4]. Hindbrain nuclei were punched with a 500 μm cannula as follows: the nucleus tractus solitarius was punched at the level of the obex, P = 6.6, and at one slice caudal to this level, P = 6.8 [13]. Two punches were obtained from each slice. The nucleus ambiguus was punched out from two slices between P = 6.0 and P = 5.6 [14], and the sensory nucleus of the trigeminal nerve was also punched from these slices. The reported values are means ± S.E.M. from 5 estimations, each estimate being based on duplicate aliquots from extracts of bilateral punches from two rats.

Tissue punches were stored frozen in closed tubes at −70°C until extraction by heating in 250 μl 0.1 M acetic acid at 95°C for 10 min. The samples were heated at 95°C for 10 min, and sonicated for 15 sec. An aliquot was taken for protein determination by a modification of the Lowry method [15]. Triton X-100 was added to the suspension, to a final concentration of 0.1%, and the mixture was centrifuged at 10,000 *g* for 3 min. Duplicate aliquots of the supernatant were taken for radioimmunoassay for dynorphin [5], using dynorphin$_{1-13}$ as standard, and for vasopressin, using an antiserum obtained from Calbiochem-Behring (San Diego, CA) and Arg^8-vasopressin as standard. Dynorphin$_{1-17}$ [8], dynorphin$_{1-13}$, and dynorphin$_{1-12}$, [5] have identical cross-reactivities in the dynorphin RIA. Larger putative precursors of dynorphin are also active. Dynorphin$_{1-11}$, dynorphin$_{1-10}$, and dynorphin$_{1-9}$ have measurable, but less than 10% cross-reactivity [5]. Shorter fragments of dynorphin, an analogue of α-neoendorphin, enkephalins, and β-endorphin do not have significant cross-reactivity [5]. Oxytocin cross-reactivity in the vasopressin assay was less than 1%.

As expected, high concentrations of vasopressin-like immunoreactivity (AVP-LI) were observed in the supraoptic and paraventricular nuclei of hypothalamus (Fig. 1). The concentrations of AVP in these nuclei are similar to levels reported in Wistar rats [9], but lower than those found in some other rat strains [4]. AVP-LI levels in the nucleus suprachiasmaticus and the nucleus anterior hypothalami were about 15% of the levels in the supraoptic and paraventricular nuclei. Lower levels were observed in all other structures examined. In contrast, dynorphin-like immunoreactivity (DYN-LI) showed a much wider distribution. DYN-LI was present in the supraoptic and paraventricular nuclei at about 1% of the concentration of AVP-LI. Similar levels of DYN-LI (about 4% of the concentrations of AVP-LI) were also found in the nucleus suprachiasmaticus. Thus, the association of dynorphin with vasopressin-containing structures [19] is confirmed by this study. However, other hypothalamic and extra-hypothalamic structures contained higher levels of DYN-LI (Figs. 1 and 2). In the hypothalamus, highest levels were observed in the anterior hypothalamic nucleus and the nucleus ventromedialis, but levels in the nucleus

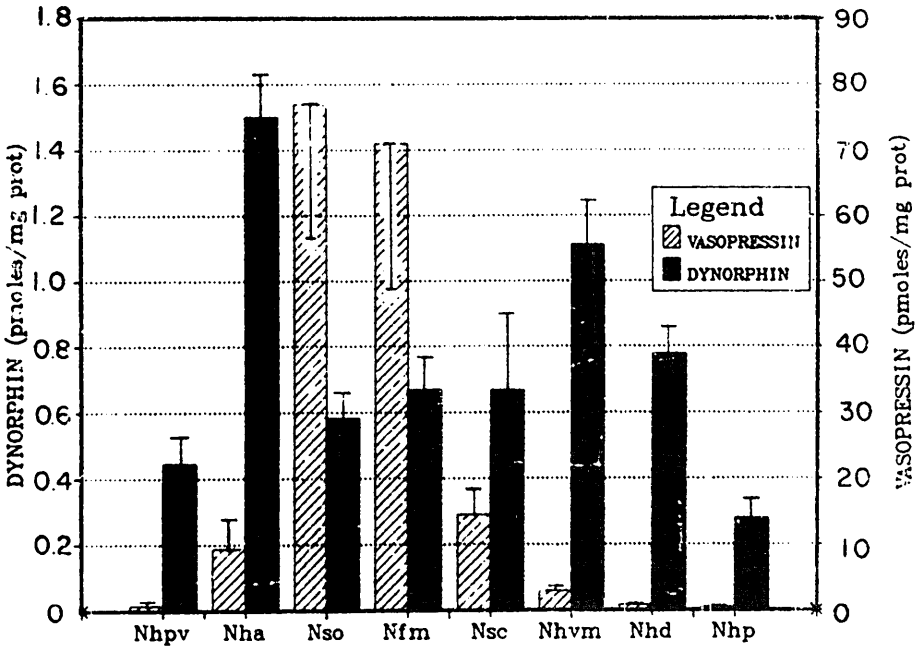

Fig. 1. Dynorphin and vasopressin immunoreactivity in selected hypothalamic nuclei. Means and S.E.M. from 5 samples, each of pooled tissues from two rats are presented. Values refer to total DYN-LI or AVP-LI, expressed relative to the protein content of each tissue. Abbreviations: Nhpv, nucleus periventricularis (hypothalami); Nha, nucleus anterior (hypothalami); Nso, nucleus supraopticus; Nfm, nucleus paraventricularis (filiformis) magnocellularis; Nsc, nucleus suprachiasmaticus; Nhvm, nucleus ventromedialis (hypothalami); Nhd, nucleus dorsomedialis (hypothalami); Nhp, nucleus posterior (hypothalami).

periventricularis and the nucleus dorsomedialis were also comparable to those in the supraoptic and paraventricular nuclei. These results differ from the initial immunocytological studies of DYN-LI distribution in brain which demonstrated intense staining of supraoptic and paraventricular magnocellular neurons, but reported only light and diffuse fiber staining in other areas [18]. The difficulty in localizing dynorphin in structures other than the vasopressin-containing magnocellular neurons by immunocytological techniques might arise from differences in the storage organelles in different cell types. Studies of the subcellular distribution of DYN-LI and AVP-LI in homogenates of neurointermediate lobe of pituitary and hypothalamus indicate that the properties of the storage organelles for DYN-LI are different in the two tissues [12]. A recent paper [20], using an antiserum raised against dynorphin$_{1-17}$, reports a wider distribution of dynorphin immunoreactive fibers in both hypothalamic and extra-hypothalamic structures. Again, in this immunocytological study, dynorphin-containing cell bodies appear to be restricted to the magnocellular neurons of the supraoptic and paraventricular and accessory nuclei [20].

The presence of high levels of DYN-LI in the anterior hypothalamus may have

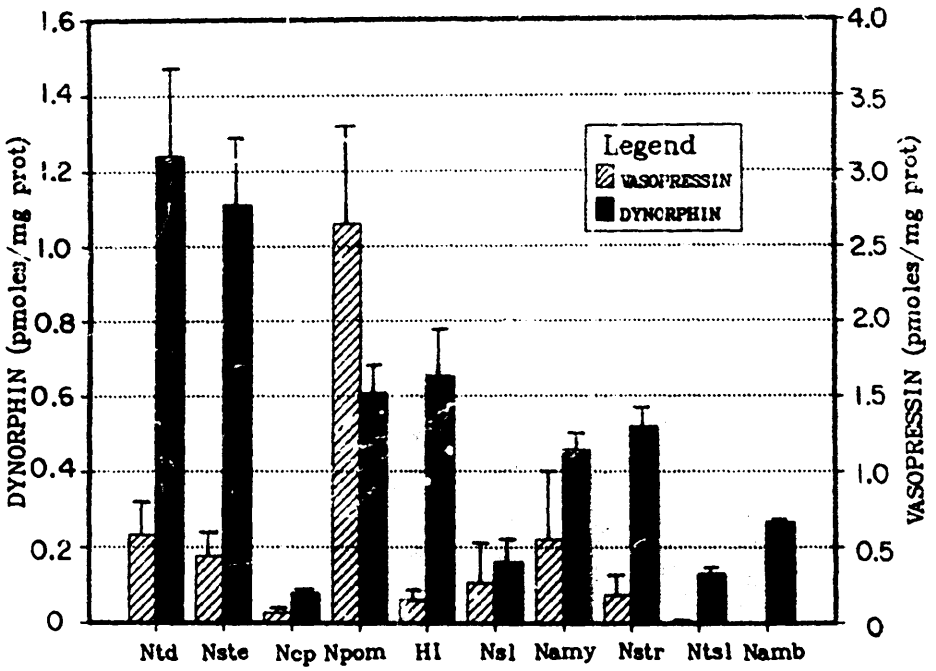

Fig. 2. Dynorphin immunoreactivity in other selected brain regions. For details refer to Fig. 1. Note that the AVP-LI scale has been expanded roughly 20-fold to record the low levels of AVP-LI present in these extrahypothalamic structures. Abbreviations: Ntd, nucleus tractus diagonalis (Broca); Nste, nucleus interstitialis stria terminalis; Ncp, nucleus caudatus putamen; Npom, nucleus preopticus medialis; HI, hippocampus; Nsl, nucleus septi lateralis; Namyg, nuclei amygdaloidei; Nstr, nucleus sensorius trigeminalis; Ntsl, nucleus tractus solitarius; Namb, nucleus ambiguus.

functional significance, since recent studies have demonstrated that localized injections of dynorphin into this region in anesthetized rats produce cardiac deceleration and a fall in blood pressure, while injections in other regions have little effect [3]. A possible role for dynorphin in the hypothalamic regulation of the cardiovascular system is therefore possible.

A number of extra-hypothalamic areas were also examined. Levels of DYN-LI comparable to the highest levels in hypothalamus were also found in the nucleus of the tractus diagonalis and in the nucleus of the stria terminalis. Levels of DYN-LI in the hippocampus, median preoptic nucleus and the amygdaloid nucleus were lower, but comparable to the levels observed in supraoptic and paraventricular nuclei. The lowest concentrations of DYN-LI were in the caudate putamen, confirming the previous report by Goldstein and Ghazarossian [6]. DYN-LI was also relatively low in the lateral septum.

Among the brainstem structures examined, levels of DYN-LI were highest in the sensory nucleus of the trigeminal nerve where they were comparable to the level observed in supraoptic and paraventricular nuclei of the hypothalamus. The association of dynorphin with a sensory nucleus is not surprising since relatively high levels

of the peptide have previously been reported in the dorsal horn of spinal cord [1]. Levels of DYN-LI in the nucleus tractus solitarius and the nucleus ambiguus were somewhat lower.

Our results demonstrate that DYN-LI is distributed widely in brain, thus confirming earlier reports of a widespread distribution in grossly dissected brain regions [6, 11]. Hypothalamic DYN-LI levels observed in the present study exceed by as much as 10-fold (assuming 100 mg protein per g tissue wet weight) the levels reported in grossly dissected anterior and posterior hypothalamus [6]. It would appear that within the hypothalamus, DYN-LI is concentrated in selected discrete nuclei. In tissues such as the caudate-putamen, which are more homogeneous at the level of analysis employed here, the observed DYN-LI levels are comparable in the two studies [6]. There is no clear parallelism in the distributions of DYN-LI that we have observed, and of β-endorphin or enkephalins as determined by immunocytological techniques. Thus the caudate-putamen and the lateral septum of the rat contain relatively high levels of enkephalins, and the septum also contains β-endorphin [17], but levels of DYN-LI are low in these tissues. In contrast, in the nucleus of the tractus diagonalis, both enkephalins [17] and DYN LI are present in relatively high amounts, while in the anterior hypothalamus enkephalin and β-endorphin levels are low [17], but DYN-LI is present in high concentration. These results are consistent with the view that each set of opioid peptides has discrete distribution and function in the central nervous system.

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