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Binding sites for melanotropins and related peptides in rat brain

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Systemic or intracerebroventricular administration of melanotropic derivatives of pro-opiomelanocortin (POMC), in particular injection of alpha-melanocyte-stimulating hormone (alpha-MSH) or gamma-MSH influences brain development, adult behavior and autonomic functions. Peptide injections may mimick either effects of circulating hormones secreted by pituitary pars intermedia, or actions of peptides released from central nerve terminals. In brain, true melanotropins are localized in POMC-containing neurons. In a separate group of neurons, a peptide (NEI) with a C-terminal region similar to alpha-MSH has been identified in the precursor of melanin-concentrating hormone (MCH; Nahon et al., 1989) these neurons are immunoreactive to alpha-MSH antibodies.

We have conducted a search for binding sites of melanotropins or related peptides in the brain of male Long Evans rats using 3H(Nva 13)-alpha-MSH synthesized according to Eberle and Zeller (1985). In vitro autoradiography revealed a high density of binding sites in hippocampus and certain cortical areas (Lichtensteiger, Schlumpf and Eberle, 1987), i.e., in regions devoid of POMC terminals but receiving projections of the MCH neuron system. Additional sites are observed in subcortical areas where an overlap with POMC terminals seems possible. So far, the characterization of binding sites focussed on a hippocampal membrane preparation. In this region, competition studies indicated the presence of high and low affinity binding sites for alpha-MSH (Kd = 8 nM and 5 μM, respectively; Kd of the tritiated analog for the high affinity site = 3.4 nM (Scatchard analysis)). Lower affinities were found for desacetyl-alpha-MSH and for gamma 1-MSH which displays only weak activity on melanocytes. Unless circulating alpha-MSH reaches the hippo-campus, these sites may represent receptors for a peptide released by the MCH/MSH-like neuron system rather than for alpha-MSH derived from POMC. Since the peptide found in the MCH precursor resembles alpha-MSH in the C-terminal part, it is interesting to note that the C-terminus of alpha-MSH, Lys Pro Val NH2, showed a relatively high affinity. ACTH 4-10 containing the central message sequence bound only to one site with low affinity. No binding was seen with Lys D-Pro Thr, an interleukin 1 beta 193-195 analog (S.H. Ferreira et al., 1988). The significance of the C-terminus for central effects of MSH-type peptides is supported by earlier observations on dopamine neurons (Lichtensteiger and Monnet, 1979) and antipyretic action (Richards and Lipton, 1984).

In conclusion, hippocampus contains low and high affinity binding sites for MSH-type peptides with different characteristics. The subcortical binding sites are presently being analyzed.

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Long-term administration of fluvoxamine antagonizes the inhibitory effect of neuropeptide Y but not the clonidine effect on isolated rat vas deferens

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It is widely accepted that neuropeptide Y (NPY) has a modulatory effect on noradrenergic transmission (AGNATI et al., 1983). The importance of noradrenergic systems in the biochemical actions of antidepressant drugs and

biochemical basis of depression is likewise accepted. Thus, it is possible that NPY play a role in the mechanism of action of antidepressant drugs. Moreover, it has been reported that central levels of NPY are decreased in affective disorders, and they increase in parallel to recovery of the symptoms (Widerlov et al., 1988). In view of this possibility, we studied the functional interaction between long-term treatment (10 mg/kg ip, twice a day for 14 days) and acute treatment (10 mg/kg ip, 12 h before experiment) with fluvoxamine (an antidepressant drug inhibitor of serotonin uptake) (FVX) and the inhibitory effect of NPY and clonidine (CND) on isolated rat vas deferens under field stimulation conditions (30 V, 0.1 Hz, width 1 msec). The results obtained show that long-term but not acute administration of FVX antagonizes this effect of NPY without affecting the inhibitory actions of CND (table 1).

Table 1

Effect of long-term and acute administration of FVX on the IC_{50} of NPY and CND in isolated rat vas deferens under field stimulation conditions (means ± S.E.M.). The results are expressed in nM.

	Saline 12 h	Saline 14 d	FVX 12 h	FVX 14 d
NPY	25.9 ± 0.48	32.8 ± 0.68	47.7 ± 1.84	180.4 ± 5.92 [a,b]
	(n = 9)	(n = 6)	(n = 4)	(n = 5)
CND	3.04 ± 0.47	2.28 ± 0.91	1.29 ± 0.31	3.72 ± 1.62
	(n = 10)	(n = 5)	(n = 9)	(n = 5)

[a] $p = 0.032$ versus FVX 12 h. [b] $p < 0.002$ versus saline 14 d. The statistical significance has been obtained using the Mann-Whitney U test.

These results show that the inhibitory actions of NPY on isolated rat vas deferens could be antagonized by FVX through a modulatory action on NPY binding sites and rule out possible implications of presynaptic alpha$_2$-adrenergic receptors.

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Induction of neuropeptide Y (NPY) precursor gene expression on membrane depolarization

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Neuropeptide Y (NPY), one of the most abundant neuropeptides, is widely distributed in the central and peripheral nervous systems and also an important neuromodulator/cotransmitter of catecholaminergic neurons. The basic functions of NPY are both the inhibition of release of neurotransmitters and hypothalamic hormones through Gi and Go proteins (presynaptically) and direct/indirect effects through the postsynaptic NPY receptors, e.g. vaso-constriction. Thus the synaptic neurotransmission (especially catecholaminergic) can be modulated by the quantitative change in NPY through these mechanisms. In order to elucidate the regulatory mechanisms of neuron-specific NPY expression, first we cloned and sequenced rat prepro-NPY cDNA. Using this cDNA probe, we have examined the tissue-specific expression of the NPY gene. The distributions of prepro-NPYmRNA (NPYmRNA) and NPY immunoreactivity in brain areas and other peripheral tissues are in good agreement, indicating that the regulation of NPY abundance is determined primarily on the level of the NPY gene expression. Next, in this paper we studied the changes of neuron-specific NPY precursor gene expression on the membrane depolarization in the clonal neural cells.

NG108-15 cells and PC12 cells were cultured at 37°C in 75 cm^2 flasks containing 25 ml medium with humidified