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Synthesis and Binding Affinity of Neuropeptide Y at Opiate Receptors

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Abstract—Neuropeptide Y and several metabolic fragments were synthesized and evaluated for binding affinity at non-selective opiate receptors. Neuropeptide Y and several C-terminal fragments were shown to bind to non-selective opiate receptors with an affinity similar to that of Leu-enkephalin.

Neuropeptide Y (NPY, Fig. 1) is a 36 amino acid C-terminally amidated peptide originally isolated from porcine brain in 1982.[1] Neuropeptide Y and its fragments are widely distributed in the human central and peripheral nervous systems. Within the central nervous system, NPY is involved in appetite, stress and blood pressure regulation.[2] While in the peripheral nervous system, NPY has been shown to be both a potent and long lasting vasoconstrictor, acting as a co-transmitter and modulator at norepinephrine neurons.[3] Recent behavioral studies also indicate NPY has an analgesic effect and shows the production of an anxiolytic effect in animal models of anxiety.[4]

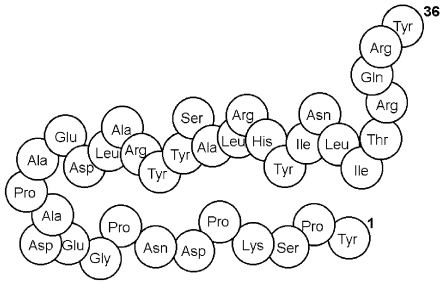

Figure 1. Structure of human neuropeptide Y.

Post-secretory processing of NPY does not terminate its biological activities like many other neuropeptides. Several neuropeptidases have been identified that process NPY producing specific fragments of the parent biomolecule.[5–7] The fragments of NPY generated from post-secretory processing show strong affinity for the subtypes of the NPY receptor. Although, a significant amount of biological data exists for the interaction of NPY and the fragments at the NPY receptors, very little is known about their interactions at other receptors in the central nervous system. These possible interactions may further define the role of neuropeptide Y as a neurotransmitter and neuromodulator within the nervous system.

Neuropeptide Y can produce a powerful antinociceptive effect following direct application to the spinal cord of rats.[8] The antinociceptive effects of NPY could possibly be mediated via presynaptic inhibition of the release of substance P and other coexisting transmitters from primary afferent nerve terminals.[9] However, like for opiates, there is little in vivo evidence for the involvement of intrinsic NPY in the direct regulation of substance P content and release in primary afferent nerve fibers. Thus, the antinociceptive effects of neuropeptide Y are particularly intriguing. At first pass, one may look to interactions at NPY receptor subtypes per se to account for these effects. However, the possibility of an interaction of NPY with opiate receptors has not been previously entertained despite recent experimental evidence that naloxone, a known opiate receptor antagonist, blocks the anxiolytic effects of NPY.[10] In addition,

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recent findings have indicated that icv application of NPY causes immunostimulation similar to that observed with Met-enkephalin.[11] Therefore, to further elucidate the role of NPY in the central nervous system (CNS) we undertook a study of the binding affinity of NPY and several metabolic fragments at opiate receptors.

In general, the original hypothesis these experiments were based on was naive. Since the first five residues of the N-terminal portion of NPY [H-Tyr-Pro-Ser-Lys-Pro] had a similar primary sequence with other known opiate peptides, like morphiceptin [H-Tyr-Pro-Phe-Pro-NH_2], the concept that NPY might also bind to the opiate receptors seemed plausible. In fact, it is clear from the present study that NPY does bind to opiate receptors and suggests a possible role for the regulation or modulation of NPY's antinociceptive effects by direct interaction of NPY at opiate receptors.

Table 1 shows the binding data for neuropeptide Y, six fragments generate by in vitro post-secretory peptidase processing,[5,6] and two reference peptides with known opiate receptor activity. As can be seen from these data the parent NPY molecule and two of the three N-terminal fragments (NPY [1–5] and NPY [1–6]) all showed similar binding affinity at non-selective opiate receptors to the known opiate receptor peptide leu-enkephalin. This confirmed our original hypothesis that primary sequence resemblance was sufficient to provide a peptide with opiate binding affinity. However, the binding of two of the three C-terminal fragments (NPY [22–36] and NPY [24–36]) of neuropeptide Y was unexpected since none of these fragments contain an N-terminal tyrosine the common 'message' residue in opiate peptides. However, the fragments do have a C-terminal amidated tyrosine residue that might account for their binding affinity at the non-selective opiate receptors. In contrast to our results with the opiate receptors, preliminary studies examining the binding affinity of opiate peptides at NPY Y1 and Y2 receptors showed no activity for either leu-enkephalin or morphiceptin at these receptor subtypes.[14]

In addition to determining the binding affinity of the peptides at the non-selective opiate receptors preliminary subtype selectivity was determined for NPY and four active fragments (Fig. 2). These data indicate that, while modest, the five peptides all show selectivity for the μ-subtype of the opiate receptor. However, at this relatively high concentration it is not clear why such modest inhibitions are observed, and may reflect the heterogeneous nature of the receptors in the non-selective opiate receptor binding assays. With this in mind, these data still, in combination with the binding affinity data at the non-selective opiate receptors, clearly indicate a potential 'crosstalk' between NPY and the opiate receptors and supports its possible significance in the regulation or modulation of the antinociceptive actions of neuropeptide Y.

Table 1. Non-selective opiate receptor binding affinities for NPY and fragments

Compd[a]	IC_{50} (μM)[b]	K_i (μM)[b]
NPY [1–36]	9.4	4.7
NPY [1–5]	15.0	7.5
NPY [1–6]	1.8	1.0
NPY [1–8]	NA	NA
NPY [22–36]	10.0	5.1
NPY [24–36]	6.9	3.9
NPY [28–36]	NA	NA
Leu-enkephaline	9.5	4.8
Morphiceptin	0.32	0.15

[a]See ref 12.
[b]Binding assays conducted by MDS Pharma Services, Bothell WA, USA, values are means of duplicate experiments conducted at 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M using a literature protocol.[13]
NA, not active.

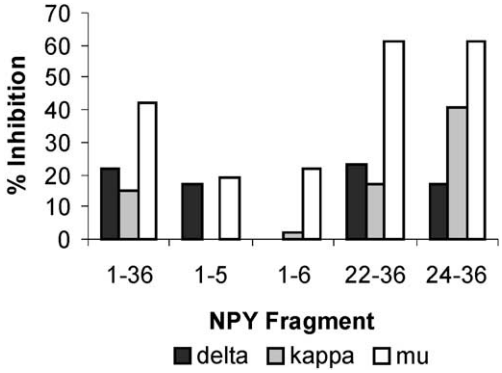

Figure 2. Opiate receptor subtype selective inhibition by NPY and four fragments. Inhibition assays conducted by MDS Pharma Services, Bothell WA, USA, values are means of duplicate experiments, at a concentration of 10^{-5} M.

This study represents efforts to determine if neuropeptide Y and its metabolic fragments act as ligands at the opiate receptors. The results presented indicate that NPY and four fragments bind to non-selective opiate receptors with modest activity similar to the endogenous opiate peptide leu-enkephalin. Additionally, in preliminary studies the parent NPY and NPY [22–36] show a strong inhibition at the μ-opiate receptor subtype. These data suggest a possible 'crosstalk' between NPY and the opiate receptors in its antinociceptive effects and warrants a further investigation of this intriguing mechanism of action.

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