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High levels of specific neuropeptide Y/pancreatic polypeptide receptors in the rat hypothalamus and brainstem

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Abstract

It is known that the paraventricular hypothalamic nucleus is responsible for some of the stimulatory effects of neuropeptide Y, peptide YY and the pancreatic polypeptides on food intake. However, specific neuropeptide Y Y_1 and Y_2 receptors were not abundantly expressed in the hypothalamus. In contrast, specific [^{125}I]human pancreatic polypeptide binding sites were detected in this hypothalamic nucleus as well as in the medial preoptic area, interpeduncular nucleus, nucleus tractus solitarius, area postrema and dorsal vagal nucleus while cortical areas and the hippocampus contained negligible levels of labeling. The ligand binding profile of the various competitors suggests that the binding sites labeled by [^{125}I]human pancreatic polypeptide are predominantly of the neuropeptide Y Y_4 and/or Y_5 subtypes. These newly cloned receptors may play a key role in the modulatory effects of neuropeptide Y and related peptides on appetite.

Keywords: Neuropeptide Y; Pancreatic polypeptide; Neuropeptide Y receptor, Y_4 and Y_5 subtype

Neuropeptide Y shares high sequence homology with peptide YY and the pancreatic polypeptides. The biological effects of this peptide family are mediated by at least six receptor subtypes (Wahlestedt and Reis, 1993; Bard et al., 1995; Gerald et al., 1996; Weinberg et al., 1996). The neuropeptide Y Y_1 receptor has high affinity for neuropeptide Y, peptide YY, [Leu^{31},Pro^{34}]neuropeptide Y and [Leu^{31},Pro^{34}]peptide YY, while long C-terminal fragments and pancreatic polypeptides are much less potent. Moreover, the biological responses induced by the activation of the neuropeptide Y Y_1 receptor subtype are blocked by (*R*)-*N*^{2}-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide (code name BIBP3226), a selective Y_1 antagonist (Rudolf et al., 1994). In contrast, the neuropeptide Y Y_2 receptor subtype has the following pharmacological profile: peptide YY ≥ neuropeptide Y ≥ neuropeptide Y-(2–36) = peptide YY-(3–36) ≥ neuropeptide Y-(13–36) = peptide YY-(13–36) ≫ [Leu^{31},Pro^{34}] substituted analogues = pancreatic polypeptides (Wahlestedt and Reis, 1993). The unique characteristic of the neuropeptide Y Y_3 receptor subtype relates to its high affinity for neuropeptide Y but not peptide YY derivatives and pancreatic polypeptides (Wahlestedt and Reis, 1993). Interestingly, the neuropeptide Y Y_4/PP_1 receptor subtype has very high affinity for pancreatic polypeptides (human, bovine and rat) and is also activated by peptide YY and [Leu^{31},Pro^{34}]peptide YY while neuropeptide Y is less potent (Bard et al., 1995). The recently cloned neuropeptide Y Y_5-'food intake' receptor subtype has a broad structure-activity relationship with high affinity for neuropeptide Y, peptide YY, neuropeptide Y-(2–36) and human pancreatic polypeptide (Gerald et al., 1996), while the most recently cloned neuropeptide Y 'Y_6' receptor has low affinity for pancreatic polypeptides but recognizes neuropeptide Y and peptide YY derivatives (Weinberg et al., 1996). Among these various receptor subtypes, the neuropeptide Y Y_1, Y_2, Y_4, Y_5 and 'Y_6' receptors have been cloned and all belong to the seven transmembrane domain receptor superfamily.

The respective anatomical localization of the neuropeptide Y Y_1-like and Y_2-like receptors has been reported for the rat (Dumont et al., 1993) and human (Widdowson, 1993) brains. In the rat central nervous system, neuropep-

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tide Y Y_1 sites are particularly abundant in cortical areas while the neuropeptide Y Y_2 receptor is more broadly distributed (Dumont et al., 1993). However, little or no information is currently available on the distribution of the other neuropeptide Y receptor subtypes in the mammalian brain. Accordingly, the aim of the present study was to investigate the discrete anatomical localization of pancreatic polypeptide binding sites in the rat brain because radiolabeled pancreatic polypeptide derivatives (especially human) demonstrate high affinity for both the neuropeptide Y Y_4 and/or Y_5 receptor subtypes.

[^{125}I]Rat pancreatic polypeptide and [^{125}I]human pancreatic polypeptide were iodinated and purified as described earlier (Dumont et al., 1993). The specific activity was assumed to be the theoretical value (2000 Ci/mmol). Rat brain sections were prepared as described by Dumont et al. (1993). Briefly, male Sprague-Dawley rats (225–250 g, Charles River, St-Constant, Quebec, Canada) were decapitated and their brains were removed and frozen in 2-methylbutane at −40°C. Sections (20 μm thick) were cut using a cryomicrotome at −17°C and were mounted on gelatin-chrome-alum-coated slides, dried overnight in a desiccator at 4°C, and then kept at −80°C until use. Adjacent coronal sections were preincubated for 60 min at room temperature in a Krebs-Ringer phosphate buffer at pH 7.4 and then incubated for 120 min in fresh Krebs-Ringer phosphate buffer supplemented with 0.1% bovine serum albumin (Sigma, St. Louis, MO, USA), 0.05% bacitracin (Sigma), 50 pM of either [^{125}I]human pancreatic polypeptide or [^{125}I]rat pancreatic polypeptide in the presence or absence of 1 μM human pancreatic polypeptide (nonspecific binding) or increasing concentrations of competitors such as human pancreatic polypeptide, rat pancreatic polypeptide, neuropeptide Y, peptide YY, [Leu^{31},Pro^{34}]peptide YY, peptide YY-(3–36) and BIBP3226. At the end of the incubation period, sections were washed in ice-cold Krebs-Ringer phosphate buffer and dried under a stream of cold air. Sections were then placed in X-ray cassettes and apposed against Hyperfilms (Amersham, Mississauga, Ontario, Canada) alongside [^{3}H]-microscales standards (Amersham) for 8 days. Films were developed using the standard procedure and measurements of autoradiographic signals were made using a MCID image analysis system (Imaging Research, St-Catherines, Ontario, Canada).

In a series of preliminary experiments, we observed that [^{125}I]human pancreatic polypeptide binding in various regions of the rat brain was highly sensitive to human pancreatic polypeptide (IC_{50}: 0.01–0.05 nM) > rat pancreatic polypeptide (0.2–17 nM) > [Leu^{31},Pro^{34}]peptide YY (0.6–20 nM) > peptide YY (> 250 nM) > neuropeptide Y (> 500 nM) > peptide YY-(3–36) (> 1000 nM) ≫ BIBP3226 (> 10000 nM). This ligand selectivity profile was clearly distinct from that of either the neuropeptide Y Y_1, Y_2, Y_3 and 'Y_6' receptor subtypes. It thus suggests that under our assay conditions [^{125}I]human pancreatic polypeptide and [^{125}I]rat pancreatic polypeptide likely label the neuropeptide Y Y_4 and/or Y_5 receptor subtypes. Further studies using, for example, [D-Trp^{32}]neuropeptide Y (Gerald et al., 1996) will be required to precisely determine the proportion of each of these two receptor subtypes that are labeled under the assay conditions used here.

As shown in Fig. 1, specific [^{125}I]human pancreatic polypeptide binding sites are discretely distributed in the rat brain. [^{125}I]Human pancreatic polypeptide labeling was found in the medial preoptic area, paraventricular nucleus of the hypothalamus, interpeduncular nucleus, nucleus tractus solitarius, area postrema and dorsal vagal nucleus, with the highest concentrations seen in the interpeduncular nucleus and area postrema. Similar results were obtained using [^{125}I]rat pancreatic polypeptide, albeit with a lower signal-to-noise ratio. Hence, in contrast to the distribution of the neuropeptide Y Y_1 and Y_2 receptors (Dumont et al., 1993), [^{125}I]human pancreatic polypeptide binding sites are not enriched in cortical and hippocampal areas. Interestingly, however, [^{125}I]human pancreatic polypeptide binding sites were detected in a few hypothalamic nuclei involved in the regulation of food intake behaviors. It thus suggests a unique role for these receptors in the potent effects of neuropeptide Y and homologues on appetite (Stanley and Leibowitz, 1984). Furthermore, in situ hybridization studies of the neuropeptide Y Y_4 (Bard et al., 1995) and Y_5 (Gerald et al., 1996) receptor mRNAs have revealed the enrichment of both messages in the rat hypothalamus, including the paraventricular nucleus.

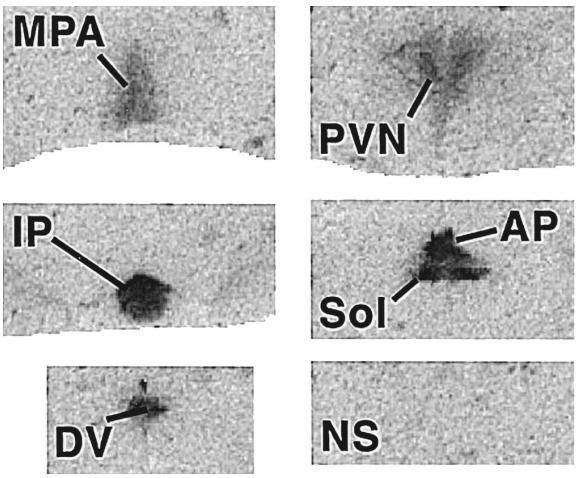

Fig. 1. Photomicrographs of the autoradiographic distribution of [^{125}I]human pancreatic polypeptide binding sites in coronal sections of the rat brain. Adjacent coronal sections were incubated with 50 pM [^{125}I]human pancreatic polypeptide in the presence and absence of 1 μM human pancreatic polypeptide (non-specific binding; NS). Abbreviations: AP, area postrema; DV, dorsal vagal nucleus; IP, interpeduncular nucleus; MPA, medial preoptic area; PVN, paraventricular nucleus of the hypothalamus; Sol, nucleus tractus solitarius. NS represents the amounts of non-specific binding detected at the level of the PVN. Similar results were obtained in all other brain regions (not shown).

In summary, specific [^{125}I]human pancreatic polypeptide and [^{125}I]rat pancreatic polypeptide are very discretely distributed in the rat brain, with enrichment in certain hypothalamic nuclei asssociated with food intake. The ligand selectivity profile of these sites suggests the labeling of the neuropeptide Y Y_4 and/or Y_5 receptor subtypes.

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