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Pharmacological characterization of neuropeptide Y-(2–36) binding to neuropeptide Y Y_1 and Y_2 receptors

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Abstract

Neuropeptide Y-(2–36) has been reported by several research groups to be a more potent orexigenic agent than intact neuropeptide Y. Therefore, it has been proposed that a novel 'Y_1 variant' may modulate ingestive behavior. To define the receptor subtype involved in neuropeptide Y-stimulated feeding behavior, we evaluated the binding properties of neuropeptide Y-(2–36) and [^{125}I]neuropeptide Y-(2–36) in established neuropeptide Y_1 and Y_2 containing cell lines and tissues. Neuropeptide Y-(2–36) displaced [^{125}I]peptide YY binding to SK-N-MC cells (neuropeptide Y Y_1 receptors) with a K_i of 3.69 nmol and SK-N-BE(2) cells (neuropeptide Y Y_2 receptors) with a K_i of 3.08 nmol. Neuropeptide Y-(2–36) also displaced [^{125}I]peptide YY binding to rat cerebral cortex, hippocampus and olfactory bulb with similar affinities. To examine the brain distribution of [^{125}I]peptide YY, [^{125}I]neuropeptide Y and [^{125}I]neuropeptide Y-(2–36), adjacent sections were labeled and the binding sites detected by autoradiography. A similar distribution of binding was observed for each radioligand in all regions examined. Therefore, neuropeptide Y-(2–36) binds non-selectively to neuropeptide Y Y_1 and neuropeptide Y Y_2 receptors, but with lower affinity than neuropeptide Y and peptide YY. The increased potency and selectivity seen with neuropeptide Y-(2–36) in feeding studies cannot be explained on the basis of a unique in vitro pharmacology.

Keywords: Neuropeptide Y; Neuropeptide Y-(2–36); SK-N-MC cell; SK-N-BE(2) cell; Autoradiography

1. Introduction

Using an assay that detected C-terminally amidated peptides, Tatemoto et al. isolated neuropeptide Y in 1982 (Tatemoto et al., 1982). Considered to be one of the most abundant peptides in the central nervous system (O'Donohue et al., 1985), neuropeptide Y belongs to a family of related peptides. These include peptide YY (Tatemoto, 1982), pancreatic peptide (Kimmel et al., 1975), and pancreatic peptide Y (Andrews et al., 1985). When injected intracerebroventricularly or into discrete brain nuclei, these peptides can elicit numerous biological responses. For example, a central injection of neuropeptide Y can dramatically increase food intake (Stanley and Leibowitz, 1985; Kalra et al., 1991; Beck et al., 1992), affect blood pressure (Boublik et al., 1989; Chalmers et al., 1989) and increase luteinizing hormone secretion (Kalra et al., 1992).

Neuropeptide Y exerts its effects through membrane bound receptors that are coupled to G-proteins (for reviews, see Gehlert, 1994; Wahlestedt and Reis, 1993). Radioligand binding studies have revealed the presence of neuropeptide Y receptors in both peripheral and central tissues. Autoradiographic studies in the central nervous system have localized these binding sites to numerous brain regions (Martel et al., 1990). Previously, subtypes of neuropeptide Y receptors had been characterized based on their affinity for C-terminal neuropeptide Y and peptide YY fragments (Sheikh et al., 1989; Wahlestedt et al., 1986, 1987). These C-terminal fragments, such as neuropeptide Y-(13–36) and peptide YY-(13–36), have an increased selectivity for neuropeptide Y Y_2 receptors. In 1990, the neuropeptide Y analog [Leu^{31},Pro^{34}]neuropeptide Y was reported by Fuhlendorff et al. as an agonist selective for the neuropeptide Y Y_1 receptor (Fuhlendorff et al., 1990). A third neuropeptide Y receptor having high affinity for neuropeptide Y, but not peptide YY was described in the adrenal medulla (Wahlestedt et al., 1992) and rat brain stem (Grundemar et al., 1991). The distribution of neuropeptide Y receptors is, for the most part, in

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agreement with the distribution of neuropeptide Y-like immunoreactivity. However, in some areas, particularly the hypothalamus, there are numerous immunoreactive terminals (Chronwall et al., 1985), but very few neuropeptide Y receptors (Martel et al., 1988). Using agonists selective for neuropeptide Y receptor subtypes, regional differences have been found in the rat brain for neuropeptide Y Y_1 and neuropeptide Y Y_2 receptors (Gehlert et al., 1992; Dumont et al., 1993).

Central administration of selective neuropeptide Y Y_1 agonists, and not neuropeptide Y Y_2 agonists, increases feeding behavior in rats (Kalra et al., 1991; Stanley et al., 1992) suggesting a role for the neuropeptide Y Y_1 receptor in food consumption. Interestingly, deletion of the amino-terminal tyrosine residue resulted in a peptide with similar consequences on ingestive behavior. In fact, neuropeptide Y-(2–36) was consistently more effective than neuropeptide Y in stimulating food intake (Jolicoeur et al., 1991; Kalra et al., 1991; Stanley et al., 1992). Furthermore, central administration of neuropeptide Y-(2–36) had no significant effect on body temperature in rats, while neuropeptide Y decreases body temperature in a dose-dependent manner (Jolicoeur et al., 1991). In a comparative autoradiographic study, neuropeptide Y-(2–36) was more potent in displacing [^{125}I]peptide YY binding in the olfactory bulb and cerebellum (Dumont et al., 1993). Collectively, these studies suggest the existence of an atypical site with high affinity for neuropeptide Y-(2–36). Presently, it is uncertain whether neuropeptide Y-(2–36) is found in the brain. Few studies have addressed the metabolic processes of neuropeptide Y or peptide YY. However, recently neuropeptide Y-(3–36) and peptide YY-(3–36) were found in porcine and human tissues, respectively (Eberlein et al., 1989; Grandt et al., 1992). Therefore, neuropeptide Y and peptide YY C-terminal fragments can occur naturally by proteolytic mechanisms.

Consequently, we evaluated the selectivity of neuropeptide Y-(2–36) for neuropeptide Y Y_1 and neuropeptide Y Y_2 receptors using cell lines and specific rat brain tissues expressing these receptors. In addition, autoradiographic studies were performed to localize [^{125}I]neuropeptide Y-(2–36) binding sites in the rat brain.

2. Materials and methods

2.1. Cell culture

The SK-N-MC and SK-N-BE(2) cells were generously provided by Dr. June Biedler from the Sloane-Kettering Memorial Institute (New York, NY, USA). These human neuroepithelioma cell lines were grown in T-150 flasks containing Dulbecco's minimal essential medium with 5% fetal calf serum (Gibco, Gaithersburg, MD, USA). The flasks were placed in a humidified incubator at 37°C containing 5% CO_2. The confluent cells were removed manually from the flasks by scraping. They were then washed with phosphate-buffered saline, pelleted by centrifugation, and stored at −70°C until assayed.

2.2. Rat brain tissue preparation

Male, 250–350 g Sprague-Dawley rats (Charles River) were anesthetized with Halothane and rapidly decapitated. The brains were quickly removed and placed on ice for microdissection of various forebrain structures. The tissues were homogenized in 50 mmol Tris (pH 7.4) with a Polytron (Brinkmann Instruments, Westbury, NY, USA) using three 10 s bursts. After an initial spin at $800 \times g$ for 10 min, membranes were isolated by pelleting the supernatant at $40\,000 \times g$ for an additional 10 min. Membranes were stored at −70°C until assayed.

2.3. Homogenate binding studies

The homogenate binding studies were conducted as previously described by Gehlert et al. (1992) with slight modification. The cell or tissue pellets were resuspended using a glass homogenizer in 25 mmol HEPES (pH 7.4) buffer containing 2.5 mmol $CaCl_2$, 1.0 mmol $MgCl_2$ and 2 g/l bacitracin. Incubations were performed in a final volume of 200 μl containing either 100 pmol [^{125}I]peptide YY (spec. act. 2200 Ci/mmol, DuPont-NEN, Boston, MA, USA) or [^{125}I]neuropeptide Y-(2–36) (spec. act. 2000 Ci/mmol, Amersham, Arlington Heights, IL, USA) and 0.2–0.4 mg protein for 2 h at room temperature. Porcine peptide YY was monoiodinated using a modification of the chloramine-T method (Hunter and Greenwood, 1962) at an undetermined tyrosine residue. [^{125}I]Neuropeptide Y-(2–36) was radioiodinated at lysine residue 4 by reacting synthetic porcine neuropeptide Y-(2–36) with *N*-succinimidyl 3-(4-hydroxy 5-[^{125}I]iodophenyl)-propionate (Bolton and Hunter reagent) (Bolton and Hunter, 1973). In the pharmacology studies, various concentrations of neuropeptide Y, [Leu^{31},Pro^{34}]neuropeptide Y, neuropeptide Y-(13–36) and neuropeptide Y-(2–36) (Peninsula, Belmont, CA, USA) were included in the incubation mixture. Using a 96-well cell harvester (TOMTEC, Orange, CT, USA), incubations were terminated by rapid filtration through GF/C filters (Wallac, Gaithersburg, MD, USA) presoaked in 0.3% polyethyleneimine (Sigma, St. Louis, MO, USA). The filters were washed with 5 ml of ice-cold 50 mmol Tris (pH 7.4) and rapidly dried at 60°C. The dried filters were treated with MeltiLex A melt-on scintillator sheets (Wallac), and the radioactivity retained on the filters counted using the Wallac 1205 Betaplate scintillation counter. Nonspecific binding was defined as the amount of radioactivity remaining on the filter after incubating in the presence of 1 μmol neuropeptide Y. The results were analyzed using the Prism software package (Graphpad, San Diego, CA, USA) or the Cheng-Prushoff equation. Protein concentrations were measured using Coomassie protein

assay reagent (Pierce, Rockford, IL, USA) with bovine serum albumin for standards (Bradford, 1976).

2.4. Autoradiographic studies

Male, 250–350 g Sprague-Dawley rats were anesthetized with Halothane, and decapitated. The brains were rapidly removed and stored at −70°C. The brains were mounted onto chucks and sectioned at 12 μm using a cryostat (Hacker, Fairfield, NJ, USA). Coronal sections were thaw mounted onto gelatin coated slides, placed at −20°C overnight and stored at −70°C until assayed. Sections were radiolabeled using a previously described protocol (Gehlert et al., 1992) with slight modifications. Sections were initially preincubated for 30 min in Krebs-Ringer buffer containing 0.4% bovine serum albumin and 0.5% bacitracin. The sections were then placed into glass jars containing either 100 pmol [^{125}I]peptide YY, [^{125}I]neuropeptide Y, or [^{125}I]neuropeptide Y-(2–36). [^{125}I]Neuropeptide Y (spec. act. 2000 Ci/mmol, Amersham, Arlington Heights, IL, USA) was radioiodinated at lysine residue 4 by reacting synthetic porcine neuropeptide Y with Bolton and Hunter reagent. Nonspecific binding was defined by incubating near-adjacent sections with the addition of 1 μmol neuropeptide Y. Following a 2 h incubation, the sections were rinsed 4 times in fresh buffer without bovine serum albumin for 5 min each and dried rapidly. The labeled sections were exposed to Hyperfilm β-max (Amersham) for one week. The film was developed in D-19 developer (Kodak, Rochester, NY, USA) for 5 min.

3. Results

3.1. Pharmacological characterization of neuropeptide Y-(2–36) using SK-N-MC and SK-N-BE(2) cells

The affinity of neuropeptide Y and various analogs to displace 100 pmol [^{125}I]peptide YY binding to cell line homogenates was examined. Fig. 1a illustrates the pharmacological profile for the neuropeptide Y Y_1 receptor expressing SK-N-MC cells and Fig. 1b for SK-N-BE(2) cells that express neuropeptide Y Y_2 receptors. The C-terminal fragment, neuropeptide Y-(2–36) was almost equipotent at displacing [^{125}I]peptide YY from these cell lines. Neuropeptide Y also inhibited binding with similar affinity in both cell lines. However, neuropeptide Y-(2–36) was 4 times less potent than neuropeptide Y at both receptors. The affinity of [Leu^{31},Pro^{34}]neuropeptide Y was similar to neuropeptide Y in SK-N-MC cells, but was 500 times less potent in the neuropeptide Y Y_2 containing cell line. In the SK-N-BE(2) cells, neuropeptide Y-(13–36) was slightly less potent than neuropeptide Y but was nearly 45 times less potent in the neuropeptide Y Y_1 containing cell line.

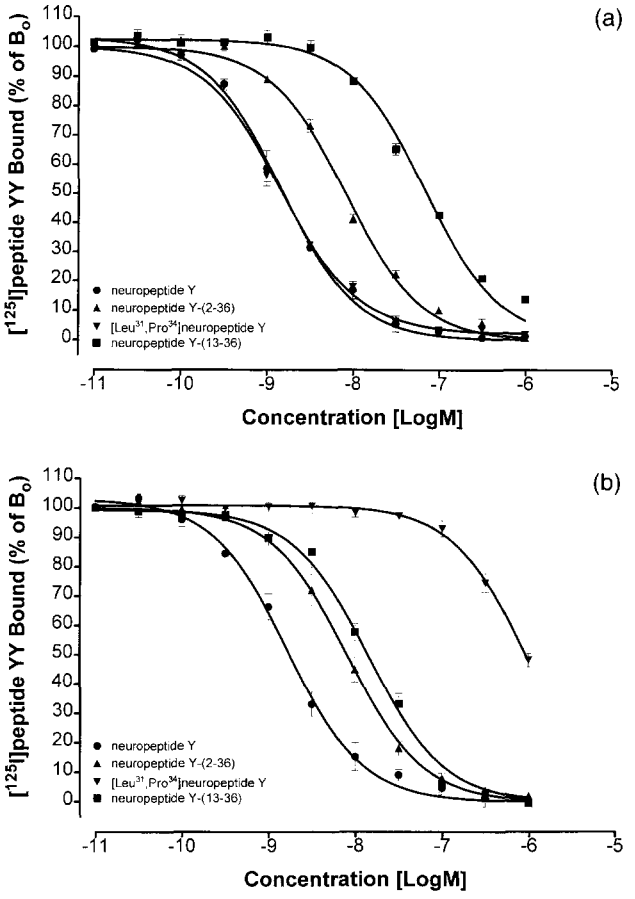


Fig. 1. Displacement of 100 pmol [^{125}I]peptide YY binding to SK-N-MC and SK-N-BE(2) cell membrane homogenates by various concentrations of neuropeptide Y (●), [Leu^{31},Pro^{34}]neuropeptide Y (▼), neuropeptide Y-(13–36) (■) and neuropeptide Y-(2–36) (▲). Specific binding is expressed as a percentage of maximal specific binding. The curves were modeled to one site and K_i values for each peptide determined using the Cheng-Prushoff equation. Neuropeptide Y, [Leu^{31},Pro^{34}]neuropeptide Y, neuropeptide Y-(13–36) and neuropeptide Y-(2–36) had K_i values of 0.79 ± 0.08, 0.75 ± 0.04, 34.9 ± 0.87, and 3.69 ± 0.13 in the SK-N-MC (panel a) cells, respectively. The SK-N-BE(2) (panel b) cells had K_i values of 0.73 ± 0.06, 369 ± 33.9, 5.57 ± 0.75, and 3.08 ± 0.36, respectively. Each value represents an average of four determinations performed in duplicate and expressed in nmol (means ± S.E.M.).

3.2. Pharmacological characterization of neuropeptide Y-(2–36) and [^{125}I]neuropeptide Y-(2–36) using homogenates prepared from specific rat brain regions

Since neuropeptide Y-(2–36) bound with similar affinity to the neuropeptide Y Y_1 and neuropeptide Y Y_2 containing cells lines, its ability to displace [^{125}I]peptide YY and [^{125}I]neuropeptide Y-(2–36) from specific rat brain regions was examined. Those areas included the cerebral cortex, hippocampus and olfactory bulb. The cerebral cortex was shown to contain mostly neuropeptide Y Y_1 receptors, while the hippocampus contains mainly neuropeptide Y Y_2 receptors (Gehlert et al., 1992). The affinities for neuropeptide Y and various analogs to inhibit

Table 1
Displacement of [^{125}I]peptide YY binding to various rat tissue homogenates by neuropeptide Y and neuropeptide Y analogs

Peptide	Cortex	Hippocampus	Olfactory bulb
Neuropeptide Y	0.67 ± 0.17	0.88 ± 0.03	0.89 ± 0.03
[Leu^{31},Pro^{34}]Neuropeptide Y	0.86 ± 0.07	38.0 ± 11.6	4.09 ± 0.29
Neuropeptide Y-(13–36)	97.5 ± 1.25	33.0 ± 1.40	29.9 ± 4.94
Neuropeptide Y-(2–36)	4.43 ± 0.28	2.15 ± 0.13	2.29 ± 0.24

Each value represents an average of three determinations performed in duplicate. The K_i values were calculated according to the Cheng-Prushoff equation and expressed in nmol (means ± S.E.M.).

[^{125}I]peptide YY binding are displayed in Table 1, while Table 2 exhibits the data for [^{125}I]neuropeptide Y-(2–36) binding. In general, the specific binding of [^{125}I]neuropeptide Y-(2–36) (approximately 60%) was consistently lower than [^{125}I]peptide YY (approximately 80%) in all tissues. In the cerebral cortex, inhibition of the binding for both radioligands had the same rank order of potency: neuropeptide Y ≥ [Leu^{31},Pro^{34}]neuropeptide Y > neuropeptide Y-(2–36) > neuropeptide Y-(13–36). The K_i values for neuropeptide Y-(2–36) in the cerebral cortex were similar. In the hippocampus, both radioligands were again displaced with a similar rank order: neuropeptide Y > neuropeptide Y-(2–36) > neuropeptide Y-(13–36) > [Leu^{31},Pro^{34}]neuropeptide Y. However, unlike the cerebral cortex, the C-terminal fragments had higher affinity in the hippocampus. Neuropeptide Y was also the most potent inhibitor of both radioligands in the olfactory bulb. Furthermore, neuropeptide Y-(2–36) bound with high affinity to the olfactory bulb followed by [Leu^{31},Pro^{34}]neuropeptide Y and neuropeptide Y-(13–36). The Hill coefficients for all the peptides were near or at 1.0, except [Leu^{31},Pro^{34}]neuropeptide Y, in these tissues (data not shown). The propensity of these four peptides to displace [^{125}I]peptide YY and [^{125}I]neuropeptide Y-(2–36) from the various brain regions (pK_i) was analyzed by linear regression (Fig. 2). Although neuropeptide Y-(13–36) in the hippocampus appears to displace [^{125}I]peptide YY and [^{125}I]neuropeptide Y-(2–36) with differing affinities, this comparison resulted in a high correlation ($r^2 > 0.91$) between the two radioligands.

Table 2
Displacement of [^{125}I]neuropeptide Y-(2–36) binding to various rat tissue homogenates by neuropeptide Y and neuropeptide Y analogs

Peptide	Cortex	Hippocampus	Olfactory bulb
Neuropeptide Y	0.48 ± 0.02	0.91 ± 0.05	1.06 ± 0.29
[Leu^{31},Pro^{34}]Neuropeptide Y	0.53 ± 0.09	43.5 ± 3.33	3.18 ± 0.84
Neuropeptide Y-(13–36)	95.9 ± 6.17	5.38 ± 0.21	25.2 ± 2.58
Neuropeptide Y-(2–36)	4.18 ± 0.26	1.14 ± 0.03	1.63 ± 0.08

Each value represents an average of three determinations performed in duplicate. The K_i values were calculated according to the Cheng-Prushoff equation and expressed in nmol (means ± S.E.M.).

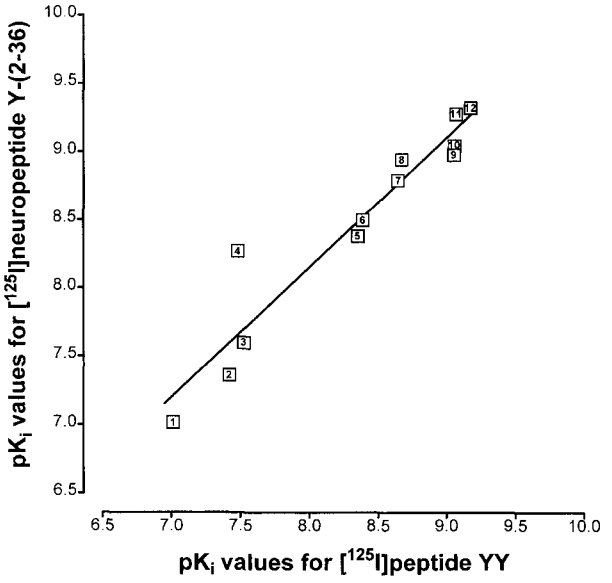
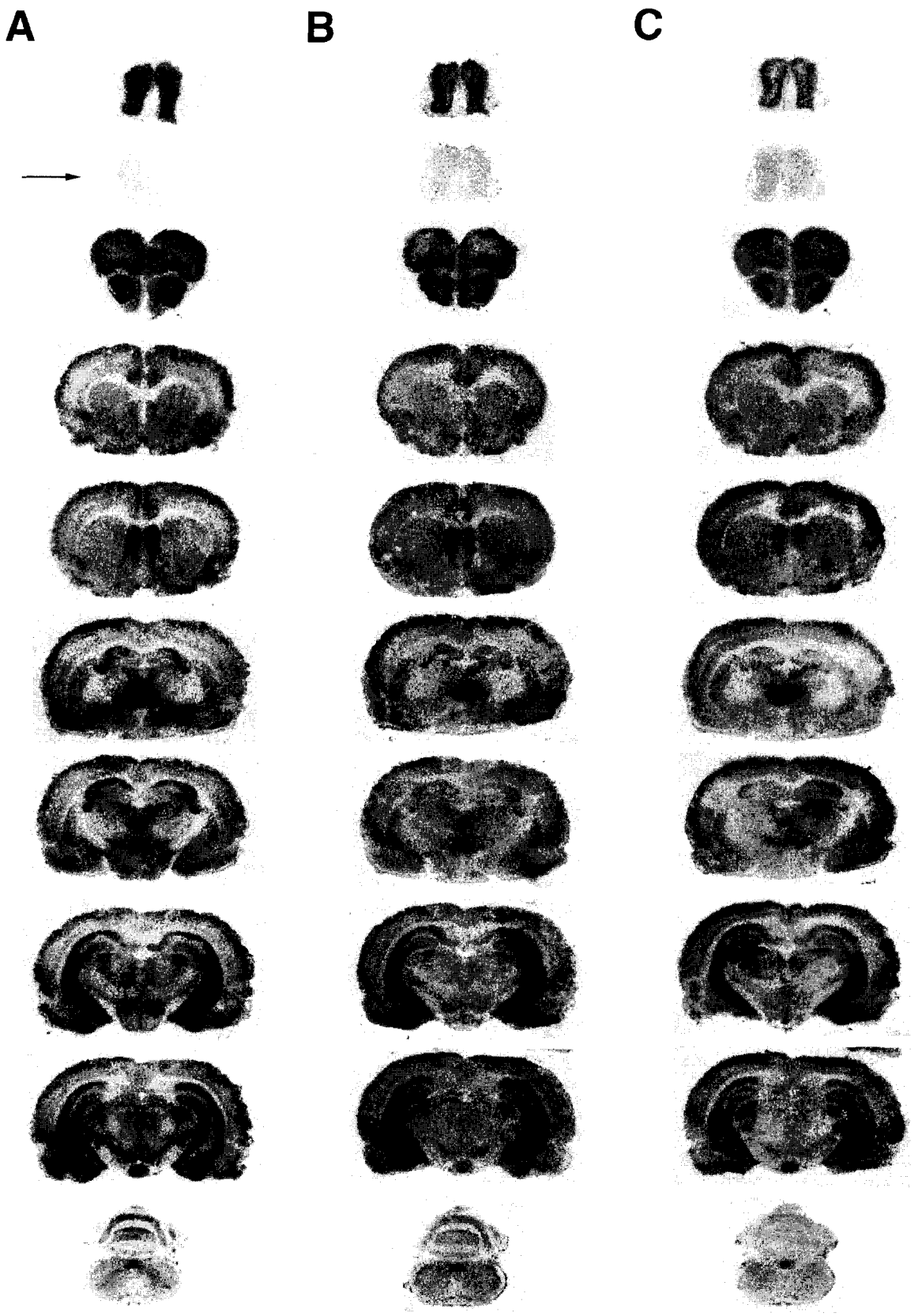


Fig. 2. Comparison of affinities for neuropeptide Y and various neuropeptide Y fragments in rat cortex, hippocampus and olfactory bulb membrane homogenates when radiolabeled with either [^{125}I]peptide YY or [^{125}I]neuropeptide Y-(2–36). Neuropeptide Y pK_i values in the cortex, hippocampus and olfactory bulb are 12, 10 and 9, respectively. [Leu^{31},Pro^{34}]Neuropeptide Y pK_i values in these same tissues are 11, 2 and 6, while neuropeptide Y-(13–36) values are 1, 4 and 3. Finally, the pK_i values for neuropeptide Y-(2–36) are 5, 8 and 7, respectively. The resulting pK_i values when compared by linear regression analysis resulted in a $r^2 > 0.91$.

3.3. Autoradiographic localization of ^{125}I-labeled peptide YY, neuropeptide Y-(2–36) and neuropeptide Y in the rat brain

A series of coronal sections through the rat brain were incubated with 100 pmol of either [^{125}I]peptide YY, [^{125}I]neuropeptide Y-(2–36) or [^{125}I]neuropeptide Y (Fig. 3) to examine the distribution of labeling in different brain structures. All three radioligands exhibited a broad distribution, binding to many distinct brain regions. In all cases, the distribution of labeling by these radioligands was identical. Significant levels of binding were seen in a variety of thalamic nuclei including the anterodorsal, central medial, and the lateral posterior nuclei. The cerebral

Fig. 3. Autoradiographic localization of ^{125}I-labeled peptide YY (A), neuropeptide Y-(2–36) (B) and neuropeptide Y (C) binding sites in the rat brain. Rat brain sections were incubated with 100 pmol of the indicated radioligand as described in Section 2. In addition, some slides were also incubated with the addition of 1 μmol neuropeptide Y to define non-specific binding. Representative autoradiograms for non-specific binding are indicated by the arrow. Autoradiograms were obtained by exposing the radiolabeled sections to X-ray film for 7 days. Overall, higher levels of non-specific binding are seen with both [^{125}I]neuropeptide Y and [^{125}I]neuropeptide Y-(2–36). Note the similar pattern of receptor binding between the three radioligands throughout the rat brain.


A
B
C

cortex, superficial (I–III) laminae also displayed a high density of labeling with each radioligand, while lower densities were seen in laminae IV–VI. In the hippocampus, significant levels of labeling were seen primarily in the striatum oriens and radiatum of CA1–3, with little binding to the molecular layer. Binding levels were higher in the more caudal regions of the hippocampus. Moderate levels of binding were observed in the mammillary nucleus and in the zona lateralis of the substantia nigra with each radioligand.

4. Discussion

One of the most pronounced actions of centrally administered neuropeptide Y is the stimulation of ingestive behavior. Historically, feeding behavior was thought to be mediated through the neuropeptide Y Y_1 receptor subtype. This was based on a study where the neuropeptide Y Y_1 selective agonist, [Leu^{31},Pro^{34}]neuropeptide Y was microinjected directly into the hypothalamus or into the third ventricle to produce a pronounced feeding response (Kalra et al., 1991). The neuropeptide Y Y_2 selective analogs, such as neuropeptide Y-(13–36), were substantially less potent. However, there are several lines of evidence that suggest an atypical neuropeptide Y Y_1 receptor mediates this response. First, there is little neuropeptide Y Y_1 binding (Dumont et al., 1993; Gehlert et al., 1992) or mRNA (Eva et al., 1990; Larsen et al., 1993) in hypothalamic areas. Secondly, Wahlestedt et al. (1993) demonstrated that reduction of central neuropeptide Y Y_1 receptor expression by antisense techniques showed no effect on food intake. Furthermore, the C-terminal fragment, neuropeptide Y-(2–36), was a more potent orexigenic agent than neuropeptide Y when injected i.c.v. (Jolicoeur et al., 1991; Kalra et al., 1991; Stanley et al., 1992). In addition, Dumont et al. (1993) reported that neuropeptide Y-(2–36) was more potent in displacing [^{125}I]peptide YY binding in the olfactory bulb and cerebellum than other brain regions. These investigators speculated that the rat brain may contain an 'atypical site' that possesses a high affinity for both neuropeptide Y and neuropeptide Y-(2–36).

In this paper, we examine whether receptor selectivity could explain the in vivo differences between neuropeptide Y and neuropeptide Y-(2–36). To test their selectivity, we evaluated the affinity of these peptides to displace neuropeptide Y Y_1 and Y_2 containing cells labeled with [^{125}I]peptide YY. The pharmacological profile of [^{125}I]peptide YY binding to SK-N-MC and SK-N-BE(2) cells is consistent with neuropeptide Y Y_1 and Y_2 receptor subtypes, respectively (Wahlestedt et al., 1992). In these cell lines, neuropeptide Y-(2–36) exhibited similar affinity for neuropeptide Y Y_1 and Y_2 receptor subtypes. This finding was further substantiated when we compared the pharmacology of [^{125}I]peptide YY and [^{125}I]neuropeptide Y-(2–36) in specific rat brain homogenates. For both radioligands, neuropeptide Y and the neuropeptide Y analogs inhibited binding with similar affinities. In fact, using linear regression analysis, their affinities were nearly identical as confirmed by a correlation coefficient greater than 0.9.

In autoradiographic experiments, [^{125}I]neuropeptide Y-(2–36) labeled brain regions known to contain Y_1 and Y_2 receptors. This radioligand exhibited substantially higher nonspecific binding when compared to [^{125}I]neuropeptide Y and [^{125}I]peptide YY. A previous study compared the localization of [^{125}I]neuropeptide Y and [^{125}I]peptide YY binding sites and found that each radioligand identified high affinity sites with differing distribution (Walker and Miller, 1988). Subsequently, it was found that [^{125}I]peptide YY bound with higher affinity to the neuropeptide Y Y_2 receptor subtype (Gehlert et al., 1992). Therefore, the comparison of [^{125}I]neuropeptide Y-(2–36) binding with [^{125}I]peptide YY and [^{125}I]neuropeptide Y resulted in a similar distribution of binding sites with some differences in intensity. For instance, all three radioligands bound to the lateral septum, however, [^{125}I]peptide YY labeled the region with higher intensity. Consistent with the preference of [^{125}I]peptide YY for the neuropeptide Y Y_2 receptor, this region of the brain contains primarily neuropeptide Y Y_2 receptors (Gehlert et al., 1992). Taken as a whole, these data are consistent with the non-selective nature of [^{125}I]neuropeptide Y-(2–36) as a radioligand for neuropeptide Y receptors. As such, this radioligand did not identify any additional regions of the brain containing novel neuropeptide Y Y_1-like receptors. Most importantly, none of these radioligands exhibited substantial binding to the paraventricular nucleus of the hypothalamus.

These data are inconsistent with the presence of a receptor with preferential high affinity for neuropeptide Y-(2–36). However, these findings do not negate the possibility of further proteolytic processing to active peptide fragments. Of particular interest, involves the degradation of neuropeptide Y and peptide YY in vivo by a novel proteolytic enzyme. Both neuropeptide Y and peptide YY are cleaved by dipeptidyl peptidase IV (Mentlein et al., 1993), an enzyme found in endothelial cells. Removal of Tyr-Pro by dipeptidyl peptidase IV reduces peptide affinity for the neuropeptide Y Y_1 receptor subtype, but preserves affinity at neuropeptide Y Y_2 receptor. These shorter fragments have been observed in human tissues and serum (Eberlein et al., 1989) and in porcine brain (Grandt et al., 1992). Since very little is known about the metabolism of neuropeptide Y-(2–36) in vivo, it is possible this fragment is metabolized to other fragments with different selectivities. Further study of proteolytic processing and receptor selectivity of neuropeptide Y fragments will be necessary to explore this hypothesis.

In conclusion, we have examined the pharmacology and autoradiographic distribution of neuropeptide Y-(2–36) in vitro. On the basis of these studies, the ability of neuropeptide Y-(2–36) to elicit a robust feeding response cannot be

explained based on selectivity for known neuropeptide Y receptor subtype pharmacology in vitro. In cell lines and rat brain tissue homogenates, neuropeptide Y-(2–36) appears to be a non-selective ligand for both neuropeptide Y Y_1 and Y_2 receptors.

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