Vol. 150, No. 1, 1988
January 15, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 25-32

PEPTIDE YY RECEPTORS IN THE BRAIN

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Received November 11, 1987

Radiolabelled ligand binding studies demonstrated that specific receptors for peptide YY are present in the porcine as well as the canine brains. Peptide YY was bound to brain tissue membranes via high-affinity (dissociation constant, 1.39×10^{-10} M) and low-affinity (dissociation constant, 3.72×10^{-8} M) components. The binding sites showed a high specificity for peptide YY and neuropeptide Y, but not for pancreatic polypeptide or structurally unrelated peptides. The specific activity of peptide YY binding was highest in the hippocampus, followed by the pituitary gland, the hypothalamus, and the amygdala of the porcine brain, this pattern being similarly observed in the canine brain. The results suggest that peptide YY and neuropeptide Y may regulate the function of these regions of the brain through interaction with a common receptor site.

Recently a novel polypeptide, peptide YY (PYY), was isolated from the porcine intestine (1,2). This peptide consists of 36 amino acid residues and is structurally similar to pancreatic polypeptide (PP) and neuropeptide Y (NPY) (3,4). Immunohistochemical studies have revealed the presence of PYY-like immunoreactivity in endocrine cells in the gastrointestinal mucosa of several species, including man, with the most pronounced reactivity in the distal intestine (5-7). A more recent study provided evidence of a PYY neuronal system with cell bodies in the rostral medulla oblongata giving rise to networks of terminal fibers widely distributed within the pons, medulla oblongata, and spinal cord (8-10). Varicose nerve fibers displaying PYY-like immunoreactivity were also observed in many parts of the hypothalamus (10). The topographical distribution of PYY-like immunoreactivity is unique and does not exhibit

0006-291X/88 $1.50

any overlap with any of the known peptide systems in these areas including that of NPY (9). These results suggest functional roles for a PYY-like peptide in the brain.

By analogy with other regulatory peptides, if PYY is to act in the brain either as a hormone or as a neurotransmitter, its action should be initiated by interaction with specific receptors. Although specific binding sites for PYY have been identified and characterized in plasma membranes prepared from rat jejunal epithelium (11), very little is currently known about the PYY receptors in the brain. We report here for the first time on the binding characteristics of PYY and its sister peptides NPY and PP, and on the receptor distribution of highly specific and selective PYY receptors in the porcine as well as in the canine brain.

MATERIALS AND METHODS

Adult mongrel dogs were anesthetized with sodium thiamylal and perfused with 0.9% saline containing aprotinin (25,000 KIU/ℓ). Brains were removed immediately after killing, frozen rapidly and dissected into olfactory bulb, cerebral (parietal) cortex, caudate nucleus, amygdala, hippocampus, thalamus, hypothalamus, cerebellar cortex, pons, medulla oblongata, and pituitary gland. Porcine brains were obtained from a local slaughterhouse and treated in the same way. Brain tissues were homogenized in five volumes of ice-cold 234 mM sucrose with a teflon-grass homogenizer. Tissue homogenates were centrifuged at 700 g for 10 min and supernatants centrifuged at 100,000 g for 15 min at 4°C. The pellets were washed with 25 mM Tris-HCl (pH 7.4), centrifuged again, and resuspended by homogenization in Tris buffer. Membrane protein was determined by the BioRad protein assay (BioRad Laboratories, Japan). Synthetic porcine PYY was radiolabelled with [^{125}I] by the chloramine-T method as described elsewhere (12).

Standard incubation buffer consisted of 25 mM Tris, 10 mM $MgCl_2$, 1 mM Phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml bacitracin, and 5 mg/ml bovine serum albumin and was adjusted to pH 7.4. For binding assays, 100 µl of membrane preparations (final protein concentration, 100 µg/ml) were incubated at 25°C for 5 h in a final volume of 1 ml containing incubation buffer and 40 pM [^{125}I] PYY. Samples were then centrifuged at 10,000 g for 3 min, supernatants aspirated, and radioactivity in the vials determined in a gamma scintillation counter. Specific binding was calculated as the difference in radioactivity bound in the presence and absence of 0.12 µM unlabelled PYY. Degradation of [^{125}I] PYY was assessed by precipitation with trichloroacetic acid and was routinely less than 5% at the termination of incubation.

Synthetic porcine PYY and NPY were obtained from Peninsula Laboratories (Belmont, CA), and highly purified porcine pancreatic polypeptide from Novo (Bagsvaerdt, Denmark).

RESULTS

Optimum Conditions for Binding Assay

Specific binding of [^{125}I] PYY to brain membranes was temperature- and time-dependent. At 25°C, steady-state binding was reached at 5 h and maintained until at least 6 h (Fig. 1), whereas at 4°C the association was much slower. At 25°C, nonsaturable binding comprised less than 5% and 20% of the total binding for porcine and canine membranes, respectively. Specific [^{125}I] PYY binding increased linearly with tissue concentrations from 50 g to 200 g/ml (Fig. 2). Peptide binding showed a relatively narrow pH optimum at pH 6-7.4. Divalent cations including Mg^{2+} decreased total [^{125}I] PYY binding to membranes. However, nonsaturable binding was lowest at 10 mM Mg^{2+} and therefore all experiments on brain membranes were carried out at this ionic strength.

Characterization of [^{125}I] PYY Binding Sites

The concentration dependence of PYY binding at equilibrium was studied at 25°C by adding increasing concentrations of unlabelled PYY to a fixed concentration of [^{125}I] PYY (Fig. 3). Scatchard analysis of the

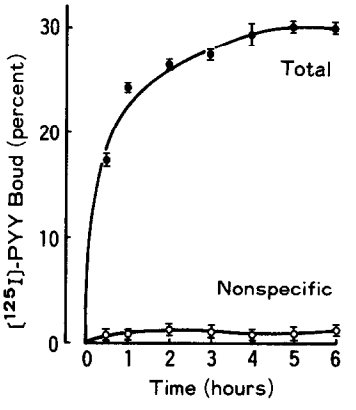

Figure 1. Time course of [^{125}I] PYY binding to porcine brain membranes (hippocampus) at 25°C. Nonsaturable binding was unchanged during the entire incubation period. Each point is the mean of triplicate determinations in a typical experiment with the vertical bars representing the SEM.

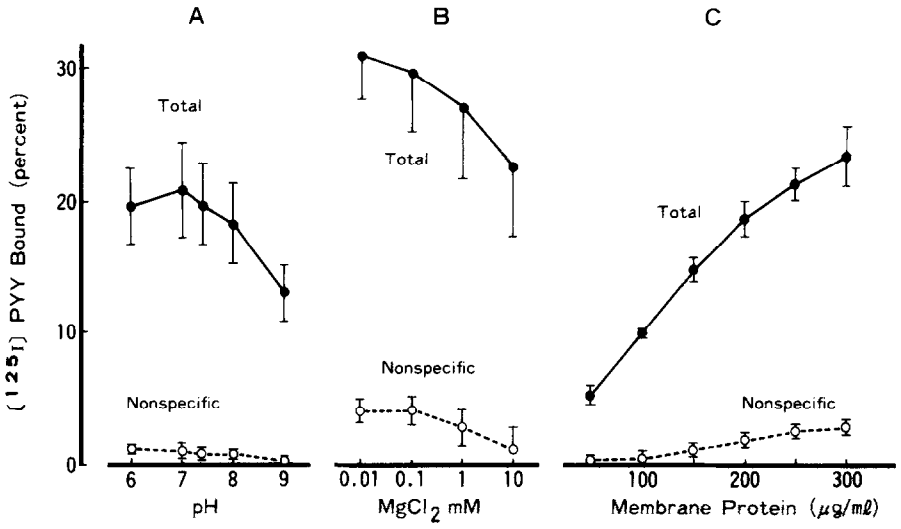

Figure 2. Dependence of [^{125}I] PYY binding to porcine brain membranes (hippocampus) on pH (A), magnesium ion concentration (B), and membrane protein concentration (C). Results are means ± SEM of three (A), four (B), and two (C) separate experiments, each determined in triplicate.

binding data yielded a curvilinear plot suggesting the occurance of an heterogeneity of [^{125}I] PYY binding sites (Fig. 4). Because other unlabelled peptides such as insulin, cholecystokinin octapeptide, methionine-enkephalin, vasoactive intestinal peptide, thyrotropin releasing hormone, and corticotropin releasing factor were unable to

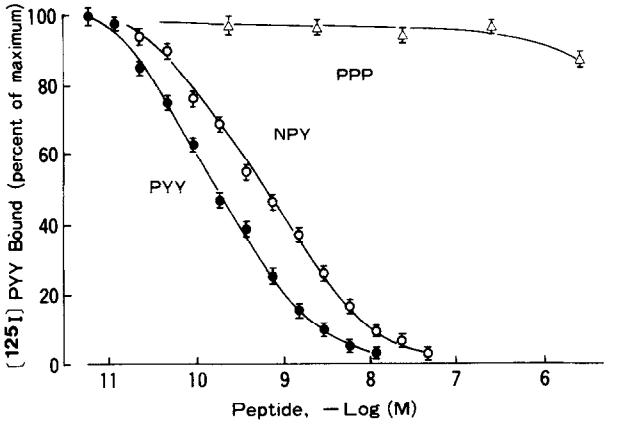

Figure 3. Inhibition of [^{125}I] PYY binding by unlabelled PYY and structurally related peptides. Various concentrations of PYY (•), NPY (o), and PP (Δ) were present as shown. Specific saturable binding was expressed as a percentage of maximal specific binding. Each point is the mean ± SEM of triplicate determinations in a typical experiment on membrane preparations from the porcine hippocampus.

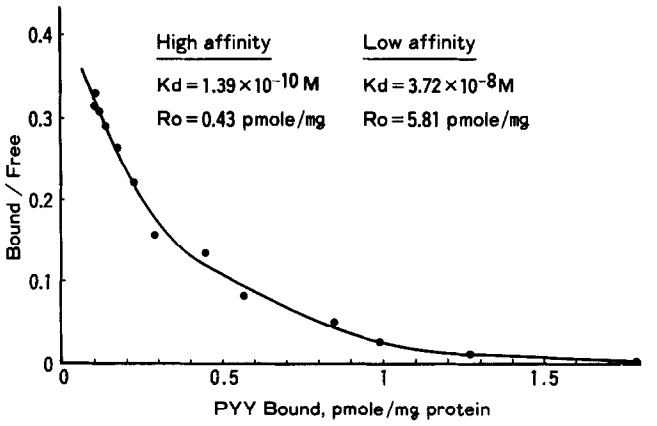

Figure 4. Scatchard analysis of differential binding of [^{125}I] PYY to porcine brain membranes (hippocampus). Displacement data from Figure 3 were subjected to Scatchard analysis, and the bound-to-free ratio of PYY has been plotted vs. bound PYY. Inset, Binding constants showing dissociation constants (Kd) and total binding capacity (Ro) for high and low affinity sites.

displace the specifically bound [^{125}I] PYY, even at concentrations up to 10^{-6} M, the interaction between brain membranes and [^{125}I] PYY was thought to be specific. Among the peptides structurally related to PYY, only NPY was much able to compete for binding sites. Unlabelled NPY, which has 25 amino acids out of 36 in common with PYY, inhibited PYY binding in proportion to its concentration (Fig.3). Half-maximal displacement occurred with 1.9 x 10^{-10} PYY and 6.2 x 10^{-10} M for NPY. NPY was thus 3 times less potent than PYY in competing for [^{125}I] PYY binding sites. Although porcine PP has 18 amino acids in common with PYY and NPY (50% homology), PP did not affect the binding of [^{125}I] PYY at concentrations up to 10^{-6} M.

Regional Distribution of the PYY Receptor

The concentration of [^{125}I] PYY binding sites differed in various brain regions (Table 1). In the porcine brain, the highest binding activity was observed in the hippocampus, pituitary gland, hypothalamus, and amygdala. Intermediate activities were observed in the caudate nucleus and brain stem (midbrain and medulla oblongata). In contrast, little binding could be detected in the cerebral cortex, olfactory bulb,

Table 1. Regional distribution of the PYY receptors in the porcine and the canine brains. Results are means ± SEM of six experiments, each determined in triplicate.

Brain region	Specific PYY binding (fmole/mg protein) pig	dog
Cerebral (parietal) cortex	2.5±0.9	5.6±1.4
Olfactory bulb	4.2±1.2	3.0±0.8
Caudate nucleus	17.7±4.9	15.1±2.2
Hippocampus	84.5±13.6	22.6±7.1
Amygdala	28.6±5.8	12.4±2.0
Thalamus	4.0±0.9	18.3±3.2
Hypothalamus	34.3±9.1	13.2±3.4
Pituitary gland	58.9±13.6	12.4±3.6
Midbrain	11.3±2.5	12.2±2.6
Pons	9.4±2.4	5.6±2.0
Medulla oblongata	16.3±3.0	7.9±0.8
Cerebellum	3.0±1.5	0.4±0.2

thalamus, or cerebellum. The distribution of [^{125}I] PYY binding in the canine brain paralleled that in the porcine brain except that the thalamus displayed high binding activity.

DISCUSSION

The present study discovered two classes of [^{125}I] PYY binding sites on brain membrane preparations. The calculated binding affinity of 0.139 nM for the high affinity component is similar to the value reported for PYY binding to membranes prepared from rat intestinal epithelium (Kd = 0.4 nM) (11). This Kd value indicates that PYY receptors in the brain can detect very low levels of the peptide.

Besides high affinity, brain PYY receptors exhibit another important property; peptide specificity. [^{125}I] PYY binding is displaced by unlabelled PYY or by NPY, but not by unrelated peptides or even by other structurally related peptides from the pancreatic polypeptide family. This indicates the strict structural requirement for recognition by PYY receptors in the brain, as do intestinal receptors in the rat (11). The mid- to N-terminal part of the PYY molecule has actually a major influence on the affinity for brain PYY receptors (unpublished data). It is particularly interesting that PYY

receptors recognize NPY, the most abundant neuropeptide in the brain (13,14), with a similar affinity. This, together with the finding that PYY is a highly potent competitor for [^{125}I]-Bolton-Hunter-NPY binding sites in the porcine brain (15), suggests that PYY and NPY regulate brain functions through interaction with common receptor site(s).

[^{125}I] PYY binding varied markedly throughout the porcine and the canine brains. Receptors were most abundant in the limbic system, pituitary gland, and hypothalamus with essentially no species variability. It is possible that these PYY receptors serve as the biochemical substrate for the common central actions of PYY and NPY, including the stimulation of feeding behavior (16-20), the lowering of rectal temperature (21), and the regulation of pituitary hormones (22-27). The molecular characterization of brain PYY receptors would help to clarify their diverse functions in the brain, and might be useful in many areas of neuroscience.

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