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Neuropeptide Y (NPY) receptors in HEL cells: comparison of binding and functional parameters for full and partial agonists and a non-peptide antagonist

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1 We have compared the binding and Ca^{2+} mobilizing properties of various full agonists, partial agonists and a non-peptide antagonist at the neuropeptide Y (NPY) receptor of human erythroleukemia (HEL) cells.

2 [^{125}I]-NPY binding to intact HEL cells was rapid, saturable, of high affinity and with a specificity typical for the Y_1-like subtype: NPY, peptide YY (PYY) and [Pro^{34}]-NPY competed for [^{125}I]-NPY binding with high affinity whereas NPY_{13-36} and NPY_{18-36} had only low affinity.

3 NPY, PYY and [Pro^{34}]-NPY potently increased intracellular Ca^{2+} in HEL cells and had equal efficacy. NPY_{13-36}, vasoactive intestinal peptide (VIP) and pancreatic polypeptide (PP) increased intracellular Ca^{2+} only poorly.

4 Whereas VIP and PP did not significantly affect NPY-stimulated Ca^{2+} mobilization, NPY_{13-36} inhibited NPY-stimulated Ca^{2+} increases and shifted the NPY concentration–response curve to the right without altering its maximal effect.

5 The agonist (pEC_{50}) potencies of the various peptides corresponded well with the affinities of these compounds in the binding assay (pK_i), whereas the antagonist potencies (pK_b) of the peptide partial agonists and the pA_2 value of the non-peptide NPY antagonist (He 90481), calculated from functional data, were lower than the respective affinities determined in the binding studies.

6 A plot of the fractional Ca^{2+} response vs the fractional receptor occupancy did not reveal any non-linear receptor-effector coupling for NPY or [Pro^{34}]-NPY; a small receptor reserve might exist for PYY.

7 We conclude that the binding and functional properties of HEL cell NPY receptors are very similar. NPY, PYY and [Pro^{34}]NPY are full agonists at these receptors, whereas NPY_{13-36} is a partial agonist.

Keywords: Neuropeptide Y; peptide YY; binding; Ca^{2+}; HEL cell; receptor reserve

Introduction

Neuropeptide Y (NPY) is a putative neurotransmitter that was first isolated in 1982 from porcine brain (Tatemoto, 1982). NPY can mediate numerous central and peripheral effects including central regulation of blood pressure and food intake, presynaptic inhibition of transmitter release, and vasoconstriction (Gray & Morley, 1986; Edvinsson *et al.*, 1987; Potter, 1988; Walker *et al.*, 1991). Many of these effects have been characterized by studying structure-activity relationships for various peptide analogues of NPY (Wahlestedt *et al.*, 1986; Chang *et al.*, 1988; Danho *et al.*, 1988; Servin *et al.*, 1989; Boublik *et al.*, 1989; Michel *et al.*, 1990). Other investigators have characterized NPY binding sites in various tissues and cell lines using similar peptide analogues of NPY (Lundberg *et al.*, 1988; Walker & Miller, 1988; Sheikh *et al.*, 1989; Martel *et al.*, 1990). Such studies have revealed that multiple NPY receptor subtypes appear to exist but an exact definition of these subtypes remains to be established (Michel, 1991).

A direct side-by-side comparison of the pharmacological properties of NPY binding sites and physiological NPY effects has rarely been performed. Such comparison, however, is important to fulfill the criteria for the acceptance of a binding site as a receptor (Laduron, 1984). Moreover, the comparison of binding and functional receptor properties provides information about the efficacy of receptor–effector coupling. It should also be kept in mind that binding studies on NPY receptors presently rely on the agonist ligand, which may not accurately define receptor number since agonist binding to NPY receptors is subject to modulation by post-receptor events such as the number and functional status of G-proteins (Feth *et al.*, 1991). Thus, a comparison of binding and functional parameters should not only rely on agonists but rather include partial and pure antagonists.

We have previously introduced the human erythroleukemia (HEL) cell line as a model system to study human NPY receptors *in vitro*; HEL cell NPY receptors couple to two independent second messenger systems, inhibition of forskolin-stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation and mobilization of intracellular Ca^{2+} (Motulsky & Michel, 1988). In the present study we have directly compared the binding and Ca^{2+} mobilizing properties of HEL cell NPY receptors using full agonists, partial agonists as well as the non-peptide antagonist He 90481 (Michel & Motulsky, 1990).

Methods

Cell culture

HEL cells were originally obtained from Dr T. Papayannopoulou (Dept. of Medicine, University of Washington, Seattle, WA, U.S.A.). They were grown in suspension culture in RPMI 1640 medium supplemented with 2 mM glutamine, 100 u ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10% foetal calf serum at 37°C with 95% air/5% CO_2. Cells were maintained at a density of 4–8 $\times$ 10^5 cells ml^{-1} by daily dilution with fresh medium.

Radioligand binding

HEL cells at a density of about 5 $\times$ 10^5 ml^{-1} were harvested by 10 min centrifugation at 450 *g* at room temperature and washed twice with phosphate-buffered saline. Thereafter they

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were resuspended at a density of 2×10^7 cells ml^{-1} in assay buffer of the following composition (mM): HEPES 10, NaCl 150, KCl 5, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and bovine serum albumin (10 mg ml^{-1}) at a pH of 7.4. The reaction was carried out in polystyrene tubes that had been precoated with Sigmacote and washed with 20 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 2 mg ml^{-1} BSA to reduce nonspecific binding. The reaction was started by adding 1.5×10^6 cells to assay buffer containing [^{125}I]-NPY at the indicated concentrations in a total volume of 500 μl and, after incubation at 25°C for 90 min, was terminated by centrifugation for 5 min at 3000 g. The cell pellet was washed twice with 1 ml phosphate-buffered saline at 4°C. The remaining radioactivity was quantified in a gamma-counter (Berthold, Wildbad, F.R.G.). Under these conditions specific [^{125}I]-NPY binding increased linearly with the number of HEL cells in the assay in a range from 0.2 to 2.5×10^6 cells per tube.

In order to achieve the various [^{125}I]-NPY concentrations, a stock solution of [^{125}I]-NPY was diluted with unlabelled NPY; thus, the total amount of c.p.m. remained constant and the specific activity decreased with increasing NPY concentrations. We defined non-specific binding as [^{125}I]-NPY binding in the presence of 200 nM unlabelled NPY.

Ca^{2+} measurements

The cytoplasmatic free Ca^{2+} concentration was quantified by use of the fluorescent indicator dye Fura-2 and a Hitachi F-2000 spectrofluorometer as previously described (Feth *et al.*, 1991). Briefly, experiments were performed in buffer of the following composition (mM): HEPES 20, NaCl 120, KH_2PO_4 5, Mg acetate 1, $CaCl_2$ 2, and 1 mg ml^{-1} glucose at pH 7.4 using approximately $6–8 \times 10^5$ cells ml^{-1}. After 1 h of loading with the dye the cells were washed twice and resuspended in fresh buffer and used for fluorescence measurements within the next hour. Fluorescence was converted to Ca^{2+} concentrations by use of a ratio method which does not take into account fluorescence coming from extracellular dye. However, we monitored such extracellular fluorescence by the Mn^{2+} ion quench method as previously described (Motulsky & Michel, 1988) and used cells only if fluoresence from extracellular dye was less than 15% of total fluoresence.

Chemicals

Human NPY and peptide YY (PYY) were from Bissendorf (Hannover, F.R.G.), human pancreatic polypeptide (PP) and vasoactive intestinal peptide (VIP) were from Bachem (Heidelberg, F.R.G.), He 90481 was kindly provided by Heumann Pharma (Nürnberg, F.R.G.), [Pro^{34}]-NPY was kindly provided by Dr T.W. Schwartz (Copenhagen, Denmark), and [^{125}I]-NPY (specific activity 74 TBq mmol^{-1}) was from Amersham (Braunschweig, F.R.G.).

Data analysis

Saturation binding experiments were analysed based on the law of mass action by fitting the experimental data to a rectangular hyperbolic function. Competition binding experiments were analysed by fitting the experimental data to a sigmoid function with a Hill-slope of -1. Maximum [^{125}I]-NPY binding was fixed at 100% and minimum [^{125}I]-NPY binding was fixed at 0%. Concentration–response relationships were analysed by fitting the experimental data to sigmoid functions; the bottom of the curve was fixed at 0 (i.e. no increase in intracellular Ca^{2+}) and the Hill slopes were fixed at 1 in order to allow optimal estimates of the EC_{50}. All of the above calculations were performed using iterative non-linear regression analysis with the InPlot program (GraphPAD Software, San Diego, U.S.A.). All data are expressed as mean ± s.e.mean.

The relationship of fractional response vs fractional receptor-occupancy was determined graphically. Percentage of the maximal response, calculated by the non-linear regression analysis of the concentration–response curves, was plotted against fractional receptor occupancy, calculated by the formula $100/(1 + K_d/[A])$. K_d is the affinity of the respective peptide as determined in the radioligand binding studies and [A] is the concentration of the peptide which elicited the corresponding fractional response.

Since the HEL cell responsiveness towards NPY varied considerably from day to day, we determined the response to 100 nM NPY in each set of experiments and expressed all other effects as % of this response (Michel *et al.*, 1990).

Results

Characterization of [^{125}I]-NPY binding sites

The binding of [^{125}I]-NPY to intact HEL cells was rapid and reached equilibrium conditions within 90 min (data not shown). Saturation binding isotherms under equilibrium conditions demonstrated saturable high affinity [^{125}I]-NPY binding to HEL cells (K_d 3.9 ± 1.0 nM, B_{max} 73.0 ± 18.8 fmol per 10^6 cells, $n = 4$, Figure 1) corresponding to approximately 44,000 binding sites per cell.

In competition experiments with NPY and related peptides we determined a rank order of potency of PYY (pK_i: 8.61) ≥ human NPY (pK_i: 8.53) ≥ [Pro^{34}]-NPY (pK_i: 8.49) ≫ $NPY_{13–36}$ ($pK_i \approx 6$) ≥ $NPY_{18–36}$ ($pK_i \approx 5$; Figure 2a and Table 1); PP and VIP did not compete for [^{125}I]-NPY binding in concentrations up to 1 μM (data not shown).

We have previously shown that He 90481 is a competitive, non-peptidic, low affinity antagonist for NPY-stimulated Ca^{2+} increases in HEL cells (Michel & Motulsky, 1990). In binding studies He 90481 competed for HEL cell [^{125}I]-NPY binding sites with a similar low affinity (pK_i 3.74 ± 0.03; Figure 2b, Table 1).

Mobilization of intracellular Ca^{2+}

In order to further characterize the NPY receptor mediating Ca^{2+} increases in HEL cells, we compared the Ca^{2+} mobilizing properties of various peptides with those of NPY. Whereas 100 nM NPY produced a significant, transient, increase in Fura-2 fluorescence, $NPY_{13–36}$, PP and VIP caused only very minor elevations which did not allow the establishment of reliable concentration–response curves. In contrast, low concentrations of PYY and [Pro^{34}]-NPY significantly increased intracellular Ca^{2+} (Figure 3, Table 1). The rank order of potency in elevation of Ca^{2+} was PYY (pEC_{50}: 8.73) ≥ NPY (pEC_{50}: 8.30) ≥ [Pro^{34}]-NPY (pEC_{50}: 8.21;

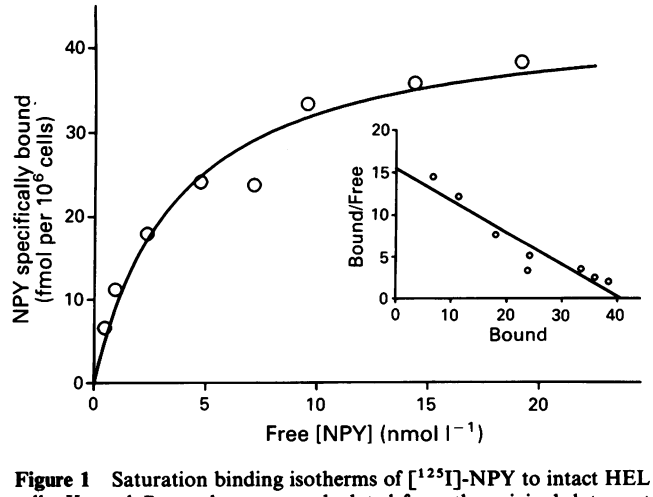

Figure 1 Saturation binding isotherms of [^{125}I]-NPY to intact HEL cells, K_d and B_{max} values were calculated from the original data, not from the Scatchard plot. Data are the average from triplicate determinations in a representative experiment. The insert shows the linearization of the data according to Scatchard (1949).

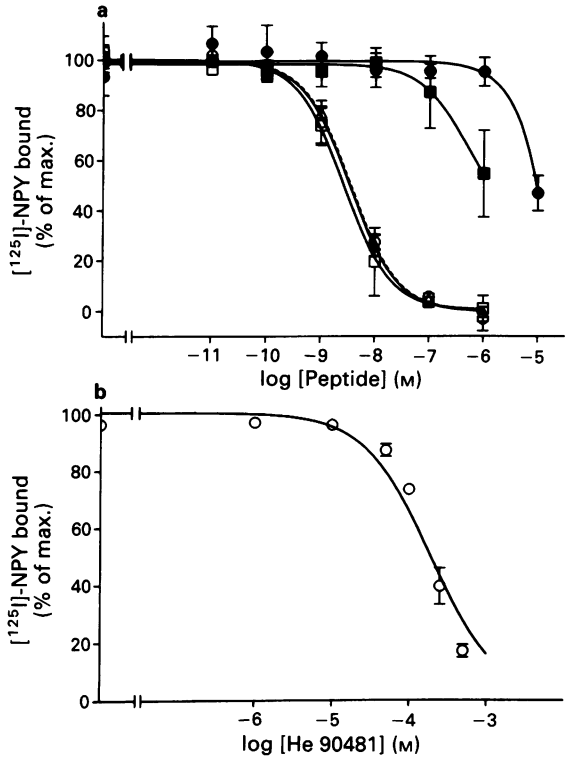

Figure 2 Competition of unlabelled peptides (a) and He 90481 (b) with [^{125}I]-neuropeptide Y (NPY) binding to HEL cells. Cells were incubated with 0.5 nM [^{125}I]-NPY in the presence of increasing concentrations of unlabelled peptides for 90 min at 25°C. In (a) effects of NPY (○), peptide YY (PYY, □), [Pro^{34}]-NPY (△), NPY_{13-36} (■), NPY_{18-36} (●) are shown. (b) Shows effects of He 90481 (○). Data are the mean of 3 experiments and vertical bars show s.d.

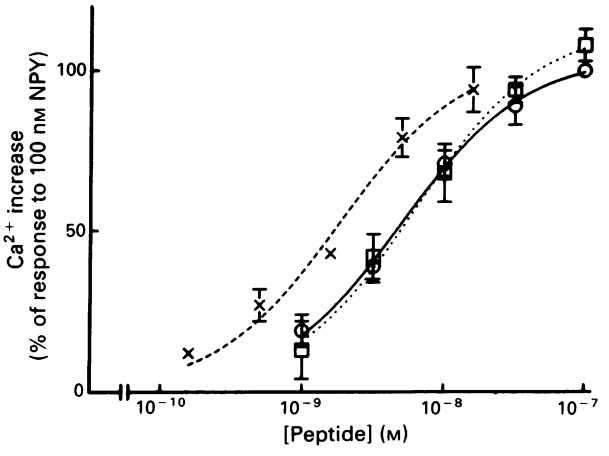

Figure 3 Concentration–response curves for peptide-mediated mobilization of intracellular Ca^{2+}. Data are the mean and vertical bars show s.e.mean of three experiments for neuropeptide Y (NPY) (○), [Pro^{34}]-NPY (□) and peptide YY (×).

Table 1), which corresponds to the potency obtained in our binding experiments. The maximum Ca^{2+} increase was similar for NPY, PYY and [Pro^{34}]-NPY (Table 1). Whereas NPY_{13-36} in concentrations up to 100 nM did not significantly elevate intracellular Ca^{2+}, 1 μM of NPY_{13-36} elevated Ca^{2+} by 9 ± 2% of the Ca^{2+} increase observed with 100 nM NPY in paired experiments (Table 2). Although VIP and PP did not inhibit NPY binding, 1 μM of these peptides elevated intracellular Ca^{2+} by 19 and 13% of the response to 100 nM NPY, respectively (Table 2).

The modest Ca^{2+} increase produced by NPY_{13-36}, VIP and PP could come from low intrinsic efficacy. Since partial agonists are, by definition, also competitive antagonists we tested the ability of these peptides to block NPY-stimulated Ca^{2+} elevations. Whereas 1 μM of either VIP or PP did not diminish the ability of NPY to raise intracellular Ca^{2+}, 1 μM of NPY_{13-36} slightly but significantly decreased the Ca^{2+} response to 100 nM NPY (Table 2). In order to test this apparent antagonism of NPY by NPY_{13-36} in more detail we compared paired concentration–response curves for NPY in the absence and presence of 1 μM NPY_{13-36}. NPY_{13-36} shifted the concentration–response curve for NPY to the right (Figure 4, Table 1) giving a pK_b value of 6.30 ± 0.16 (n = 3).

Studies on receptor reserve for Ca^{2+} mobilization in HEL-cells

Using the above data, we have plotted the fractional response for NPY, PYY and [Pro^{34}]-NPY vs the fractional receptor occupation by these peptides (Figure 5). The data points obtained for NPY and [Pro^{34}]-NPY did not differ significantly from the line of identity. The data points for PYY, however, were consistently above the line of identity and were

Table 2 Agonist and antagonist effects of pancreatic polypeptide (PP), vasoactive intestinal peptide (VIP) and NPY_{13-36} on Ca^{2+} mobilization in HEL cells

	Ca^{2+} rise by peptide	*Inhibition of Ca^{2+} rise induced by 100 nM NPY*
PP	19 ± 3%	3 ± 6%
VIP	13 ± 2%	−16 ± 6%
NPY_{13-36}	9 ± 2%	13 ± 4%*

All peptides were tested for agonist (left column) and antagonist effects (right column) at 1 μM. Agonist effects are expressed as % of the response elicited by 100 nM neuropeptide Y (NPY) in paired cuvettes. Antagonist effects are expressed as % inhibition of the response to 100 nM NPY. Data are the mean ± s.e.mean of 3–5 experiments (*P < 0.05 vs 100 nM NPY alone in paired experiments).

Table 1 Affinity of neuropeptide Y (NPY) and other peptides for [^{125}I]-NPY binding sites, together with their potency and efficacy at stimulating or inhibiting mobilization of intracellular Ca^{2+} in HEL cells

	Binding	*Ca^{2+} mobilization*		
	pK_i	*pK_b*	*pEC_{50}*	*Maximum (%)*
NPY	8.53 ± 0.09		8.30 ± 0.08	105 ± 1
PYY	8.61 ± 0.08		8.73 ± 0.17	108 ± 9
[Pro^{34}]-NPY	8.49 ± 0.04		8.21 ± 0.18	117 ± 10
NPY_{13-36}	≈6	6.30 ± 0.16		
NPY_{18-36}	≈5	6.53 ± 0.12[a]		
He 90481	3.74 ± 0.03	4.43[b]		

Data are the mean ± s.e.mean of 3–5 experiments as shown in Figures 2, 3 and 4. Maximal responses are expressed as % of the response to 100 nM NPY, as determined in the same set of cuvettes. For He 90481 a pA_2 value is shown instead of a pK_b value; only approximate pK_i values are shown for NPY_{13-36} and NPY_{18-36} since the complete competition was not achieved by the peptide concentrations used. [a] Data taken from Michel *et al.* (1990). [b] Data taken from Michel & Motulsky (1990).

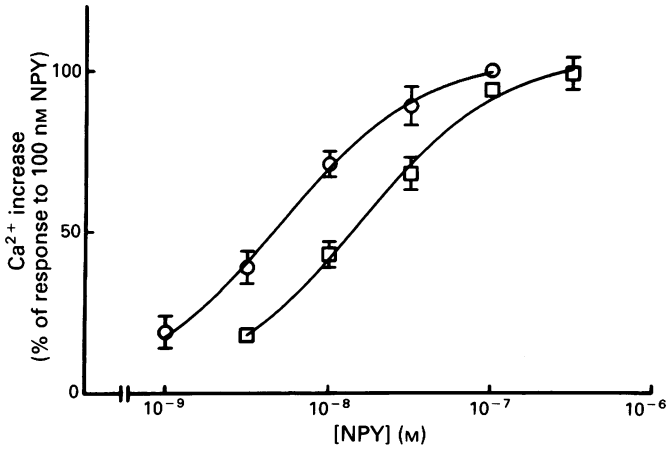

Figure 4 Antagonist properties of neuropeptide Y_{13-36} (NPY_{13-36}). Concentration–response curves for NPY-stimulated Ca^{2+} mobilization were obtained in the absence (○) or presence of 1 μM NPY_{13-36} (□). Data are the mean and vertical bars show s.e.mean of 3 experiments.

fitted better by a rectangular hyperbolic function, indicating the possible existence of a small receptor reserve for PYY-stimulated Ca^{2+} increases. Thus, 50% of maximal Ca^{2+} mobilization was achieved with approximately 30% receptor occupation.

Discussion

We have previously introduced HEL cells as a model to study human NPY receptors (Motulsky & Michel, 1988). The ability of 28 peptide analogues of NPY to stimulate Ca^{2+} elevations in this cell line (Michel *et al.*, 1990) correlates well with their ability to raise blood pressure in conscious unrestrained rats following intra-arterial injections (Boublik *et al.*, 1989). Moreover, the order of potency for Ca^{2+} increases by selected peptides from this group was similar to that for potentiating the field stimulation-induced vasoconstriction in the rabbit ear artery (Michel *et al.*, 1990). Thus, HEL cells appear to contain an NPY receptor subtype which is pharmacologically similar to that responsible for direct vasoconstriction in most vascular beds, as reflected by the blood pressure increases and for

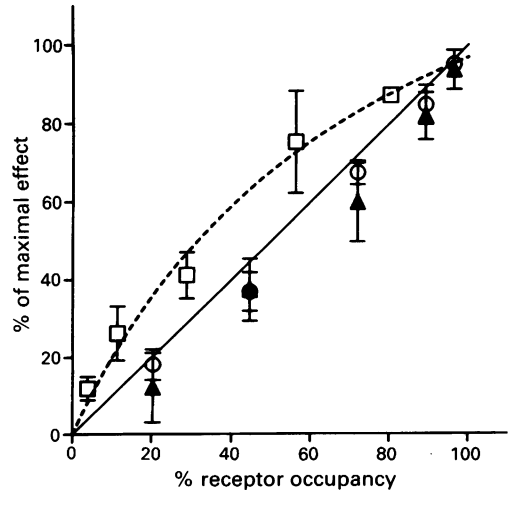

Figure 5 Receptor occupancy vs. fractional response of peptide-stimulated mobilization of intracellular Ca^{2+}. Neuropeptide Y (NPY) (○), peptide YY (□), [Pro^{34}]-NPY (▲). For assessment of the data see Methods.

potentiation of the vasoconstriction by other hormones. The value of HEL cells as a model system to study human NPY receptors has also been documented by the development of a peptide and a non-peptide antagonist of NPY receptors based on Ca^{2+} mobilization in HEL cells (Michel & Motulsky, 1990; Tatemoto, 1990). In order to evaluate further the value of this cell line for studies on human NPY receptors, we have now characterized HEL cell [^{125}I]-NPY binding sites and defined the NPY receptor subtype present in these cells; these data were used to assess the existence of a receptor reserve for Ca^{2+} mobilization via the HEL cell NPY receptor.

HEL cells exhibited high-affinity binding of [^{125}I]-NPY which had the pharmacological specificity to be expected from NPY receptors: VIP and PP had very low affinity, whereas human NPY and PYY had high affinity. Moreover, the affinity of the peptide analogues of NPY was in good agreement with their potency for mobilization of intracellular Ca^{2+}. Thus, [^{125}I]-NPY appears to label functional NPY receptors in HEL cells. The observation that HEL cells have more than 40,000 NPY receptors but less than 6000 α_2-adrenoceptors (McKernan *et al.*, 1987) might explain why NPY-stimulated Ca^{2+} increases in this cell line are consistently greater (Motulsky & Michel, 1988; Michel *et al.*, 1990) than those elicited by α_2-adrenoceptor agonists (Michel *et al.*, 1989; Motulsky & Michel, 1989).

We have previously shown that the C-terminal NPY fragment NPY_{18-36} possesses only low potency in HEL cells (Michel *et al.*, 1990), indicating the possible presence of a Y_1-like sub-type. The variable potency of C-terminal NPY fragments among assay systems, however, suggests that the definition of NPY receptor subtypes should not be based solely on a single C-terminal NPY-fragment (Michel, 1991). Therefore, we have now tested a second C-terminal fragment (NPY_{13-36}) as well as the Y_1-selective [Pro^{34}]-NPY (Krstenansky *et al.*, 1990). NPY_{13-36} and NPY_{18-36} had only very low affinity for [^{125}I]-NPY binding sites, whereas [Pro^{34}]-NPY had a similarly high affinity as NPY. This fits our functional data in which NPY_{13-36} (this study) and NPY_{18-36} (Michel *et al.*, 1990) had a low potency whereas [Pro^{34}]-NPY had a high potency. Moreover, all three peptides exhibited steep monophasic competition curves indicating interaction with a single homogeneous class of binding sites. Since PYY had a similar potency and affinity for HEL cell NPY receptors, they obviously do not belong to the PYY-insensitive Y_3-like subtype (Michel, 1991). Thus, we conclude that HEL cells express a homogeneous population of Y_1-like NPY receptors.

An additional finding of our binding experiments was that NPY_{18-36} had a ten fold lower affinity for HEL cell NPY receptors than the longer C-terminal fragment NPY_{13-36}. Since both fragments have similar affinities for the Y_2-like NPY receptors in rat brain and vas deferens (Martel *et al.*, 1990), it may be concluded that NPY_{18-36} has a greater selectivity for the Y_2-like subtype. This notion is supported by our recent literature survey which detected a greater discriminatory power of NPY_{18-36} in various assay systems (Michel, 1991).

Since the poor agonistic effects of NPY_{13-36}, VIP and PP could come from low affinity and/or from low efficacy and since NPY_{18-36} is a partial agonist at HEL cell NPY receptors (Michel *et al.*, 1990), we tested the possible antagonistic effects of these peptides. Since 1 μM VIP or PP did not inhibit the NPY-stimulated Ca^{2+} increases, they do not appear to have antagonistic effects at HEL cell NPY receptors. Since they also exhibited very low affinity in the binding studies, we conclude that their modest Ca^{2+} increasing effects are not mediated via NPY receptors but rather by a different (perhaps non-specific) mechanism. In contrast, 1 μM NPY_{13-36} produced a small but significant inhibition of NPY-stimulated Ca^{2+} increases and had detectable affinity for [^{125}I]-NPY binding sites. Similar to NPY_{18-36} (Michel *et al.*, 1990), NPY_{13-36} shifted the NPY concentration-response curve to the right without affecting the maximal response to NPY. These data

suggest competitive antagonism with a K_b value of approximately 500 nM, which is in a similar range as our previously observed K_b value of 297 nM for NPY_{18-36} (Michel *et al.*, 1990). In this context it should be noted that NPY_{18-36} behaves as a pure antagonist with low affinity at the putative Y_3-like PYY-insensitive NPY receptor in rat heart (Balasubramaniam & Sheriff, 1990). Thus, C-terminal NPY fragments apparently not only distinguished NPY receptor subtypes by their affinity, but also by their efficacy with low affinity and intermediate efficacy (partial agonism) at Y_1-like receptors (Wahlestedt *et al.*, 1986; Michel *et al.*, 1990), high affinity and efficacy (full agonism) at Y_2-like receptors (Wahlestedt *et al.*, 1986; Michel *et al.*, 1990), and intermediate affinity and low efficacy (pure antagonism) at Y_3-like receptors (Balasubramaniam & Sheriff, 1990).

We have previously demonstrated that He 90481 is a low affinity, competitive, non-peptidic antagonist for NPY-stimulated Ca^{2+} mobilization in HEL cells (Michel & Motulsky, 1990). Our binding studies confirm that He 90481 competitively binds to NPY receptors with low affinity. It should be noted, however, that the affinity of He 90481 in the binding studies was even lower than its previously obtained pA_2 value. Similarly, the affinity of the partial agonists NPY_{13-36} and NPY_{18-36} for the [^{125}I]-NPY binding sites was consistently lower than their presently (NPY_{13-36}) or previously (Michel *et al.*, 1990) described pK_b values. Moreover, the K_b value of NPY_{18-36} at the Y_3-like NPY receptor in rat heart was also significantly lower than its affinity for the NPY binding sites in this tissue (Balasubramaniam & Sheriff, 1990). In contrast, the potencies of the full agonists NPY, PYY and [Pro^{34}]-NPY agree well with their affinity in the binding studies. Taken together, these data suggest that competition for agonist binding might underestimate the potency of partial and pure antagonists.

Finally, we tested whether a receptor reserve exists for the Ca^{2+} increases elicited by NPY, PYY and [Pro^{34}]-NPY by comparing the fractional receptor occupancy and the fractional Ca^{2+} mobilization response. Since NPY receptors similar to many other G-protein coupled receptors exhibit high and low affinity states for agonists (Walker & Miller, 1988; Feth *et al.*, 1991), the question arises whether to use the high or the low affinity for calculation of receptor occupancy. On the one hand, agonists at G-protein coupled receptors are thought to act via the high affinity state of the receptor. On the other hand, steady state EC_{50} values for agonists at G-protein coupled receptors generally correlate better with their low affinity state. This is because the majority of receptors is in this state in intact cells, where the GTP concentration is high and the high-affinity state is induced following the agonist binding (Thomsen *et al.*, 1988). Thus, we used the low affinity state measurements obtained in the intact cells (where the GTP concentration is high) for our calculations of receptor occupancy. Our data do not reveal evidence for non-linear receptor–effector coupling for the full agonists NPY and [Pro^{34}]-NPY. Thus, no receptor reserve appears to exist for these two peptides. In contrast, the data points for PYY were consistently above the line of identity and were fitted well by a hyperbolic function indicating a possible small receptor reserve for this peptide. This idea is further supported by our previous observation that NPY was equally potent for inhibition of cyclic AMP accumulation and Ca^{2+} mobilization in HEL cells, whereas PYY was 18 fold more potent for inhibition of cyclic AMP accumulation than for Ca^{2+} mobilzation (Michel *et al.*, 1990). The requirement of a greater efficacy for receptor coupling to Ca^{2+} mobilization compared to coupling to inhibition of cyclic AMP accumulation was also documented by the greater efficacy of NPY_{18-36} and [D-Tyr^{36}]-NPY for cyclic AMP accumulation inhibition than Ca^{2+} mobilization (Michel *et al.*, 1990). Similar data were also found when comparing the efficacy of α_2-adrenoceptor agonists at HEL cell cyclic AMP accumulation inhibition and Ca^{2+} mobilization (Michel *et al.*, 1989). Taken together these data suggest that PYY has a greater intrinsic efficacy at HEL cell NPY receptors than NPY itself. If this would also be true for NPY receptors in other model systems, it might explain why different ratios of NPY/PYY potency have previously been obtained by other investigators in functional assay systems and raises the question as to whether PYY or NPY is the 'endogeneous' ligand at HEL cell NPY receptors.

In conclusion, our data demonstrate that [^{125}I]-NPY binding sites in HEL cells identify a homogeneous population of functional NPY receptors of the Y_1-like subtype. NPY_{13-36} is a partial agonist at these receptors. Although no receptor reserve exists for Ca^{2+} mobilization by NPY and [Pro^{34}]-NPY, a possible small receptor reserve for PYY indicates that this peptide might have a greater intrinsic efficacy at this receptor.

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