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The rabbit ileum: A sensitive and selective preparation for the neuropeptide Y Y_5 receptor

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Abstract

The rabbit ileum shows high sensitivity to neuropeptide Y. Relaxations are obtained in this tissue with human pancreatic polypeptide > peptide YY ≫ [Leu^{31},Pro^{34}]neuropeptide Y > rat pancreatic polypeptide > human neuropeptide Y in this order of potency that is indicative of a Y_5 receptor. Effects of neuropeptide Y and congeners are not affected by neuropeptide Y Y_1 receptor antagonist (BIBP 3226), but are reduced by the neuropeptide Y Y_5 receptor antagonist JCF 104 (2-(naphtalen-1-yl)-3-phenylpropane-1,2-diamine). Rabbit ilea provide sensitive and selective neuropeptide Y Y_5 receptor preparations. © 1997 Elsevier Science B.V.

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Cloning of novel neuropeptide Y receptors associated with feeding behavior in rats (Gerald et al., 1996; Hu et al., 1996), has been followed by pharmacological in vitro characterization on cells transfected with neuropeptide Y Y_1, Y_2, Y_4 or Y_5 receptor gene-sequences (Gerald et al., 1996) or with rat and human neuropeptide Y Y_5 receptor DNA (Hu et al., 1996). Using several neuropeptide Y receptor agonists and BIBP 3226, the selective neuropeptide Y Y_1 receptor antagonist (Rudolf et al., 1994), it was shown that the neuropeptide Y Y_5 receptor is equally sensitive to neuropeptide Y, peptide YY or pancreatic polypeptide and is not blocked by BIBP 3226, while Y_1 receptors are blocked by BIBP 3226 and are almost insensitive to pancreatic peptide (Gerald et al., 1996). In vitro hybridization with mRNA has shown the presence of neuropeptide Y Y_5 receptors in the brain, but little in peripheral organs (Gerald et al., 1996; Weinberg et al., 1996), despite findings that intestinal pancreatic polypeptide, peptide YY and neuropeptide Y inhibit intestinal activities presumably through specific receptors (Larhammar, 1993). In this line of thoughts, we investigated segments of intestine of various animals and found that the rabbit ileum relaxes in response to peptide YY and shows a pharmacological profile indicative of a neuropeptide Y Y_5 functional site. Ileums were obtained from Albino New Zealand rabbits weighing between 1.25 and 1.5 kg. When suspended in Tyrode solution under the conditions currently used for pharmacological assays (Regoli and Barabé, 1980), under 1 g of tension, the ileum shows spontaneous activity with regular spikes which are inhibited by pancreatic polypeptide or peptide YY in a concentration-dependent manner. The spike activity derives from the continuous release of acetylcholine since it is eliminated by tetrodotoxin (10^{-6} M) or by atropine (10^{-6} M), but not by capsaicin (10^{-5} M), L-NMMA (10^{-5} M) or indomethacin (10^{-6} M). In the presence of tetrodotoxin or atropine, pancreatic polypeptide and peptide YY are inactive, while noradrenaline is still inhibitory, suggesting that pancreatic polypeptide, peptide YY and congeners (Table 1) inhibit acetylcholine release by acting on receptors localized in the cholinergic nerves. These receptors were characterized with agonists and antagonists (Table 1). It was found that

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Table 1
Pharmacological profile of neuropeptide Y Y_5 receptors: apparent affinities of agonists (EC_{50}) and antagonists (IC_{50})

Compound	EC_{50} (nM)		α^E	IC_{50} (μM)	K_b (nM)
	Rabbit ileum	Rat Y_5 [a]			
Agonists					
hNPY	115.1 ± 16.0	0.96 ± 0.19	0.5		
hPYY	2.0 ± 0.2	1.0 ± 0.3	1.0		
hPP	0.4 ± 0.3	1.4 ± 0.5	1.0		
rPP	29.3 ± 5.3	170 ± 30	1.0		
[Leu^{31},Pro^{34}]NPY	8.7 ± 1.6	1.2 ± 0.3	1.0		
NPY-(13-36)	> 1000	20 ± 3	—		
C_2-NPY	> 1000	290 ± 30	—		
[D-Trp^{32}]NPY	> 1000	45 ± 12	—		
PYY-(3-36)	> 1000	4.2 ± 1.3	—		
Antagonists					
BIBP 3226				I	I
JCF 104				1.7	—

EC_{50}, The concentration of agonist required to produce 50% of the maximum effect. α^E, The maximal response obtainable with a full agonist (in this study, hPYY). IC_{50} (μM), The concentration of antagonist that reduced the effect of a double dose of agonist to that of a single dose. EC_{50} (nM) and K_b (nM) indicate affinities measured in biological assays using peptide YY as agonist (Gerald et al., 1996). I, Inactive at 10^{-5} M.

[a] Data taken from Gerald et al. (1996) for comparative purposes.

human pancreatic polypeptide and peptide YY are the most active compounds, followed by [Leu^{31},Pro^{34}]neuropeptide Y, while human neuropeptide Y is weak and acts as a partial agonist, which induces only 50% of the maximal response of peptide YY. The rat pancreatic polypeptide is much weaker than human pancreatic polypeptide and some fragments (neuropeptide Y-(13-36), peptide YY-(3-36)) or synthetic neuropeptide Y analogues (C2-neuropeptide Y, [D-Trp^{32}]neuropeptide Y) are inactive at micromolar concentrations. The effects of peptide YY and human pancreatic polypeptide are not blocked by BIBP 3226 while they are reduced by JCF 104 (2-(naphtalen-1-yl)-3-phenylpropane-1,2-diamine), the compound described in the patent by Gerald (1996) and shown to inhibit food intake in the rat. The pharmacological profiles described and compared in Table 1 suggest that the rabbit ileum contains a functional relaxing site that fulfills the requirements for a neuropeptide Y Y_5 receptor, despite some differences with the profile described by Gerald et al. (1996) for the rat neuropeptide Y Y_5 receptor.

The relaxation of the rabbit ileum in response to the human pancreatic polypeptide cannot be mediated by neuropeptide Y Y_1 receptors because of the high affinity of the human pancreatic polypeptide, the inefficiency of BIBP 3226 and the block by JCF 104, which is inactive on the neuropeptide Y Y_1 receptor of the rabbit saphena vein (L.H. Pheng, unpublished). It cannot be a neuropeptide Y Y_2 receptor because it is highly sensitive to the human pancreatic polypeptide and the rat pancreatic polypeptide as well as to [Leu^{31},Pro^{34}] neuropeptide Y and is insensitive to the selective neuropeptide Y Y_2 receptor agonist C_2-neuropeptide Y. It cannot be a neuropeptide Y Y_4 receptor because the rabbit ileum is sensitive to peptide YY and responds (although partially) to human neuropeptide Y. Moreover, the human pancreatic polypeptide is more potent than the rat pancreatic polypeptide. Differences between the neuropeptide Y Y_5 receptor of the rabbit ileum and that of the rat brain, namely the weakness of the human neuropeptide Y, may be explained by the fact that neuropeptide Y acts as a partial agonist, a feature that is easily detected with bioassays but not with the binding. We have however no explanation for the weakness of peptide YY (3-36) and [D-Trp^{32}]neuropeptide Y except that we have studied the neuropeptide Y Y_5 receptor of the rabbit which may be different from that of other species.

In conclusion, rabbit ileum shows high sensitivity to neuropeptide Y. Relaxations are obtained in this tissue with human pancreatic peptide > peptide YY ≫ [Leu^{31},Pro^{34}] neuropeptide Y > rat pancreatic peptide > human neuropeptide Y, in this order of potency that is indicative of a neuropeptide Y Y_5 receptor. Effects of neuropeptide Y and congeners are not affected by neuropeptide Y Y_1 receptor antagonist (BIBP 3326) but are reduced by the neuropeptide Y Y_5 receptor antagonist JCF 104. Thus, the rabbit ilea provide sensitive and selective neuropeptide Y Y_5 receptor preparations that may be useful to study the functional sites which are involved in the control of food intake in the rat and in man.

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