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The dog saphenous vein: a sensitive and selective preparation for the Y_2 receptor of neuropeptide Y

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Abstract

The dog saphenous vein responds to neuropeptide Y with a dose-dependent contraction and this vasopressor effect is not altered by the removal of the endothelium nor by the neuropeptide Y Y_1 receptor antagonist, BIBP 3226 ((*R*)-*N*^{2}-(diphenylacetyl)-*N*-[(*n*-hydroxyphenyl)methyl]-argininamide). The dose-response curves obtained with neuropeptide Y, peptide YY and with C-terminal fragments such as neuropeptide Y-(2–26), neuropeptide Y-(13–36) and peptide YY-(3–36) have similar slopes and maxima. EC_{50} values of these compounds vary between 30 ± 10 and 89 ± 47 nM. The neuropeptide Y Y_1 receptor-selective agonist [Leu^{31},Pro^{34}]neuropeptide Y and human pancreatic polypeptide are inactive up to 1 μM. This pharmacological profile suggests that the contraction of the dog saphenous vein induced by neuropeptide Y and its homologues or fragments is mediated by a neuropeptide Y Y_2 receptor type. Moreover, this neuropeptide Y Y_2 receptor appears to be localized in the venous smooth muscle, where it exerts a direct myotropic effect that may be useful for the pharmacological characterization of new compounds acting as agonists or antagonists of the neuropeptide Y Y_2 receptor.

Keywords: Neuropeptide Y; Bioassay; Saphenous vein, dog; Neuropeptide Y Y_2 receptor

1. Introduction

Two different receptors for neuropeptide Y were proposed 10 years ago by Wahlestedt et al. (1986) on the basis of results obtained with naturally occurring peptides (neuropeptide Y, peptide YY) and neuropeptide Y fragments. This proposal was validated a few years later (a) by the identification of selective agonists for both neuropeptide Y Y_1 (Fuhlendorff et al., 1990) and Y_2 (Wahlestedt et al., 1986) receptors, (b) by the discovery of an antagonist (BIBP 3226) ((*R*)-*N*^{2}-(diphenylacetyl)-*N*-[(*n*-hydroxyphenyl)methyl]-argininamide), which interacts with the neuropeptide Y Y_1 but not with the neuropeptide Y Y_2 receptor (Rudolf et al., 1994) and (c) by the cloning of both the neuropeptide Y Y_1 (Herzog et al., 1992) and the neuropeptide Y Y_2 (Rose et al., 1995) receptors.

The most commonly used bioassay for the neuropeptide Y Y_2 receptor is the rat vas deferens stimulated electrically (Wahlestedt et al., 1986), where neuropeptide Y inhibits the release of the endogenous transmitter (noradrenaline) by acting on its prejunctional neuropeptide Y Y_2 receptor. The myotropic effect of neuropeptide Y is therefore indirect since it derives from the activation, by noradrenaline, of α_1 smooth muscle receptors. The limitations of such a preparation are obvious, especially when maximum effects have to be measured to build complete concentration–response curves and estimate agonist (pD_2) or antagonist (pA_2) affinities. Moreover, it has been suggested that this preparation is not a pure Y_2 system (Jorgensen et al., 1990). It may contain the neuropeptide Y_4 receptor subtype since the [Leu^{31},Pro^{34}]neuropeptide Y and the pancreatic polypeptide show activities in this preparation.

In the present study, we have looked for a vascular preparation in order to provide a new bioassay for the characterization of vascular smooth muscle contractile receptors of the Y_2 type. Such a functional site has already been shown to be present in the pig spleen circulation (Modin et al., 1991). In the dog, such a site is found in the saphenous vein, where it mediates concentration-dependent contractions in response to neuropeptide Y and shows a typical Y_2 receptor pharmacological profile.

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2. Materials and methods

2.1. Chemicals

The following peptides: human neuropeptide Y, porcine peptide YY, human peptide YY-(3–36), porcine neuropeptide Y-(13–36) and porcine [Leu^{31},Pro^{34}]neuropeptide Y were synthesized and purified in our laboratory; human pancreatic polypeptide was purchased from Bachem (USA), the neuropeptide Y Y_1 receptor antagonist, BIBP 3226 was obtained from Karl Thomae (Germany) and methysergide, indomethacin, phentolamine, diphenhydramine, propranolol and noradrenaline bitartrate were purchased from Sigma (USA). All compounds were dissolved in oxygen-free water and kept in concentrated solutions (1 mg/ml) at −20°C until used. Noradrenaline was dissolved in water containing ascorbic acid.

2.2. Bioassays

Mongrel dogs of either sex, weighing between 20 and 50 kg, were killed in the laboratory of the Animal Protection Branch in Sherbrooke with carbon dioxide, according to the rules of the Canadian Council for Animal Care. The saphenous vein (3–4 cm from the medial genicular vein) was taken out and a small glass canula (diameter approximately 2 mm) was pushed into the lumen for facilitating the preparation of helical strips (segment of 3 mm large and 3 cm long) which were suspended in 10 ml organ baths containing oxygenated (with 5% CO_2 and 95% O_2) Krebs solution (see Rioux et al. (1973) for the composition of the Krebs) at 37°C. The endothelium was removed from all preparations by rubbing the lumen side with a Whatman filter paper. The vein strips were stretched with a tension of 2.0 g and changes of tension produced by contractile agents were recorded isometrically with Grass (FT 03C) transducers and displayed on a Grass (Model 7D) polygraph. The contractile responsiveness of the tissues was tested after 90 min of equilibration, by adding a single dose of noradrenaline (10^{-7} M) or neuropeptide Y (10^{-8} M) to the organ baths. Four out of five preparations derived from different animals responded to neuropeptide Y: the unresponsive tissues were discarded. Concentration–response curves to neuropeptide Y and related peptides were measured to estimate their apparent affinities. In some tissues, contracted with neuropeptide Y (10^{-7} M), the absence of endothelium was assessed by the lack of relaxing effect of acetylcholine (2.6×10^{-6} M). Results are presented in terms of EC_{50} (concentration of agonist inducing 50% of the maximal response) and are given as means ± S.E.M. of at least 4 determinations. EC_{50} values were calculated by fitting the concentration–response curves data into the logistic equation:

$$\text{Effect} = E_{max} \times [A]^n / [A]^n + [EC_{50}]^n$$

where E_{max} is the maximal effect, [A] is the agonist concentration and n is the slope parameter.

3. Results

Tracings in Fig. 1 indicate that the dog isolated saphenous vein responds to porcine peptide YY (0.2–1000 nM) with concentration-dependent contractions which reach stable plateaux and a maximum tension of 2.0–2.5 g. The effect is rapidly reversible after washing out the peptide and the full response is generally recovered within 40–60 min. The contractions induced by human neuropeptide Y (10–1000 nM) reach also the maximum, while those evoked by porcine neuropeptide Y are weaker (Fig. 1). The effect of peptide YY was not modified by BIBP 3226 (10^{-6} M) as well as by 10^{-6} M of phentolamine, propranolol, methysergide or diphenhydramine (not shown). Indomethacin however slightly potentiated the contractile effect of human neuropeptide Y or porcine peptide YY in this preparation.

Neuropeptide Y and peptide YY as well as some of their C-terminal fragments, porcine neuropeptide Y-(2–36),

Table 1
Pharmacological parameters obtained with neuropeptide Y (NPY), pancreatic polypeptide (PP), peptide YY-related peptides in the dog saphenous vein and adipocytes, and the rat vas deferens: comparison with affinities measured in LMTK$^-$ mouse fibroblast cells

Agonist	Dog saphenous vein		Dog adipocytes [a]	Rat vas deferens [b]	Rat Y_2-transfected LMTK$^-$ cells [c]
	EC_{50} (nM)	α^E	K_i (nM)	EC_{50} (nM)	EC_{50} (nM)
Porcine PYY	30 ± 10	1.0	0.05 ± 0.01	2.4 ± 1.1	0.58 ± 0.05
Human NPY	77 ± 4	1.0	0.20 ± 0.09	44 ± 2	1.2 ± 0.2
Porcine NPY	62 ± 17	0.8	–	25 ± 3	2.7 ± 0.1
Human PP	> 1000	–	–	–	> 1000
Human PYY-(3–36)	72 ± 8	1.1	0.57 ± 0.27	4.1 ± 2.4	0.64 ± 0.19
Porcine NPY-(2–36)	30 ± 3	1.0	0.79 ± 0.34	–	1.2 ± 0.4
Porcine NPY-(13–36)	89 ± 47	0.6	4.90 ± 3.0	340 ± 60	2.2 ± 0.7
Porcine [Leu^{31},Pro^{34}]NPY	> 1000	–	45 ± 33	280 ± 40	> 1000

EC_{50} represents the concentration of peptide inducing 50% of the maximal response. α^E, intrinsic agonist activity expressed as a fraction of the maximum response to peptide YY. Data are means ± S.E.M. of 4–15 experiments.
[a] Wieland et al. (1995); [b] Dumont et al. (1994); [c] Gerald et al. (1996).

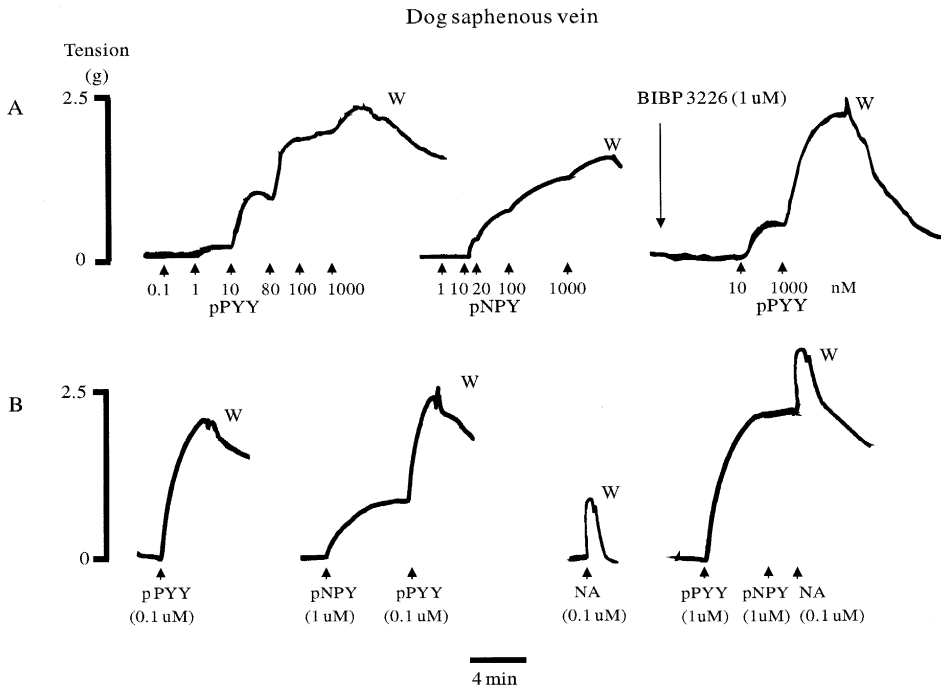

Fig. 1. Original recordings showing: (A) the cumulative concentration–response curves of porcine peptide YY (pPYY), porcine neuropeptide Y (pNPY) and porcine peptide YY in the presence of BIBP 3226; (B) the effect of porcine peptide YY in the absence and presence of a maximal concentration of porcine neuropeptide Y and the addition of porcine neuropeptide Y as well as of noradrenaline (NA) at the top of a maximal contraction elicited by porcine peptide YY. Ordinate, changes of tension in g; abscissa, time in min; W, washout.

porcine neuropeptide Y-(13–36) and human peptide YY-(3–36) show strong activities and are as active as neuropeptide Y (Fig. 1 and Table 1), while porcine [Leu^{31},Pro^{34}]neuropeptide Y and human pancreatic polypeptide are inactive and do not antagonise the effect of porcine peptide YY. The preparation maintains a good sensitivity for hours, when enough time (40–60 min) is left between two cumulative curves. Concentration–response curves obtained with various neuropeptide Y-related peptides (Fig. 2) indicate that three of the five peptide agonists have the same maximum effect (see also α^E in Table 1) while porcine neuropeptide Y appears to be a partial agonist. To clarify this point, an average concentration of porcine peptide YY was tested alone and in the presence of a maximal concentration of porcine neuropeptide Y. Porcine peptide YY was able to evoke the maximal response, suggesting that porcine neuropeptide Y does not exert any antagonistic effect on porcine peptide YY. In the presence of porcine peptide YY (1 μM), porcine neuropeptide Y did not have any effect, while noradrenaline evoked its full contraction. Interestingly, human neuropeptide Y appears to be more active than the corresponding porcine sequence. The apparent affinities of the various peptides, given in terms of EC_{50} values, are presented in Table 1 and indicate that the most potent peptides are porcine peptide YY and the selective neuropeptide Y Y_2 receptor agonist, the human peptide YY-(3–36). The neuropeptide Y Y_1 receptor antagonist described by Rudolf et al. (1994), BIBP 3226, is completely inactive (Fig. 1) against the contraction elicited by porcine peptide YY or neuropeptide Y in this preparation.

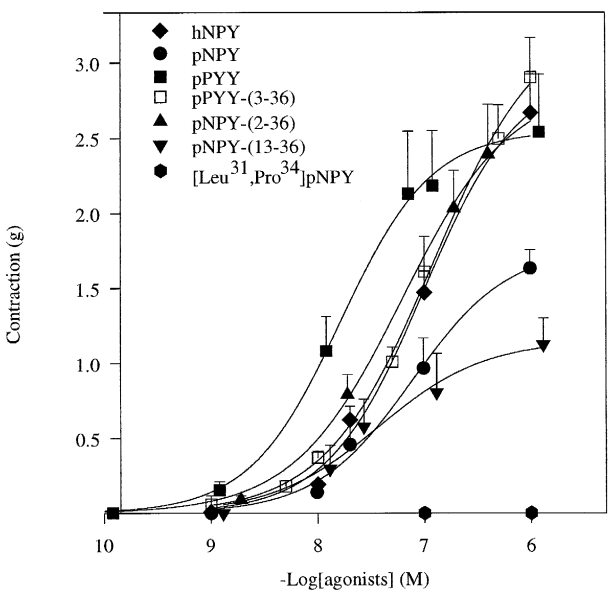

Fig. 2. Concentration–response curves of porcine peptide YY, human neuropeptide Y and porcine neuropeptide Y as well as related fragments and analogues in the dog saphenous vein. Each point represents the mean ± S.E.M. of 4–15 determinations. Ordinate, contraction in g; abscissa, concentration (M) of agonist.

4. Discussion

A systematic screening of tissues, particularly vessels, of various species led to the identification of the dog saphenous vein as a sensitive neuropeptide Y Y_2 receptor preparation. Already Hunter et al. (1996) have reported that the neuropeptide Y content in some dog veins (saphenous and portal veins) is higher than in other tissues and neuropeptide Y could be released in greater amounts than from other vessels: however, these authors did not observe any direct or indirect biological effect of exogenous neuropeptide Y on the isolated saphenous vein suspended in vitro. Different experimental conditions could be invoked to explain the discrepancy between the present results and those of Hunter et al. (1996). Firstly, Hunter et al. used rings instead of strips as we did. Secondly, we have observed that not all sections of the vein respond to neuropeptide Y: it is therefore possible that Hunter et al. used a part of the vein that does not contain receptors for neuropeptide Y. In the present study, the segment of 3–4 cm from the medial genicula vein (see Section 2) was used and found to contract in the presence of neuropeptide Y.

Similar to other veins, for instance the saphenous vein of the rabbit (Cadieux et al., 1993), the dog saphenous vein responds to peptides related to neuropeptide Y with concentration-dependent contractions that reach stable plateaux and allow for the recording of cumulative concentration–response curves, well reproducible, if enough time is allowed between repeated determinations. Such a characteristic is very useful for comparing agonist potencies and for estimating antagonist affinities. The contractions induced by neuropeptide Y are independent of endothelium: as mentioned in Section 2 all preparations used in the present study were endothelium-denuded; moreover, the contractions induced by neuropeptide Y are not mediated by the release of noradrenaline, 5-hydroxytryptamine or histamine. Arachidonic acid metabolites appear to attenuate the contractile effect of neuropeptide Y in some tissues since indomethacin (not shown) was found to slightly potentiate the contractile effect of neuropeptide Y. The present results also indicate that human neuropeptide Y (and porcine peptide YY) have higher intrinsic activities than porcine neuropeptide Y and porcine neuropeptide Y-(13–36). We have no explanation for these findings. Thus, the new preparation allows for the distinction between potent and less potent agonists among various neuropeptide Y-related peptides that interact with the neuropeptide Y Y_2 receptors. In fact, some peptides (porcine neuropeptide Y and especially porcine neuropeptide Y-(13–36)) have simply lower activities than human neuropeptide Y or porcine peptide YY. Thus, the order of potency of neuropeptide Y-related peptides in the dog Y_2 receptor is as follows: peptide YY ≈ peptide YY-(3–36) ≈ neuropeptide Y ≈ neuropeptide Y-(13–36) ≫ [Leu^{31},Pro^{34}]neuropeptide Y ≈ pancreatic polypeptide, and is similar (although not identical) to the order of potency of agonists which has been determined on cloned rat Y_2 receptor, where: peptide YY ≈ peptide YY-(3–36) ≈ neuropeptide Y ≈ neuropeptide Y-(13–36) ≫ [Leu^{31},Pro^{34}]neuropeptide Y > pancreatic polypeptide.

On the other hand, the order of potency in the rat vas deferens is: peptide YY ≈ peptide YY-(3–36) ≈ neuropeptide Y ≫ neuropeptide Y-(13–36) ≈ [Leu^{31},Pro^{34}]neuropeptide Y.

The present results are also in agreement with the finding of Wieland et al. (1995) who compared the affinities of neuropeptide Y-related peptides in dog adipocytes by binding assays (see comparison in Table 1). As for antagonists, the neuropeptide Y Y_1 receptor antagonist BIBP 3226 (Rudolf et al., 1994) is inactive, as it is to be expected from a tissue that contains only Y_2 receptors. The dog saphenous vein contraction in response to neuropeptide Y-related peptides is not mediated by neuropeptide Y Y_3 or Y_4 receptors because porcine peptide YY is the most active agonist; it is not mediated by neuropeptide Y Y_5 receptors, since human pancreatic polypeptide is inactive.

In conclusion, the dog saphenous vein is a sensitive preparation which can be used as a pure neuropeptide Y Y_2 receptor system for the pharmacological characterization of new compounds interacting, as agonists or antagonists, with the neuropeptide Y Y_2 receptor. From the pharmacological profile, described in this paper, the dog saphenous vein appears to be a reliable neuropeptide Y Y_2 receptor system, useful in neuropeptide Y pharmacology, because the effect of neuropeptide Y is a direct contractile one, it is easily quantified over a large extent of concentrations and is not antagonized by BIBP 3226.

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