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Differential vascular effects of neuropeptide Y(NPY) selective receptor agonists

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Abstract. Neuropeptide Y (NPY) increases blood pressure either directly or indirectly by potentiating the effect of various vasoconstrictors. Only one (the Y_1-receptor) of two subtypes of receptors (Y_1 and Y_2) is thought to mediate the vascular smooth muscle contraction. To test this hypothesis we challenged isolated rat mesenteric arteries that had a functional endothelium with (1–36) NPY and with specific Y_1-receptor ([Leu^{31}, Pro^{34}] NPY) and Y_2-receptor ([Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY) agonists. The Y_1-receptor agonist elicited a contractile response similar to that of NPY, whereas the Y_2-receptor agonist had no effect on wall tension. We also found that the presence of a functional endothelium has no influence on the contractile response to NPY. From these data we conclude that the direct contractile effect of NPY in the mesenteric artery is mediated by stimulation of Y_1-receptors and is not endothelium-dependent.
Key words. Arterial contractility; Y_1- and Y_2-receptors; endothelium; norepinephrine; acetylcholine.

Neuropeptide Y is a 36 amino-acid peptide which belongs to the pancreatic polypeptide family[1,2]. This peptide is found in the central and peripheral nervous system of mammals, including humans and is thought to play an active role in cardiovascular regulation[3]. In the periphery, NPY can increase blood pressure by a direct effect[4] as well as by potentiating the contractile effect of various vasoconstrictors, including norepinephrine and angiotensin II[5,6].

Two major subtypes of NPY receptors have been isolated and characterized so far[7–10]. The Y_1-receptor seems to be located only postsynaptically; its activation requires the entire NPY peptide or at least large parts of the N- and C-terminus[11,12]. One peptide, [Leu^{31}, Pro^{34}] NPY, has been shown to be a specific Y_1-receptor agonist[13]. It binds to cells possessing Y_1-receptors alone and induces intracellular responses which are similar to those evoked by NPY. Y_1-receptor stimulation causes vascular smooth muscle contraction[9].

The second subtype of receptor, the so-called Y_2-receptor, is the major NPY receptor present in the central nervous system. In the peripheral nervous system, it is located predominantly presynaptically[8]. Activation of the Y_2-receptor inhibits norepinephrine release by sympathetic nerve endings[9]. Recently a NPY analog ([Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY) has been developed and demonstrated to be highly specific for the Y_2-receptor[14].

The purpose of this study was first to compare in vitro the vascular effects of (1–36) NPY, the Y_1-receptor agonist [Leu^{31}, Pro^{34}] NPY and the Y_2-receptor agonist [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY.

Materials and methods

Male Wistar rats (Iffa Credo, Domaine des Oncins, L'Arbresle, France), weighing 230 to 280 g, were used for this study. They were housed in a room with a constant temperature of 23 °C. Ordinary rat chow and water were provided ad libitum. The protocol and procedures used in the present study were approved by the governmental review committee for animal experiments. The animals were anesthetized with 50 mg pentobarbital (Nembutal) administered i.p. After laparotomy, third generation branches originating from the superior mesenteric artery were prepared. Two tungsten wires (25 µm diameter) were inserted through the lumen of the arterial segments (length 2 mm, diameter 150 µm) which were subsequently mounted on a myograph[15]. One wire was attached to a support carried by a micromanipulator (MR 50, Micro-Controle, Evry, France), the other to a support fixed on the arm of a force transducer (DSC-6, Kistler-Morse, Cambridge, England). This procedure lasted about 20 min. The vessels were equilibrated for 2 h in a physiological salt solution (mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5 and glucose 5.5) that was continuously aereated with 95% O_2 and 5% CO_2 and maintained at 38.5 °C. The vessels were then stretched as described by Freslon and Guidicelli[16]. Briefly, the distance between the threads of tungsten wire was increased with the micromanipulator in order to generate a tension of 100 mmHg (representing the force exerted by the vessel on the transducer divided by the length of the

vessel). Then the vessels were challenged with increasing norepinephrine doses (10^{-8} to 10^{-5} M) (Arterenol, Hoechst AG, Frankfurt am Main, Germany).

In a first set of experiments we used isolated mesenteric arteries to see whether the presence or absence of a functional endothelium is a prerequisite for the direct effect of NPY. The presence of endothelium was confirmed by the ability of acetylcholine (10^{-7} to 10^{-5} M) (Sigma Chemical Company, St. Louis, Missouri, USA) to relax vessels precontracted with 10^{-5}M norepinephrine by more than 50%. To remove the endothelium, the internal wall of the vessel was rubbed with a tungsten wire. Thereafter, the vessels were equilibrated again for one hour before starting the challenge with various concentrations of (1–36) NPY, from 10^{-12} M to 10^{-7} M (Sigman Chemical Company, St. Louis, MO, USA), the doses being increased each time after a 10 min washout.

In a second set of experiments we compared the vascular effects of (1–36) NPY with those of the Y_1-receptor agonist and the Y_2-receptor agonist. The Y_1-receptor agonist [Leu^{31}, Pro^{34}] NPY (Peninsula Laboratories, Heidelberg, Germany) was purchased. The Y_2-receptor agonist [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY was synthesized and purified as described elsewhere[14]. This peptide has a high affinity and selectivity for the Y_2-receptor as demonstrated by binding to cell lines bearing only this subtype of NPY receptor. Like NPY, this Y_2 agonist inhibits forskolin-induced cyclic AMP accumulation. It is also able to inhibit the norepinephrine release in the vas deferens, a preparation used to test the Y_2 presynaptic effects[14]. A small amount of this latter peptide was available for the studies so that the experiments could only be performed in 5 rats. The 3 peptides were always tested in the same order, the doses being increased after a 10 min washout period, i.e. first (1–36) NPY (10^{-10} to 10^{-7} M); then, after a washout period of 30 min, the Y_1-receptor agonist [Leu^{31}, Pro^{34}] NPY (10^{-10} to 10^{-7} M) and finally, after another 30 min washout period, the Y_2-agonist [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY (10^{-10} to 10^{-7} M).

The tension of the arterial segment is expressed in milliNewton per millimeter (mN/mm). The data are reported as means ± SEM.

Results

Figure 1 shows the peak increase in arterial tension induced by increasing doses of norepinephrine in 5 vessels with and in 7 vessels without a functional endothelium. Acetylocholine (10^{-7} to 10^{-5} M) was added to the top of the higher dose of norepinephrine (10^{-5} M). Acetylcholine caused a dose-dependent relaxation of the precontracted vessels with a functional endothelium but had no significant effect on the vessels whose internal walls had been rubbed with the wire.

The arteries were then challenged with increasing concentrations of (1–36) NPY. Using NPY at 10^{-9} and 10^{-8} M, there was no significant difference between the contractile responses obtained in the arteries with (n = 5) or without (n = 7) a functional endothelium. The lower concentrations (10^{-12} to 10^{-10} M) had no significant contractile effect whether the endothelium was present or not (not shown). The highest dose (10^{-7} M) caused similar responses to those induced by 10^{-8} M (not shown).

In the second set of experiments only vessels (n = 5) with a functional endothelium were used. The contractile response to NPY and [Leu^{31}, Pro^{34}] NPY were similar, with no effect being observed at 10^{-10} M and the maximum being reached at 10^{-8} M. [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY had no effect at all in this vascular

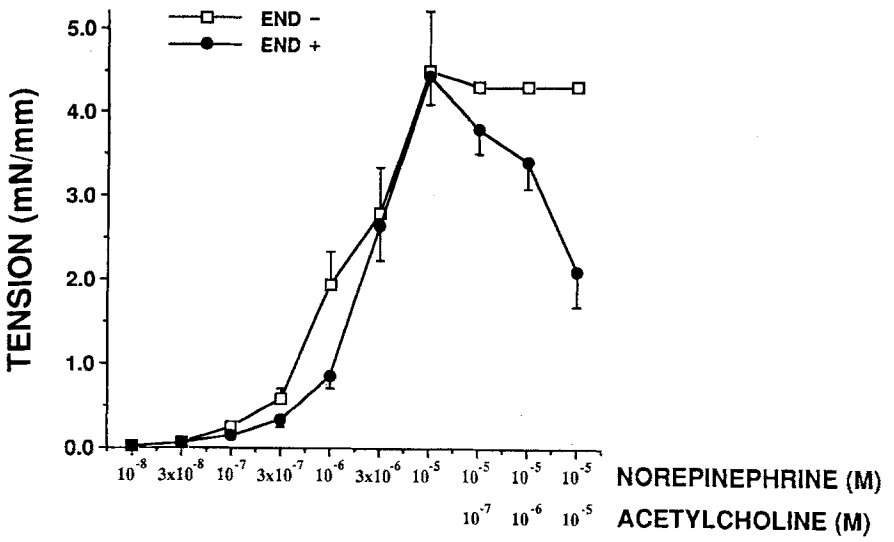

Figure 1. Effect of acetylcholine (Ach) on the tension of 5 mesenteric arteries with (END+) and 7 arteries without (END–) a functional endothelium. All arteries were precontracted with norepinephrine (NE) (mean ± SEM).

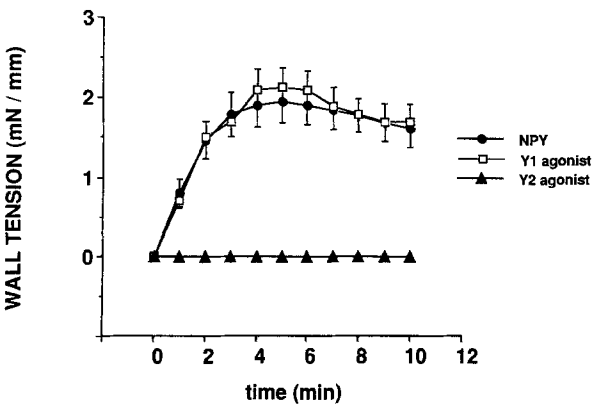

Figure 2. Effect of an equimolar concentration (10^{-7} M) of (1–36) NPY, [Leu^{31}, Pro^{34}] Y_1-agonist) and [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY (Y_2-agonist) on the wall tension of 5 mesenteric arteries with an intact endothelium (Mean ± SEM).

preparation. Figure 2 depicts the time course of the contractile responses obtained with the 3 test agonists at a concentration of 10^{-8} M.

Discussion

This study confirms that the rat mesenteric arterial bed is highly sensitive to NPY, a peptide with direct vasoconstrictor properties[17]. It also shows that (1–36) NPY and [Leu^{31}, Pro^{34}] NPY produce, at equimolar concentrations, a contractile response of similar magnitude. This is of interest since [Leu^{31}, Pro^{34}] NPY binds specifically to Y_1-receptors, i.e. the receptors located on vascular smooth muscle cells[13]. It is still debated whether the presence of a normally functioning endothelium influences the direct vasoconstrictor action of NPY[17,18]. In our hands, the response of the mesenteric artery to NPY was not altered after removal of the endothelium. It is relevant that [Ahx$^{5-24}$, γ-Glu^2-ε-Lys^{30}] NPY lacks any effect on the mesenteric artery. This peptide is the most specific Y_2-receptor agonist described to date[14]. Y_2-receptors are present essentially at the presynaptic side of sympathetic terminals. The present findings support therefore the concept that the direct vasoconstrictor effect of NPY is Y_1-mediated. In a previous study, another Y_2-receptor agonist, (13–36) NPY, caused a contraction of the porcine spleen, suggesting that Y_2-receptors were also present post-synaptically[19]. The same NPY fragment was however devoid of effect on systemic pressure and renal artery resistance. The existence of Y_2-receptors on the vasculature of some organs can consequently not be ruled out.

In summary, these data indicate that the direct constrictor effect of NPY in the mesenteric artery is mediated by stimulation of Y_1-receptors and does not depend on the presence of an intact endothelium. This vascular bed can therefore serve to characterize the effects of peptides or compounds with NPY agonistic or antagonistic properties.

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