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Chemical sympathectomy reveals pre- and postsynaptic effects of neuropeptide Y (NPY) in the cardiovascular system

Y. Mabe, R. Pérez, K. Tatemoto* and J. P. Huidobro-Toro

*Laboratory of Pharmacology, Department of Physiological Sciences, Faculty of Biological Sciences, Catholic University of Chile, P.O. Box 114-D, Santiago 1 (Chile), and *Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto (California, USA), 3 February 1987*

Summary. Intravenous injection of neuropeptide Y (NPY) caused short-lasting dose-dependent pressor responses in anesthetized rats. NPY was equipotent with noradrenaline in producing proportional pressor effects. Chemical sympathectomy, following the administration of 100 mg/kg 6-hydroxydopamine (6-OHDA), significantly potentiated the systemic pressor effects elicited by NPY or noradrenaline. Pretreatment with 2 nmol NPY enhanced the noradrenaline-induced pressor response in control rats. NPY did not change the basal tension of isolated rat aortic strips but significantly potentiated the contractile activity induced by 16 nM noradrenaline. This effect of NPY was not observed in aortic strips from rats pretreated with 6-OHDA. The presence of pre- and postsynaptic sites of action for NPY in the cardiovascular system of the rat is discussed.
Key words. Vascular system; NPY responses; NPY-pressor responses; NPY-induced supersensitivity; aortic contractions.

Neuropeptide Y (NPY), a 36-amino acid peptide with structural homology with avian pancreatic polypeptide, is frequently found co-stored with noradrenaline in discrete brain nuclei including the human brain medulla[1] and a variety of sympathetically innervated organs such as the heart, spleen, vas deferens and blood vessels[2,3]. Although the physiology of NPY remains to be established, because of its co-distribution with noradrenaline in central and peripheral synapses, particularly in nerve terminals around blood vessels[4,5], it is anticipated that the peptide plays a role in the noradrenergic transmission in the vascular system. In fact, it is known that NPY contracts blood vessels, increasing systemic blood pressure via a non-adrenergic mechanism[6,10]. NPY enhances the contractile effects of noradrenaline and other pressor substances in isolated vascular smooth muscles[11,12] and potentiates the hypertensive response caused by preganglionic sympathetic nerve stimulation[13]. The aim of this study was to document further the pre- and postjunctional effects of NPY on the cardiovascular system. To distinguish between pre- and postsynaptic sites of action, we sympathectomized rats using 6-hydroxydopamine (6-OHDA) and examined the pressor activity of NPY. In addition, we used isolated aortic strips from normal and 6-OHDA-treated rats to examine whether NPY interacts with noradrenaline in this vessel. Results discuss the presence of pre- and postsynaptic NPY sites in the vascular territory.

Materials and methods. Blood pressure monitoring. Male Sprague Dawley rats (250–280 g) were anesthetized with 40 mg/kg sodium pentobarbital i.p.; systemic blood pressure was recorded continuously via a catheter inserted into the carotid artery. The catheter was connected to a strain gauge transductor; continuous blood pressure recordings were inscribed on a Grass polygraph (for details see Mabe et al.[10]). Drugs were administered i.v. via a cannula placed on the femoral vein. Groups of 6 rats each were used; one group was treated with 100 mg/kg 6-OHDA i.v. 48 h prior to blood pressure monitoring; the control group was injected i.v. with the solvent (0.3 ml of 1% ascorbic acid dissolved in saline). Results compare the mean increase in systolic or diastolic blood pressure induced by NPY and noradrenaline in control and 6-OHDA-treated rats.

In a second series of experiments, noradrenaline pressor dose-response curves were performed in 10 normal rats before and 10 min after a bolus dose of 10 µg NPY/rat (2 nmol). Results plot the increase in systolic blood pressure caused by noradrenaline before and after treatment with NPY.

Isolated aortic strips preparation; noradrenaline-NPY interactions. Adult male Sprague-Dawley rats were sacrificed by cervical dislocation. The thorax and abdominal cavity were opened; the aorta was dissected from the heart to the mid-abdomen. The tissue was placed on a Petri dish containing Tyrode solution to prepare spiral strips of the aorta 2–3 cm long. Special precaution was taken not to damage the aorta endothelium during the tissue handling. The artery was immediately mounted on a 30-ml organ bath to record isometric contractions. The buffer was maintained at 37 °C and bubbled with 95% O_2/5% CO_2 to maintain the pH close to 7.4. Tissues were given a resting tension of 5 mNewton (mN) that was maintained throughout the experiment. The tissues were washed with fresh oxygenated Tyrode solution every 20 min to prevent accumulation of toxic metabolic end products[14]. The composition of the Tyrode solution was as follows (mM): NaCl 118; KCl 5.4; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 11.1 and $NaHCO_3$ 23.8. The recording of isometric contractions was done with a Grass FT-3C force displacement transducer coupled to a Grass polygraph.

Tissues were equilibrated for 90 min prior to drug additions. Different concentrations of noradrenaline were tested; 16 nM noradrenaline provided the most consistent and reproducible contractile responses to the application of the catecholamine. The noradrenaline-induced aortic contractions were examined in strips from control rats before and after tissue incubation with 10 µg NPY (190 nM) for 3 min.

In a parallel series of experiments, aortic strips from rats pretreated 48 h before with 100 mg/kg 6-OHDA i.v. were prepared (these rats were not the same as those used in blood pressure monitoring). Groups of aortic strips were challenged with 16 nM noradrenaline, while another group was tested with 16 nM noradrenaline in the presence of 190 nM NPY. Results compare the contractile activity of noradrenaline in control and sympathectomized rats and the effects of NPY in the two populations of rats.

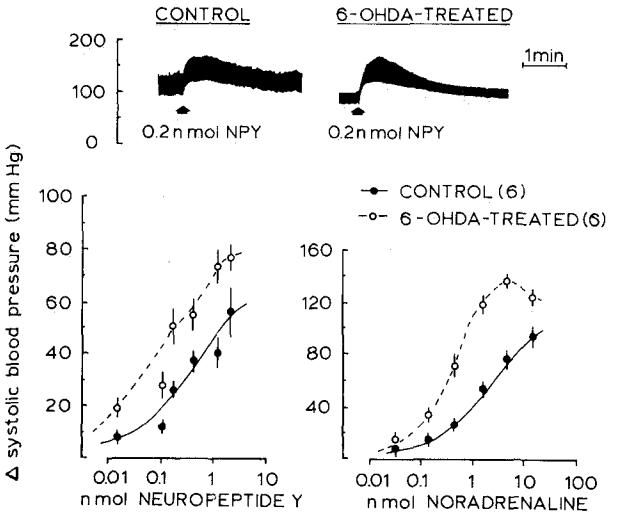

Figure 1. Pressor dose-response curves of neuropeptide Y (NPY) and noradrenaline (NA) in control and sympathectomized rats. *Upper panel:* tracing of the pressor effect produced by 0.24 nmol NPY injected i.v. in a control rat and a rat treated with 6-hydroxydopamine (6-OHDA) (100 mg/kg, 48 h prior to blood pressure monitoring). Calibration in mmHg. *Lower panel:* mean increase in systolic blood pressure caused by NPY and NA. Bars indicate the SE. Systolic blood pressure in the control group was 133 ± 8 mmHg, a value that was significantly lowered by the 6-OHDA treatment (90 ± 4 mmHg, $p < 0.01$).

Drug sources. Natural porcine NPY was purified by K. Tatemoto. Noradrenaline HCl and 6-OHDA HBr were purchased from Sigma Chemical Co. (St. Louis, MO). Drugs were dissolved in saline immediately prior to use. 6-OHDA solutions were prepared in saline containing 1% ascorbic acid to avoid drug oxidation.

Statistical methods. Analysis of variance was utilized in all cases. Significance was set at a p-value less than 0.05.

Results. NPY-induced pressor responses in control and sympathectomized rats. I.v. administration of NPY caused increases in systolic and diastolic pressure proportional to the dose. On a molar basis, NPY proved almost equipotent as compared to noradrenaline. The duration of the NPY-induced pressor activity varied with the dose, but ranged between 2 and 5 min. No tachycardia was associated with the short-lasting hypertension. Chemical sympathectomy did not abolish the NPY or the noradrenaline-induced pressor effects. On the contrary, the pressor effect of NPY was significantly potentiated both in magnitude and duration (6/6 rats treated with 6-OHDA). Supersensitivity to NPY and noradrenaline following sympathectomy was evidenced by a displacement of the dose-response curves to the left (fig. 1).

Synergism of the noradrenaline-induced pressor response following NPY. The i.v. bolus application of 2 nmol NPY/rat caused a rapid increase of systolic and diastolic blood pressure of 48.3 ± 5.2 mm Hg. 10 min after the application of NPY, when arterial blood pressure returned to control values, the injection of noradrenaline caused increases in blood pressure that were significantly larger than those obtained prior to NPY. As shown in figure 2, the noradrenaline dose-response curve was significantly shifted to the left in a parallel fashion.

NPY potentiation of the noradrenaline-induced aortic contractions. Noradrenaline contracted the isolated rat aortic strip in a concentration-dependent fashion (data not shown). The application of NPY did not modify the muscular tension of the aortic strip but increased significantly the contractions developed by noradrenaline. Figure 3A illustrates the contractile activity induced by 16 nM noradrenaline in the absence and in the presence of 190 nM NPY. In contrast to the effect of NPY obtained in aortic strips from control rats, the NPY-induced potentiation of the noradrenaline response was not observed in aortic strips from rats pretreated with 6-OHDA (fig. 3B).

Discussion. It is likely that the pressor effect of NPY can be explained, at least in part, by an increase in peripheral resistance, as was originally described in the submandibular glands[6]. In addition to vascular actions, NPY has been shown to increase inotropism slightly in the heart of certain rodents[15, 16]; however, no clear-cut changes in chronotropism have been reported[2, 16]. The pressor action of NPY appears to be mainly of peripheral origin since essentially the same magnitude of increase in blood pressure was observed following the administration of 2 nmol NPY in pithed rats[13] as in the present study. At present, two mechanisms can be invoked to explain the influences of NPY on the adrenergic activity of the vascular system; firstly, the activation of a postsynaptic NPY receptor, which appears to be linked to influxes of extracellular calcium[7, 10, 17] and secondly, a presynaptic feed-back mechanism controlling the release of catecholamines from peripheral sympathetic nerves[18, 19].

It is well documented that 6-OHDA destroys adrenergic nerve terminals causing a depletion of both noradrenaline and NPY-like immunoreactivity in a variety of sympathetically innervated organs[2, 20]. The finding that the NPY-induced pressor response is potentiated in the absence of adrenergic nerve terminals must imply that the pressor effect of NPY is not of presynaptic origin. It is thus reasonable to postulate the existence of postsynaptic NPY receptors located in the smooth muscle membranes of the vascular tree. It is clear that the pressor effect of NPY is not mediated via activation of alpha 1-adrenoceptors, since the effect of NPY in isolated blood vessels or in the whole animal is not modified by alpha 1-adrenoceptor blocking drugs[6, 7, 10]. Supersensitivity to the pressor effects of both NPY and noradrenaline developed as a result of the denervation caused by the neurotoxin upon destruction of the noradrenergic synapses where these neurotransmitters are presumably co-

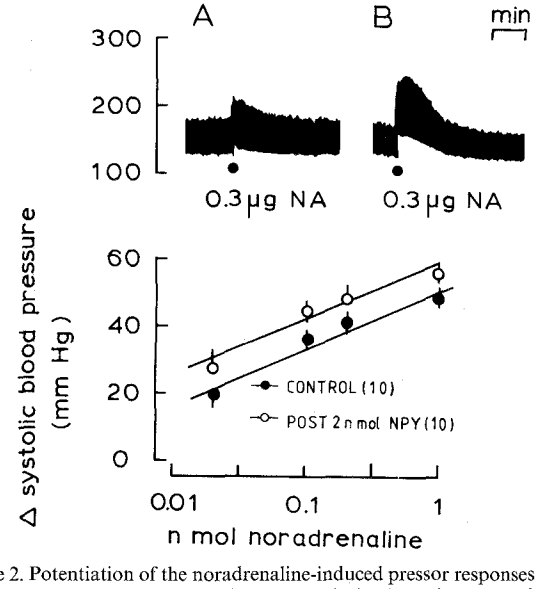

Figure 2. Potentiation of the noradrenaline-induced pressor responses by NPY. *Upper panel:* pressor effect induced by the i.v. bolus injection of 0.3 µg noradrenaline (NA) before and 10 min following the administration of 10 µg (2 nmol) NPY. *Lower panel:* Noradrenaline pressor response curve in control and rats treated with a bolus dose of 10 µg (2 nmol) NPY. Symbols refer to the mean increase in systolic blood pressure. Analysis of variance indicated that the noradrenaline dose response curve obtained following NPY was statistically different ($p < 0.05$) as compared to the control curve.

stored. Several mechanisms can be adduced to explain the mechanism of the supersensitivity. Among them, denervation may cause inhibition of the degradation or re-uptake of noradrenaline, up-regulation of the NPY and of the alpha adrenoceptors; it may modify the affinity or increase the efficacy of the intracellular transduction mechanism of these neurotransmitters at their receptor sites.

Perhaps one of the most revealing findings about the possible role of NPY in the peripheral circulation is the discovery that it potentiates the noradrenaline-induced contraction in isolated blood vessels as well as the pressor effect induced by stimulation of preganglionic sympathetic nerve fibers. In this connection, the present results demonstrate a significant potentiation of the pressor effect of noradrenaline 10 min after pretreatment with a bolus dose of NPY. Although the synergism is modest in terms of absolute magnitude, it was consistently observed and lasted for at least 30 min following the administration of NPY. A similar finding was recently reported in the isolated coronary circulation[9]. The mechanism of the potentiation awaits further investigations but it clearly indicates that NPY and noradrenaline may exert a coordinated action in the control of the peripheral vascular tone.

With regard to the study on the superfused aortic strips, present results show that NPY potentiates the noradrenaline-induced contractions. The magnitude of the potentiation is moderate in relation to what has been described in the rabbit gastroepiploic artery, or rat femoral artery[11, 12, 21]. Since the NPY-induced potentiation of the noradrenaline vasoconstriction is totally absent following sympathectomy, it is reasonable to postulate that in addition to a postsynaptic site of action, NPY must also activate presynaptic sites. The mechanism of this presynaptic effect of NPY is as yet unknown, but it could be related to the release of neurochemicals that facilitate the contractile action of noradrenaline. These results should however be interpreted with caution. First of all, several authors have provided information in support of the conclusion that the rat aorta is sparsely innervated if at all[22, 23]. The low density of adrenergic terminals in the aorta might explain the lack of supersensitivity to noradrenaline in the aortas from 6-OHDA-treated rats. Furthermore, a presynaptic inhibitory mechanism has recently been described in the rat portal vein and femoral artery; the activation of these sites by NPY leads to a reduced release of ^{3}H-noradrenaline[18, 19]. Based on this notion, a possible presynaptic mechanism adduced to explain the noradrenaline-NPY interaction in the isolated aorta could be related to the blockade of release of an inhibitory neurotransmitter by NPY.

In conclusion, NPY acts at both pre- and postsynaptic sites in the vascular territory of the rat. It is likely that resistance vessels are enriched with postsynaptic NPY receptors; the activation of these sites leads to vasoconstriction mediated via a non-adrenergic mechanism. Blood vessels like the aorta, have apparently only presynaptic NPY sites that promote the contractile activity of the catecholamine. Other blood vessels, particularly veins, have presynaptic NPY receptors whose activation decreases the release of noradrenaline.

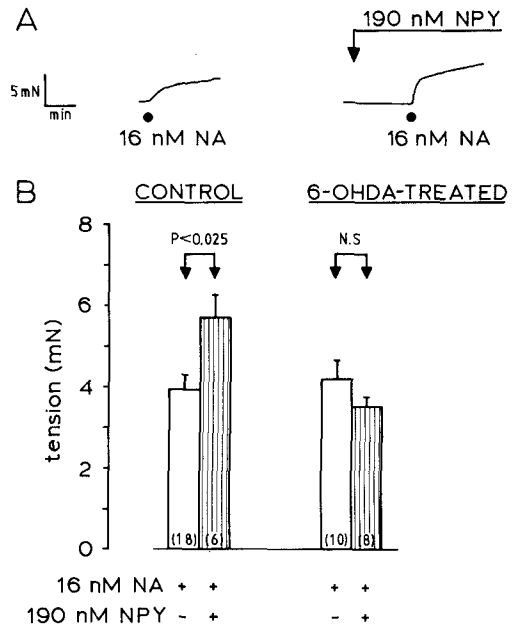

Figure 3. Noradrenaline-induced aortic strip contractions: interaction with NPY. *Upper panel:* contractile effect induced by 16 nM noradrenaline in the aorta of a control rat in the absence and in the presence of 190 nM NPY. *Lower panel:* mean tension developed by aortic strips from control and 6-OHDA treated rats to the application of 16 nM noradrenaline in the absence (open columns) and in the presence of 190 nM NPY (shaded column). Bars indicate the SE; numbers in the column denote the times the experiment was repeated in separate preparations.

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