Br. J. Pharmacol. (1992), **105**, 181–183

Changes in plasma catecholamine and neuropeptide Y levels after sympathetic activation in dogs

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1 Plasma levels of noradrenaline (NA) and neuropeptide Y (NPY) were evaluated in two experimental models associated with an increase in sympathetic tone: conscious dogs which were subject to either sinoaortic denervation or acute administration of the α_2-adrenoceptor antagonist yohimbine.

2 Dogs that had undergone sinoaortic denervation exhibited a two fold increase in plasma NA without any change in NPY levels.

3 Yohimbine (0.05 mg kg^{-1} i.v. as a bolus) produced similar effects. A higher dose of yohimbine (0.5 mg kg^{-1} i.v.) increased both plasma NA (7 fold) and NPY (6.5 fold) levels.

4 The present results indicate that changes in plasma catecholamines and NPY are not always concomitant. They suggest that the simultaneous release of NA and NPY is only observed under *in vivo* conditions for a marked increase in sympathetic tone.

Keywords: Catecholamines; noradrenaline; neuropeptide Y; sympathetic nervous system; yohimbine; sinoaortic denervation

Introduction

Neuropeptide Y (NPY) is a 36-amino acid residue peptide which was originally isolated from the porcine brain. It possesses potent vasoconstrictor activity and is co-stored with noradrenaline (NA) in peripheral perivascular sympathetic nerves and the chromaffin cells of the adrenal medulla (see Lundberg *et al.*, 1989; 1990 for reviews).

Activation of the sympathetic nervous system has been shown to be associated *in vitro* with an increase in NPY release (Lundberg *et al.*, 1990). Cardiovascular diseases, such as acute myocardial infarction, angina pectoris and left heart failure are associated with sympathetic activation and elevated concentrations of circulating NPY (Lundberg *et al.*, 1990; Hulting *et al.*, 1990). However, the co-release of NPY and catecholamines has been poorly investigated in intact subjects.

Thus, the aim of the present study was to investigate whether an enhancement of sympathetic tone (reflected by plasma catecholamine levels) is necessarily associated with an increase in plasma NPY levels. Two different experimental models were selected: conscious dogs subject to sinoaortic denervation, a model associated with a marked increase in sympathetic tone (Damase-Michel *et al.*, 1987; Valet *et al.*, 1989a, b); and conscious dogs treated with yohimbine, a drug known to activate the sympathetic nervous system (Valet *et al.*, 1989c) through the blockade of α_2-adrenoceptors located on sympathetic nerve endings (Goldberg & Robertson, 1983).

Methods

Sinoaortic denervation

Six mongrel dogs of either sex weighing 16 to 24 kg were studied: sinoaortic denervation was performed as previously described (Damase-Michel *et al.*, 1987; Valet *et al.*, 1989a, b). Briefly, sinoaortic denervation involved two successive surgical procedures under α-chloralose (80 mg kg^{-1}, i.v.) anaesthesia. First, the carotid sinus nerve at the carotid bifurcation and the aortic depressor nerve in the cervical region were cut on the right side. The same procedure was repeated on the left side 4 weeks later. The effectiveness of baroreceptor denervation was assessed by the failure of noradrenaline (2 μg kg^{-1}, i.v.) and phenylephrine (0.1, 1.0 and 10.0 μg kg^{-1}, i.v.) to induce bradycardia. Moreover, nitroglycerin (1, 3, 10 and 30 μg kg, i.v.) induced a dose-dependent decrease in blood pressure in these animals without any change in heart rate, thus confirming the absence of any baroreceptor reflex. Each dog was studied before sinoaortic denervation and 1 month after sinoaortic denervation.

Effect of yohimbine

These experiments were performed on 6 or more mongrel dogs, each animal receiving two doses of yohimbine. The two experiments were separated by at least one week. No sham injection of saline was performed since previous studies have indicated that this had no effect on the cardiovascular parameters or on plasma catecholamine levels (Damase-Michel *et al.*, 1987).

Yohimbine hydrochloride (Sigma, Paris, France) was chosen for its ability to increase plasma catecholamine levels. Two doses (0.05 and 0.5 mg kg, i.v.) were selected. The drug was injected as a bolus after a 30 min rest period and blood pressure, heart rate, plasma NA, adrenaline (Ad) and NPY concentrations were measured before, and 15 min after injection.

Blood pressure and heart rate measurements in conscious dogs

Several days before any experiment, dogs were trained to stand still for 2 or 3 h on a Pavlov table and to become accustomed to i.v. infusion, recording and blood samplings. Systolic and diastolic blood pressures were recorded by means of a catheter, introduced into the abdominal aorta via the left femoral artery under local anaesthesia (xylocaine, 5% Lab. Roger Bellon, Neuilly sur Seine, France), connected to a Gould P23ID transducer and a Honeywell recorder. The heart rate was obtained with a heart period meter triggered by blood pressure.

Blood sampling and catecholamine assays

Arterial blood was collected from the catheter previously introduced into the abdominal aorta to prevent any stress. It was collected on lithium heparin with sodium metabisulphite (10 mM), centrifuged at 2000 *g* for 10 min at 0°C and plasma

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was stored at −80°C. Catecholamines were isolated selectively from the sample at 0°C, in darkness, by adsorption to activated alumina, then eluted with 0.1 M acetic acid. Dihydroxybenzylamine was used as internal standard. NA and Ad were assayed by a Waters high-pressure liquid chromatography apparatus using electrochemical detection: the working electrode potential was set at 0.65 V against a Ag/AgCl reference electrode. Catecholamines were separated on a C18 column (3.9 × 150 mm) at a constant flow rate of 1 ml min^{-1}. The electrochemical detector response was linear for concentrations ranging from 10 pg ml^{-1} to 100 ng ml^{-1}. In these conditions, the detection limit is 10 pg ml^{-1} (Valet *et al.*, 1989a).

Radioimmunoassay of neuropeptide

Plasma concentrations of NPY were determined by a sensitive and specific radioimmunoassay (Peninsula, England) using a specific antiserum, which was raised in rabbit against porcine NPY. Duplicate 100 µl aliquots of plasma were obtained from the dog as indicated above. We used arterial plasma since Howe *et al.* (1986) found no change in venous plasma NPY content from SHR stroke-prone rats but an increase in arterial plasma NPY concentrations. Plasma samples were incubated with [^{125}I]-NPY and a specific rabbit antiserum. Bound antigen was separated by a second antibody method, and its radioactivity was measured in a gamma counter. The antiserum shows no cross-reactivity to structurally related peptides, such as peptide YY or pancreatic polypeptide (respectively less than 0.003% and 0.002%). The response was linear for concentrations ranging from 5 to 1280 pg/100 µl. The detection limit was 5 pg/100 µl.

Statistical analysis

All data are presented as mean values ± s.e.mean. Statistical analysis was performed with the Wilcoxon test for paired comparisons. The level of significance was $P < 0.05$.

Results

Dogs with sinoaortic denervation

Sinoaortic denervation significantly increased systolic blood pressure (+45%), diastolic blood pressure (+79%) and heart rate (+69%) (Table 1). Under our experimental conditions, these cardiovascular values remained stable since they did not vary more than 30 mmHg and 40 beats min^{-1} respectively during a 3 h recording session (Valet *et al.*, 1989a). One month after sinoaortic denervation, NA plasma levels significantly rose (+83%) without any significant change in Ad values. Plasma NPY levels measured before and 1 month after sinoaortic denervation did not vary significantly (Table 1). In fact, they increased by 40% but because of the size of the standard error of the mean, this was not statistically significant.

Yohimbine administration

Acute administration of 0.05 mg kg^{-1} i.v. yohimbine significantly increased systolic (+30%) (but not diastolic) blood pressure, heart rate (+26%) and plasma NA (+120%) (but not Ad) levels. Plasma NPY levels did not change. The higher dose of yohimbine (0.5 mg kg^{-1} i.v.) increased systolic (+29%) and diastolic (+29%) blood pressures and also increased plasma NA (+500%), Ad (+377%) and NPY (+570%) levels (Table 1).

Discussion

The present study was undertaken in order to investigate whether NPY is always co-released with catecholamines when sympathetic tone increases *in vivo*. In fact, conflicting results occur in the literature since some authors demonstrate that there is differential release of NPY and catecholamines *in vivo* and that high frequencies of stimulation are required for NPY release *in vivo* in animals (Allen *et al.*, 1984; Zukowska-Grojec *et al.*, 1988; Lundberg *et al.*, 1989) or in man (Morris *et al.*, 1986; Pernow *et al.*, 1988). In contrast, Castagné *et al.* (1987) found that NPY release closely parallels changes in circulating NA during stress in the rat, whereas Lundberg *et al.* (1985) suggested that NPY is co-released together with NA upon sympathetic activation during physical exercise in man. More recently, increased plasma NPY and NA levels were described in hypertensive patients (Solt *et al.*, 1990) and a positive relationship was found between plasma NA and NPY levels during myocardial ischaemia in man (Ullman *et al.*, 1990).

Plasma variations of catecholamines (especially NA) are known to reflect changes in the activity of the sympathetic nervous system (Hjemdahl, 1988). Thus, in the present paper, two experimental conditions characterized by an increase in sympathetic tone (sinoaortic denervation or acute administration of yohimbine) were investigated. The present results clearly indicate that changes in plasma catecholamines and NPY are not always concomitant. In fact, in this study, there was no relationship between plasma NA and NPY levels whatever the experimental model used.

In dogs subject to sinoaortic denervation, the rise in sympathetic tone is not associated with a significant increase in NPY levels. To explain these results, three hypotheses can be proposed. Firstly, sinoaortic denervation could elicit an insufficient stimulation of sympathetic tone (2 fold) to induce an increase in NPY release. These results can be related to the data from Lundberg *et al.* (1990): NA and NPY are only co-released upon high frequency stimulation whereas low average frequency released only NA. Secondly, it is possible that the persistent rise in sympathetic tone observed in sinoaortically denervated dogs is unable to induce an increased NPY release

Table 1 Effect of sinoaortic denervation (SAD) or yohimbine administration on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), noradrenaline (NA), adrenaline (Ad) and neuropeptide Y (NPY) plasma levels in conscious dogs

		SBP (mmHg)	*DBP* (mmHg)	*HR* (beats min^{-1})	*NA* (pg ml^{-1})	*Ad* (pg ml^{-1})	*NPY* (pg 100 μl^{-1})
SAD dogs	Before	185 ± 9	81 ± 3	77 ± 5	498 ± 56	181 ± 34	17 ± 9
	1 Month after SAD	269 ± 13*	145 ± 13*	130 ± 10*	910 ± 91*	239 ± 48	24 ± 16
Yohimbine 0.05 mg kg^{-1}, i.v.	0 min	174 ± 9	60 ± 3	72 ± 4	275 ± 48	234 ± 24	24 ± 4
	15 min	227 ± 11*	76 ± 4	91 ± 5*	605 ± 61*	369 ± 66	30 ± 5
Yohimbine 0.5 mg kg^{-1}, i.v.	0 min	180 ± 9	75 ± 5	80 ± 4	325 ± 75	242 ± 51	20 ± 8
	15 min	233 ± 14*	97 ± 6*	114 ± 17	2214 ± 645*	1155 ± 454*	134 ± 46*

Statistical analysis was performed by the Wilcoxon test for paired comparisons. Mean values ± s.e.mean are given. $n = 6$ aminals in each experiment.
*$P < 0.05$ when compared with control values.

which was shown to be preferentially facilitated by intermittent bursts of stimulation (Lundberg *et al.*, 1989). Finally, one can also postulate that a long term rise in sympathetic tone could lead to a depletion of the NPY pool in sympathetic nerve endings.

The second part of the study used yohimbine, an α_2-adrenoceptor antagonist. As already mentioned, the drug clearly increased NA and Ad levels (Valet *et al.*, 1989c) through the blockade of central and/or presynaptic α_2-adrenoceptors (Goldberg & Robertson, 1983). The higher dose ($0.5\,mg\,kg^{-1}$) induced a marked (7 fold) increase in plasma NA values associated with similar enhancement in plasma NPY values (6.5 fold). This agrees with the levels of yohimbine-induced NPY released in the pithed pig (Dahlöf *et al.*, 1986) and with the fact that α_2-adrenoceptors also regulate NPY release from the sympathetic nerve endings (Lundberg *et al.*, 1990). However, it should be noted that this dose of yohimbine elicited a very marked (7 fold) increase in plasma NA levels above the values measured in sinoaortically denervated dogs. Thus, we selected a lower dose ($0.05\,mg\,kg^{-1}$) in order to induce a 2 fold increase in plasma NA, i.e. a similar rise to that observed in the dogs with sinoaortic denervation. Under these conditions, plasma NPY values did not change.

In conclusion, these results demonstrate that, in the intact conscious dog, an increase in sympathetic tone is not necessarily associated with a rise in NPY release. They suggest that the simultaneous release of NA and NPY is only observed under *in vivo* conditions for a marked increase in sympathetic tone. These data suggest the existence of a threshold level of a sympathetic stimulation beyond which NA and NPY are co-released.

The authors acknowledge the technical help of Mr Bernou and Mrs G. Portolan and the secretarial assistance of Mrs Bontemps.

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(Received January 11, 1991
Revised June 13, 1991
Accepted September 17, 1991)