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Effects of neuropeptide Y injected into A_1 noradrenergic nucleus on blood pressure and catecholamines in plasma of cats[1]

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ABSTRACT The microinjection of neuropeptide Y (NPY) 0.75 and 1.5 μg into A_1 noradrenergic nucleus of cats produced dose-dependent falls in mean arterial blood pressure of 2.0 ± 0.6 and 4.9 ± 2.4 kPa, respectively. NPY microinjection (1.5 μg) also produced marked decreases in noradrenaline (5 ± 4 pmol · ml^{-1}) and adrenaline (23 ± 8 pmol · ml^{-1}), but no significant change in dopamine in blood plasma. The results suggest that the depressor effect of NPY in A_1 noradrenergic nucleus may be realized by not only reducing the release of noradrenaline in sympathetic terminals around the peripheral vascular beds but also inhibiting the sympathetico-adrenal system.

KEY WORDS neuropeptide Y; microinjections; brain stem; blood pressure; norepinephrine; epinephrine; dopamine

Neuropeptide Y (NPY), a 36-amino acid peptide[1], has been found to be present in many regions of the mammalian central nervous system[2]. Its coexistence with noradrenaline (NA) and adrenaline (Adr) in brainstem nuclei[3] concerned with cardiovascular control, plus its presence in sympathetic perivascular nerves[4], has led to a proposed involvement in cardiovascular regulation. In the periphery, NPY has long-lasting peripheral pressor actions and enhances the vasoconstriction evoked by exogenous NA. The central cardiovascular effects of NPY are at present confusing. In this study, we investigated the cardiovascular effects of NPY in A_1 noradrenergic nucleus, since its A_1 noradrenergic cell group was involved in the tonic control of sympathetic discharge[5] and was surrounded by NPY-IR terminals[3].

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MATERIALS AND METHODS

Cats weighing 3.0 ± *s* 0.6 kg of both sexes were anesthetized with iv a mixture of α-chloralose (0.05 g · kg^{-1}) and urethane (0.5 g · kg^{-1}) in distilled water. The femoral artery was cannulated with polyethylene tubing for recording blood pressure and collecting blood sample. The trachea was connnected to artificial ventilator with room air. Rectal temperature was maintained at 37.0-38.5℃ with a heating blanket.

Microinjection into A_1 noradrenergic nucleus The dorsal surface of the medulla oblongata was exposed by a limited craniotomy, and the cats were rested for at least 2 h before experiments. NPY (Sigma) was dissolved in 0.9% saline (pH 7.0). NPY or saline were injected into A_1 noradrenergic nucleus in a volume of 1 μl during 1 min with 10 μl microsyringe. Stereotaxic coordinates[6] were: 13.5 mm posterior to the interaural line, 4.2 mm lateral to the midline, and 9.5 mm ventral to the interaural line. At the end of the experiments, 1 μl of 2% pontamine sky blue was injected. Cats were killed and the brains were put in 10% formalin. After 7-10 d, 40-μm sections were cut for identification of the injection site.

Determination of plasma catecholamine concentrations by reverse phase HPLC and electrochemical detection Cat plasma (2.0 ml), extracted by alumina, was added to glass centrifuge tubes containing 50 mg of acid-washed alumina, 100 μl of 5% $Na_2S_2O_5$, 100 μl of 5% EDTA · 2Na, and 1.0 ml of Tris-HCl 1.5 mol · L^{-1} (pH 8.6). The tubes were capped and shaken for 15 min on the shaker. The alumina was washed thrice with 5 ml of double distilled water. The

catecholamine was eluted with 100 μl of HCl 0.1 mol · L^{-1}. A 25 μl aliquot of samples was injected.

The chromatography consisted of a Model 6000A solvent delivery system, a Model U6k injector (Waters Associates), a pre-column (Bandapak C_{18}) and a reversed-phase column (Bio-SIL ODS-10, 4 mm ID × 250 mm). The column eluent was monitored with an electrochemical detector (Bio-RAD, Model 1340). The detector pontential was +0.72 V and the sensitivity was 10 nA · V^{-1}. The flow-rate was maintained at 10 ml · min^{-1}. The mobile phase was 1 volume of methanol and 9 volumes of potassium phosphate 0.1 mol · L^{-1} (pH 3.4), ionpair reagent (IPR-B_7) 0.2 mmol · L^{-1} and EDTA · 2Na 0.1 mmol · L^{-1}. The column and buffer were used at 18-20℃.

Reagents NA (Serva), Adr (Sigma), dopamine (DA, Fluka), and IPR-B_7 (Tianjin Second Factory of Chemical Reagents) were used. Alumina was activated[7]. All other reagents were filtered through a 0.45-μm membrane filter before use.

Statistics All data were presented as $\bar{x} \pm s$. The significance of the difference between groups were calculated using *t* test.

RESULTS

Effects on mean arterial blood pressure (MAP) Basal MAP was 16.6 ± 1.8 kPa. Unilateral microinjection of NPY 0.75 μg into A_1 noradrenergic nucleus produced a significant and substained fall in MAP ($n=8$). A maximal fall in MAP of 2.0 ± 0.6 kPa ($P<0.05$) was recorded at 47 ± 21 min postinjection (Tab 1). The blood pressure was restored 90-180 min after NPY injection.

In another 9 anesthetized cats, basal MAP was 16.1 ± 1.6 kPa. NPY 1.5 μg injected into A_1 noradrenergic nucleus produced a hypotensive effect. A maximal fall in MAP of 4.9 ± 2.4 kPa ($P<0.01$) was recorded at 24 ± 13 min postinjection (Tab 1). The hypotensive effect was recovered between 90 and 150 min after NPY injection.

Basal MAP in control ($n=8$) was 15.7 ± 1.2 kPa. A small decrease ($P>0.05$) in MAP was seen at 10 ± 10 min after microinjection of saline into A_1 noradrenergic nucleus (Tab 1). NPY microinjection into regions peripheral to A_1 noradrenergic nucleus produced no hemodynamic responses.

Effects on catecholamine contents in plasma Basal levels of NA, Adr, and DA in blood plasma were 12 ± 11, 46 ± 23, and 18 ± 32 pmol · ml^{-1}, respectively. Unilateral microinjection of NPY 1.5 μg into A_1 noradrenergic nucleus produced a significant lowering of NA (5 ± 4 pmol · ml^{-1}) and Adr (23 ± 8 pmol · ml^{-1}) levels at 5 min postinjection compared to control, but no signigicant change in DA (9 ± 22 pmol · ml^{-1}) level in plasma (Tab 1). NPY microinjection into regions peripheral to A_1 noradrenergic nucleus or microinjection of saline into the nucleus produced no change in NA, Adr, DA levels in plasma.

Tab 1. Effects of neuropeptide Y (NPY) injected into A_1 noradrenergic nucleus on mean arterial blood pressure (MAP) and contents of noradrenaline, adrenaline, and dopamine in plasma. $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

NPY		MAP / kPa		Plasma catecholamines / pmol · ml^{-1}								
				Noradrenaline			Adrenaline			Dopamine		
μg	*n*	Before	After	*n*	Before	After	*n*	Before	After	*n*	Before	After
0	8	15.7 ± 1.2	15.0 ± 1.3	7	5 ± 4	5 ± 6	7	46 ± 36	51 ± 32	7	9 ± 22	8 ± 16
0.75	8	16.6 ± 1.8	14.6 ± 1.8**									
1.5	9	16.1 ± 1.6	11.2 ± 2.6***	9	12 ± 11	7 ± 8***	6	46 ± 23	23 ± 18***	7	18 ± 32	9 ± 10*

DISCUSSION

A_1 noradrenergic nucleus consists of a lot of noradrenergic neurones[8]. The importance of A_1 noradrenergic nucleus in central cardiovascular control is well established and involves inhibition of sympathetic outflow[5]. Immunohistochemical evidence has revealed NPY-IR terminals around A_1 cells[3]. The present study demonstrated that in the anesthetized cat, NPY microinjection into A_1 noradrenergic nucleus produced a dose-dependent fall in MAP, compared to control, and the depressor effect exhibits regional specificity. In our experiments, marked decreases in NA and Adr in blood plasma accompany the depressor effect produced by NPY microinjection into A_1 noradrenergic nucleus. The decrease in NA in blood plasma suggests that NPY microinjection into A_1 noradrenergic nucleus could inhibit sympathetic ouflow reducing the release of NA in sympathetic terminals around the peripheral vascular beds. It is important to note that in our study Adr in blood plasma was decreased by NPY applied into A_1 noradrenergic nucleus. The decrease in Adr in blood plasma indicates activities of adrenal medulla were inhibited by NPY applied into A_1 noradrenergic nucleus.

In conclusion, the results of this study indicate that NPY has a depressor effect in A_1 noradrenergic nucleus of cats and the depressor effect may be realized by not only reducing the release of NA in sympathetic terminals around the peripheral vascular beds but also inhibiting the sympathetico-adrenal system.

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A_1 核内注射神经肽 Y 对猫血压和血浆内儿茶酚胺的影响

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提要 猫 A_1 去甲肾上腺素核内微量注射神经肽 Y (NPY)平均动脉压呈剂量依赖性下降，降压峰值分别为 2.0±0.6 和 4.9±2.4 kPa. 同时，血浆内去甲肾上腺素和肾上腺素明显下降，下降幅值分别为 5±4 和 23±8 pmol · ml^{-1}. 但多巴胺无明显变化. 该效应可能与外周血管床周围交感末梢内去甲肾上腺素的释放减少有关，也可能通过抑制交感—肾上腺系统而实现.

关键词 神经肽 Y; 微量注射; 脑干; 血压; 去甲肾上腺素; 肾上腺素; 多巴胺 儿茶酚胺