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Increase of release of neuropeptide Y *in vitro* from platelets of spontaneously hypertensive rats[1]

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AIM: To investigate the pathophysiological role of platelet neuropeptide Y (NPY) in hypertension. **METHODS:** The content of NPY in plasma and platelets from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKR) were measured by radioimmunoassay. **RESULTS:** There was no difference in NPY concentration between SHR plasma and WKR plasma (2.1 ± 1.0 *vs* $1.8 + 1.0$ $\mu g \cdot L^{-1}$), but higher amounts of NPY in SHR platelets than in WKR platelets (32 ± 6 *vs* 22 ± 9 $\mu g \cdot L^{-1}$, $P < 0.05$). The platelet aggregation and the release of NPY from platelets induced by thrombin and collagen were increased in SHR; ADP did not cause NPY release from platelets, although it produced severe aggregation in both SHR and WKR. **CONCLUSION:** The content of NPY in platelets and thrombin- and collagen-induced NPY release from platelets are higher in SHR than in WKR.

KEY WORDS blood platelets; platelet aggregation; neuropeptide Y; inbred SHR rats; inbred WKY rats; thrombin; collagen

Neuropeptide Y (NPY) is a tyrosine-rich 36 amino acid peptide widely distributed in many autonomically innervated organs, including blood vessels[1,2]. NPY is a potent vasoconstrictor and, more importantly, it can potentiate other vasoconstrictors as well[2,3]. Thus, NPY may be important in regulation of both systemic blood pressure and local blood flow. It is conventionlly thought that NPY found in plasma is released by neurons. Recent studies, however, showed that NPY could be synthesized in rat megakaryocyte and stored in platelets[4]. NPY levels are significantly higher in platelets than in plasma. The NPY in platelets can be released during collagen-stimulated platelet secretion[5]. Thus, circulating NPY in rats may represent both neuronal and platelet release. The content of NPY was higher in spontaneously hypertensive rats (SHR) than in Wistar-Kyoto rats (WKR)[6]. In the present paper, we describe release of NPY from platelets induced by ADP, thrombin, and collagen, and compare the differences between SHR and WKR.

MATERIALS AND METHODS

Four SHR and 4 WKR (♂, 16—17 wk old), matched by body weight ($350 \pm s$ 50 g), were used as experimental rats. Systolic blood pressure was measured using a polythysmograph by the tail-cuff method[7] one day prior to the experiment. Systolic blood pressure was higher in SHR than in WKR (24.6 ± 0.6 kPa *vs* 18.8 ± 0.4 kPa, $P < 0.05$).

Rats were anesthetized with urethane (1.0 g $\cdot kg^{-1}$, ip), and blood was collected from the abdominal aorta into ice-chilled tubes containing 3.8 % sodium citrate (1:9 volume). Platelet-rich plasma (PRP), platelet poor plasma (PPP), and platelet samples were prepared by differential centrifugation. The platelet pellet was washed once with buffer containing: NaCl 123, glucose 30, sodium citrate 13 mmol $\cdot L^{-1}$, pH 6.8, and resuspended in Tyrode's buffer containing: NaCl 140, KCl 3, $MgSO_4$ 1, HEPES 10, glucose 10 mmol $\cdot L^{-1}$, pH 7.4. Platelet number in PRP or the resuspended platelet suspension (RPS) was counted

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under a phase-contrast microscope and was adjusted to 10^{12} platelets · L^{-1} by adding PPP or Tyrode's buffer. Samples were kept at −30 C before use.

Platelet aggregation was measured by the turbidimetric method[8]. PRP or RPS was equilibrated at 37 C for 3 min prior to addition of ADP (Sigma), thrombin (Beijing Biochemical Products Co), or acid-soluble collagen (Sigma, prepared by the method of Cazenave[9]). Aggregation curves were recorded for 4 min and analyzed in terms of percent of maximal increase in light transmittance (LM 14-104, Da Hua Instrument Co. Shanghai). PRP or RPS was centrifuged at 2200×g for 10 min and the supernatant was stored at −30 C for NPY determination.

NPY was measured after cold acid-ethanol extraction of plasma samples by radioimmunoassay with [^{125}I]NPY (Amersham)[10]. The NPY antiserum (a gift from Prof J K McDonald, Emory University, USA) showed negligible cross reaction to peprides of similar size and structure. In addition to measurement in the post-aggregation plasma, the peptide was quantified in PRP or RPS which was not aggregated but platelets were disrupted during the extraction. NPY-immunoactivity was also measured in PPP prepared from PRP. Quantities of NPY in plasma samples taken during platelet aggregation were expressed as % of NPY content in PRP or RPS over the control. Radioactivity was measured by a γ-counter (Wizard 1470, Pharmacia).

The significance between groups was determined by *t* test.

RESULTS

NPY contents in plasma and RPS No significant difference in plasma NPY content was found between SHR (2.1±1.0 μg·L^{-1}) and WKR (1.8±1.0 μg·L^{-1}), but NPY content was higher in RPS from SHR than in RPS from WKR (32±6 *vs* 22±9, $P<0.05$).

ADP-induced platelet aggregation and NPY release ADP produced only a reversible, primary aggregation response in PRP from both SHR and WKR. ADP 1 μmol·L^{-1} caused a minor aggregation response in WKR (2.2±1.6 %) and only shape change in SHR. When ADP 2.5 μmol·L^{-1} was used, a marked aggregation response occurred, but no difference was seen between SHR (35±14 %) and WKR (37±13 %). ADP 1 or 2.5 μmol·L^{-1} yielded no release of NPY.

Thrombin- or collagen-induced aggregation and NPY release in RPS Thrombin induced concentration-dependent aggregation and release of NPY in RPS from both SHR and WKR. With thrombin 200 U·L^{-1}, platelet aggregation was not produced (only a shape change was seen), but platelet NPY release was obvious in both SHR and WKR, and was greater in SHR than in WKR. With thrombin 500 U·L^{-1}, both platelet aggregation and NPY release were greater in SHR than in WKR. Thrombin 2000 U·L^{-1} caused maximal aggregation and NPY release, with no abvious difference between SHR and WKR. (Fig 1)

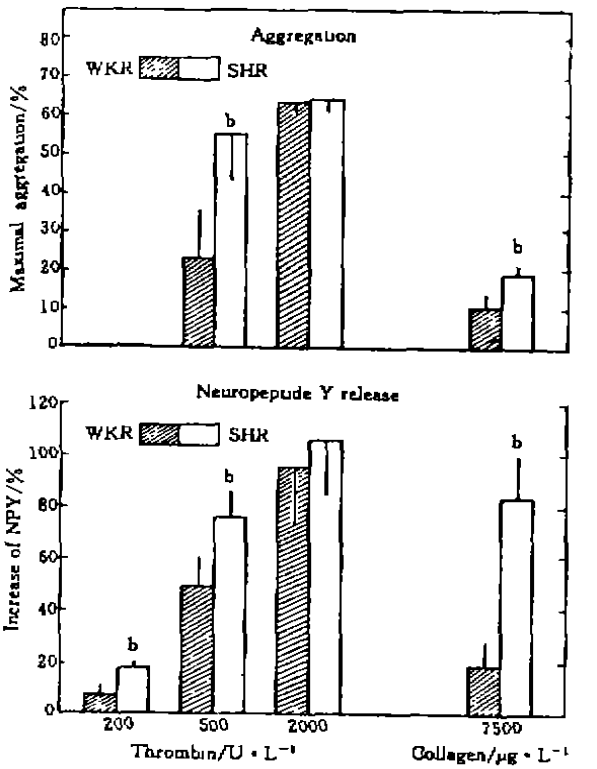

Fig 1. Comparison of thrombin- or collagen-induced aggregation and NPY release in washed platelets from SHR and WKR. $n=4$ mice. $\bar{x}\pm s$. $^bP<0.05$.

Both the platelet aggregation and the release of NPY induced by acid-soluble collagen 7.5 mg·L^{-1} were higher in SHR than in WKR (Fig 1).

DISCUSSION

In this study not only aggregation but also NPY release from thrombin and collagen-induced platelets was greater in SHR than in WKR. These results suggest that circulating NPY levels may be increased more in SHR than in WKR during vascular endothelium damage which causes subendothelial collagen exposure, or during high coagulant states when endogenous thrombin is released. NPY released from platelets potentiated collagen response induced by other co-released substances, such as 5-HT and some α_1-adrenergic receptor agonists[11]. Thus the increased local or circulating level of NPY during these pathophysiological situations may contribute to the increase of vascular reactivity and higher rate of vasospasm in spontaneous hypertension.

In the present study, we found no difference in plasma NPY content between SHR and WKR, but higher levels of NPY in SHR platelets than in WKR platelets. These results are in agreement with the studies[6]. Higher levels of NPY in SHR platelets may be one reason for increased NPY release during platelet activation. But the precise mechanisms for increased NPY release from SHR platelets remain to be determinated.

Our findings suggest that higher NPY levels in SHR platelets and increased release of NPY from SHR platelets during platelet activation may be involved in the pathophysiology of hypertension.

REFERENCES

1 Chronwall BM, Dimaggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL. The anatomy of neuropeptide-Y-containing neurones in rat brain. Neuroscience 1985; **15**: 1159—81.

2 Edvinsson L, Emson P, McCulloch J, Tatemoto K, Uddman R. Neuropeptide Y: immunocytochemical localization to and effect upon feline pial arteries and veins *in vitro* and *in situ*. Acta Physiol Scand 1984; **122**: 155—63.

3 Abel PW, Han C. Effects of neuropeptide Y on contraction, relaxation, and membrane potential of rabbit cerebral arteries. J Cardiovasc Pharmacol 1989; **13**: 52—63.

4 Ericsson A, Hemsen A, Lundberg JM, Persson H. Detection of neuropeptide Y-like immunoreactivity and messneger RNA in rat platelets: the effects of vinblastine, reserpine, and dexamethasone on NPY expression in blood cells. Exp Cell Res 1991; **192**: 604—11.

5 Myers AK, Farhat MY, Vaz CA, Keiser HR, Zukowska-Grojec Z. Release of immunoreactive-neuropeptide Y by rat platelets. Biochem Biophys Res Commun 1988; **155**: 118—22.

6 Ogawa T, Kitamura K, Kawamoto M, Eto T, Tanaka K. Increased immunoreactive neuropeptide Y in platelets of spontaneously hypertensive rats (SHR). Biochem Biophys Res Commun 1989; **165**: 1399—05.

7 Huzoor-Akbar, Anwer K. Evidence that the rat is not an appropriate model to study the role of prostaglandins in normal or abnormal platelet aggregation. Thromb Res 1986; **41**: 555—66.

8 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; **194**: 927—29.

9 Cazenave JP, Packham MA, Mustard JF. Adherence of platelets to a collagen-coated surface: development of a quantitative method. J Lab Clin Med 1973; **82**: 978—9.

10 Han C, Wang X, Fiscus RR, Gu J, McDonald JK. Changes in cardiac neuropeptide Y after experimental myocardial infarction in rat. Neurosci Lett 1989; **104**: 141—46.

11 Yang YF, Yang LH, Shi GP, Han QD. Platelet neuropeptide Y and its vasoconstriction effect in rat. Acta Physiol Sin 1993; **45**: 400—4.

自发性高血压大鼠血小板神经肽 Y 释放增多

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目的：比较自发性高血压大鼠(SHR)和 WKR 大鼠血浆和血小板中神经肽 Y (NPY)的含量，

以及 ADP、凝血酶和胶原引起血小板聚集和 NPY 释放的差别. 方法: 应用放射免疫分析法测定血小板及血浆中 NPY 的含量. 结果: SHR 和 WKR 大鼠血浆 NPY 含量无明显差别, 分别为 2.1±1.0和1.8±1.0 $\mu g \cdot L^{-1}$, 而 SHR 血小板中 NPY 含量(32±6 $\mu g \cdot L^{-1}$)显著高于 WKY 大鼠(22±9 $\mu g \cdot L^{-1}$). 凝血酶和胶原可引起血小板聚集和 NPY 释放, SHR 显著高于 WKR 大鼠; 而 ADP 虽可引起血小板聚集, 却不引起 NPY 释放. 结论: SHR 血小板中 NPY 含量增高、释放增多.

关键词 血小板; 血小板聚集; 神经肽 Y; 近交 SHR 大鼠; 近交 WKY 大鼠; 凝血酶; 胶原

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Effects of anisodamine against myocardial ischemia-reperfused injuries and antilipid peroxidation

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AIM: Anisodamine (Ani), an alkaloid first isolated in China. To study the relationship between the protective effects of Ani on myocardial cells of reperfused injuries and the antilipid peroxidation. **METHODS**: Coronary ligation for 15-min followed by 10 min reperfusion was performed in anesthetized rats. **RESULTS**: Ani 1, 3, 5 $mg \cdot kg^{-1}$ iv 1 min prior to reperfusion could dose-dependently lower the release of creatine kinase (282±29, 252±53, 226±50), counteract the increase of malondialdehyde content (3.3±1.3, 3.2±1.6, 3.1±1.2) in the reperfused myocardium and preserve the SOD activity (41±7, 46±8, 55±8). Ani completely abolished the drop in the contents of principal unsaturated fatty acids (oleic, linoleic, and arachidonic acids) of the membrane lipids in the reperfused myocardium. SOD 75 $U \cdot kg^{-1}$ iv 1 min prior to reperfusion exerted similar effects like Ani 3 $mg \cdot kg^{-1}$. **CONCLUSION**: It is suggested that the antilipid peroxidative effect of Ani may contribute to its protection against reperfusion-induced myocardial injuries.

KEY WORDS anisodamine; superoxide dismutase; myocardial reperfusion injury; creatine kinase; malondialdehyde; unsaturated fatty acids; lipid peroxidation; atropine derivatives

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Anisodamine (Ani), an alkaloid first extracted from *Hyoscyamuse niger* L in China, is very similar to atropine in chemical structure. It showed protective effects against certain experimental arrhythmias[1] and of ischemia-reperfusion arrhythmias[2, 3] and improved the cardiac performance during reperfusion[3]. We observed that the protective effects of Ani against reperfusion induced arrhythmia may have something to do with reduced myocardial lipid peroxidation[4].

In order to ascertain the relationship between the protective effects of Ani on myocar-