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Platelet eicosanoids and the effect of captopril in blood pressure regulation

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Abstract

We investigated the eicosanoid synthesis of platelets of Wistar and of Okamoto spontaneously hypertensive rats (SHR), and the effect of captopril in vitro, using [^{14}C]arachidonic acid as a tracer substrate and chromatographic determination. Lipoxygenase activity was elevated, while the formation of cyclooxygenase products was reduced in SHR platelets, compared to those of Wistar rats. This difference might play a role in the pathomechanism of hypertension in SHR. In SHR with lower blood pressure, captopril reduced thromboxane synthesis, while in SHR with higher blood pressure thromboxane synthesis was unchanged, but the synthesis of prostaglandin D_2, a potent vasodilator, and of 12-L-hydroxy-5,8,10-heptadecatrienoic acid, a stimulator of endothelial prostacyclin formation, was increased. We may conclude that, in spite of the missing angiotensin converting enzyme in platelets, a direct effect on platelet eicosanoid synthesis could contribute to the blood pressure decreasing effect of captopril. © 1997 Elsevier Science B.V.

Keywords: Spontaneously hypertensive rat (SHR); Platelet; Captopril; Thromboxane; 12-L-Hydroxy-5,8,10-heptadecatrienoic acid; Lipoxygenase metabolite

1. Introduction

The regulation of blood pressure depends on a number of genetic and environmental factors (Ganten, 1993). Peripheral vascular resistance and blood volume play important roles in the maintenance of blood pressure (Laragh, 1993). Besides the autonomic nervous system, vasoactive peptides and eicosanoids alone or in collaboration can modify vascular tone (Carretero and Scicli, 1991; Schrör, 1993; Kjeldsen et al., 1994). Various vasoactive substances (eg. serotonin, eicosanoids, etc.), released from activated platelets can contribute to the process of vasoregulation (Nityanand et al., 1993; Islim et al., 1995).

Angiotensin II, produced by angiotensin converting enzyme from angiotensin I, induces vasoconstriction and smooth muscle cell proliferation, and plays an important role in the pathomechanism of hypertension (Goldfarb, 1994).

Among the most frequently used drugs in the therapy of hypertension are angiotensin converting enzyme inhibitors. They lower high blood pressure both by inhibiting angiotensin II synthesis, and by inhibiting the degradation of bradykinin (Vanhoutte et al., 1993). Besides these effects, other mechanisms might also be involved in the mechanism of action of angiotensin converting enzyme inhibitors. Vanhoutte et al. (1993) reported that angiotensin converting enzyme inhibitors indirectly increase the vascular synthesis of nitric oxide and prostacyclin. Furthermore, certain angiotensin converting enzyme inhibitors can influence the metabolism of arachidonic acid. Captopril has been shown to increase the plasma concentration and urinary excretion of vasodilator prostaglandins in the human (Swartz and Williams, 1982; Witzgall et al., 1982; Silberbauer et al., 1983; Dzau and Swartz, 1987) and to enhance prostacyclin formation in isolated rat aorta (Düsing et al., 1983). Abe et al. (1980) also suggested that the eicosanoids are involved in the antihypertensive effect of captopril in hypertensive patients. Captopril contains a free sulfhydryl group, which has been implicated in the observed effects on prostaglandin synthesis (Van Gils et al., 1987). Arachidonate metabolites play an important role in

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hemostasis, and the effects of captopril may also influence both platelet–vascular interaction and vasoregulation.

In contrast to vascular tissue, the platelets do not contain angiotensin converting enzyme. They can metabolize angiotensin I via a carboxypeptidase to Des–Leu^{10}–angiotensin I, which is an endogenous angiotensin converting enzyme inhibitor (Snyder et al., 1985; Snyder and Wintroub, 1986; Santucci et al., 1988; Scharpé et al., 1990).

Among the angiotensin converting enzyme inhibitors, captopril is suitable for in vitro experiments because it is not a pro-drug (Kripalani et al., 1980). The present study was designed to investigate whether captopril has a direct effect on the arachidonate cascade of platelets isolated from Okamoto spontaneously hypertensive rats with either lower or higher blood pressure.

2. Materials and methods

2.1. Chemicals

Arachidonic acid (grade I), 12-L-hydroxy-5,8,10-heptadecatrienoic acid and 12-hydroxy-5,8,10,14-eicosatetraenoic acid unlabeled standards were purchased from Sigma, St. Louis, MI. [^{14}C]arachidonic acid (specific activity: 2035 MBq/mM) was obtained from Amersham (England). Serum-free tissue culture Medium 199 was purchased from Sigma, St. Louis, MI. Silica gel thin-layer plates (0.25 mm) were obtained from Merck, Darmstadt. Prostaglandin E_2, prostaglandin D_2, thromboxane A_2, thromboxane B_2 (the stable metabolite of thromboxane A_2), prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) were generously provided by Upjohn Co, Kalamazoo. Captopril was obtained from Squibb Laboratories.

2.2. Animals and isolation of platelets

Male spontaneously hypertensive Okamoto and Wistar rats (body weight: 162 ± 3 g, 8-weeks-old) were used in this study. Genetically hypertensive Okamoto rats were divided into two groups: Okamoto spontaneously hypertensive rats, with lower blood pressure (blood pressure: 144 ± 3 mm Hg) and Okamoto spontaneously hypertensive rats (SHR), with higher blood pressure (blood pressure: 214 ± 9 mm Hg). Under light ether anaesthesia, blood was drawn from the abdominal aorta and was diluted with phosphate buffer (pH 7.4) containing EDTA (5.8 mM) and glucose (5.55 mM). Platelet-rich plasma was collected after the whole blood had been centrifuged at 200 *g* for 10 min at room temperature. The platelets were sedimented from the supernatant by centrifugation at 2000 *g* for 10 min. The pellet was contaminated with red blood cells, which can metabolize arachidonic acid by the lipoxygenase pathway and release 12-hydroxy-5,8,10,14-eicosatetraenoic acid and leukotrienes (Kobayashi and Levine, 1983). Therefore erythrocytes were lysed with hyposmic ammonium chloride (0.83%, 9 parts) containing EDTA (0.02%, 1 part) at 4°C for 15 min. The platelets were then washed with phosphate buffer (pH 7.4, containing 5.8 mM EDTA and 5.55 mM glucose) and centrifuged at 2000 *g* for 10 min at room temperature. During the separation procedure activation of platelets was inhibited by using Ca^{2+}-free medium and siliconised glassware. The washed platelet suspension was free from other cellular elements of the blood (red blood cells, leukocytes, etc.) and plasma proteins. After the last centrifugation the platelets were resuspended (10^8 platelets/ml) in serum-free tissue culture Medium 199. Animal experiments were performed with the permission of the Ethical Committee for the Protection of Animals in Research (Albert Szent-Györgyi Medical University, Szeged, Hungary).

2.3. Analysis of eicosanoids

Platelets (10^8 cells/ml in each sample) were incubated at 37°C for 5 min, and captopril (concentration range 10^{-12}–10^{-8} M) was then added to the incubation mixture. The enzyme reaction was started by the introduction of tracer substrate, [^{14}C]arachidonic acid (3.7 kBq, 0.172 pmol), into the incubation mixture. The platelets are not activated by such a low concentration of arachidonic acid. Ten minutes later the enzyme reaction was stopped by bringing the pH of the incubation mixture to 3 with formic acid. The samples were then extracted with ethyl acetate (2×3 ml) and the organic phases were pooled and evaporated to dryness under nitrogen. The residues were reconstituted in 150 μl ethyl acetate and quantitatively applied to silica gel G thin-layer plates. The plates were developed to a distance of 15 cm in the organic phase of ethyl acetate:acetic acid:2,2,4-trimethylpentane:water (110:20:30:100) by means of overpressure thin-layer chromatography (Labor MIM, Hungary) (Abdel-Halim et al., 1980). Each 3-mm band of the chromatograms was then scraped off and the radioactivity was determined in a liquid scintillation counter (TRI-CARB 2100TR, Canberra Packard, USA) as disintegrations per minute (DPM), using 5 ml toluene containing 0.44% w/v 2,5-diphenyloxazole, 0.02 w/v% 1,4-di-[2-(5-phenyl)-oxazoyl]benzene and 10 v/v% ethanol. The radiolabelled products of arachidonic acid were identified with unlabelled authentic standards, which were detected with anisaldehyde reagent (Kiefer et al., 1975). For the separation of lipoxygenase products of the arachidonate cascade, high-performance liquid chromatography (HPLC, ISCO 2350, USA) was also applied, with a reversed phase column (4.6×250 mm), connected to a guard column (4.6×25 mm), both packed with Nucleosil C^{18} (5 μm particles). The eluent consisted of acetonitrile:water (700:300), and phosphoric acid was added to adjust the pH to 4. In our pre-experiments we determined the absolute amounts of lipoxygenase products (9.65 ± 1.42 nmol/l) in the incubation mixtures with a

UV detector (Hewlett Packard 1050, USA) at 235 nm wavelength, following HPLC separation. In the same sample we also determined the amount of radiolabelled lipoxygenase fraction (98.13 ± 13.24 fmol/l) with a liquid scintillation counter. According to our determinations the ratio between the platelet lipoxygenase pool and the labelled platelet lipoxygenase pool was 100000:1.

Statistical analysis was carried out by using SPSS for Windows (version 6.1.2). Analysis of variance was performed, followed by Duncan's multiple range post hoc test.

3. Results

The eicosanoid synthesis of platelets isolated from SHR (lower or higher blood pressure) and from Wistar rats was investigated. The platelets of SHR with lower blood pressure synthesized significantly more eicosanoids. The total amount of platelet eicosanoids (i.e. the lipoxygenase metabolites and the cyclooxygenase products of arachidonic acid together) was elevated, when compared to that in Wistar animals (Table 1). The lipoxygenase pathway of the arachidonate cascade was significantly more active in the platelets of SHR, independently of the blood pressure. On the other hand, the cyclooxygenase pathway was significantly decreased only in the platelets of SHR with higher blood pressure, as compared with that of Wistar animals (Table 1). These differences resulted in an increase in the lipoxygenase dominance of the arachidonate cascade of platelets isolated from SHR. The ratio of the lipoxygenase metabolites to the cyclooxygenase products of arachidonic acid was significantly ($P < 0.05$) elevated from 2.89 ± 0.14 in Wistar rats to 4.35 ± 0.30 and 4.47 ± 0.14 in SHR with lower and higher blood pressure, respectively. Synthesis of the vasodilator, as well as of the vasoconstrictor cyclooxygenase metabolites was equally decreased in platelets of the SHR with higher blood pressure, as compared with Wistar platelets (Table 1). Therefore, the ratio of vasoconstrictor to vasodilator cyclooxygenase products did not differ significantly among the groups studied (1.39 ± 0.09, 1.37 ± 0.06 and 1.36 ± 0.05 in the Wistar rats, SHR with lower and higher blood pressure, respectively). Concerning the cyclooxygenase metabolites of platelets isolated from SHR with lower blood pressure, only the synthesis of prostaglandin E_2 was significantly lower than that of the Wistar or the SHR animals with higher blood pressure (Table 1). On the other hand, platelets isolated from SHR with higher blood pres-

Table 1
The eicosanoid synthesis of platelets of Wistar and Okamoto spontaneously hypertensive rats (SHR), with either lower or higher blood pressure

Products	Wistar rats	SHR with lower blood pressure	SHR with higher blood pressure
Total amount of platelet eicosanoids	98.26 ± 5.26	120.11 ± 8.49[a]	111.11 ± 4.37
Lipoxygenase metabolites	72.98 ± 4.51	97.55 ± 7.78[a]	90.85 ± 3.99[a]
Cyclooxygenase metabolites	25.27 ± 1.09	22.55 ± 1.25	20.30 ± 0.53[a]
Vasoconstrictor metabolites	14.44 ± 0.67	13.05 ± 0.94	11.68 ± 0.33[a]
Vasodilator metabolites	10.69 ± 0.72	9.50 ± 0.37	8.62 ± 0.32[a]
12-Hydroxyheptadecatrienoic acid	4.79 ± 0.40	4.55 ± 0.24	3.43 ± 0.15[a,b]
Prostaglandin D_2	2.84 ± 0.11	2.53 ± 0.18	2.29 ± 0.12[a]
Thromboxane B_2	12.00 ± 0.51	11.37 ± 0.88	10.08 ± 0.24[a]
Prostaglandin E_2	2.01 ± 0.14	1.37 ± 0.09[a]	1.87 ± 0.11[b]
Prostaglandin $F_{2\alpha}$	2.45 ± 0.32	1.88 ± 0.17	1.60 ± 0.16[a]
6-keto prostaglandin $F_{1\alpha}$	1.19 ± 0.12	1.05 ± 0.05	1.03 ± 0.07

Results are reported as means ± standard error of the mean ($n = 9$), in 10^3 DPM. Platelets were incubated with [^{14}C]-arachidonic acid as a tracer substrate for 10 min at 37°C. The metabolites were extracted, separated by overpressure thin-layer chromatography and quantitated with a liquid scintillation counter. Statistically significant ($P < 0.05$) differences are indicated: [a]compared to Wistar animals; [b]compared to SHR with lower blood pressure.

Table 2
The effect of captopril on the arachidonate cascade of the platelets of Okamoto spontaneously hypertensive rats (SHR), with lower blood pressure

Products	Control	Captopril (M)				
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}
Total amount of platelet eicosanoids	120.11 ± 0.49	112.21 ± 6.89	118.80 ± 3.01	108.84 ± 2.44	120.64 ± 3.29	117.70 ± 3.27
Lipoxygenase metabolites	97.55 ± 7.78	91.13 ± 6.61	97.95 ± 3.13	89.71 ± 2.50	99.79 ± 2.92	98.563 ± 3.40
Cyclooxygenase metabolites	22.55 ± 1.25	21.08 ± 0.53	20.86 ± 0.73	19.13 ± 0.83[a]	20.85 ± 0.87	19.13 ± 0.87[a]
Vasoconstrictor metabolites	13.05 ± 0.94	11.53 ± 0.28	11.92 ± 0.51	10.11 ± 0.50[a]	10.55 ± 0.63[a]	10.02 ± 0.51[a]
Vasodilator metabolites	9.50 ± 0.37	9.55 ± 0.42	8.93 ± 0.28	9.02 ± 0.48	10.30 ± 0.46	9.11 ± 0.51

Results are reported as means ± standard error of the mean ($n = 9$), in 10^3 DPM. Platelets were incubated with [^{14}C]-arachidonic acid as a tracer substrate for 10 min at 37°C. The eicosanoids were extracted, separated by overpressure thin-layer chromatography and quantitated with a liquid scintillation counter. Statistically significant ($P < 0.05$) differences are indicated: [a]compared to the corresponding control value.

Table 3
The effect of captopril on the arachidonate cascade of the platelets of Okamoto spontaneously hypertensive rats (SHR), with higher blood pressure

Products	Control	Captopril (M)				
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}
Total amount of platelet eicosanoids	111.11 ± 4.37	108.79 ± 5.09	117.38 ± 4.82	105.12 ± 3.35	130.77 ± 8.55[a]	117.15 ± 3.03
Lipoxygenase metabolites	90.85 ± 3.99	85.31 ± 5.30	92.23 ± 4.73	82.70 ± 9.19	106.17 ± 7.33[a]	93.99 ± 3.10
Cyclooxygenase metabolites	20.30 ± 0.53	23.49 ± 0.63	25.15 ± 1.53[a]	22.42 ± 1.25	24.93 ± 1.67[a]	23.15 ± 1.05
Vasoconstrictor metabolites	11.68 ± 0.33	13.31 ± 0.45	14.19 ± 1.14	12.56 ± 0.99	13.78 ± 0.81	13.44 ± 0.74
Vasodilator metabolites	8.617 ± 0.32	10.18 ± 0.32[a]	10.97 ± 0.56[a]	9.864 ± 0.42	10.82 ± 0.70[a]	9.71 ± 0.40

Results are reported as means ± standard error of the mean ($n = 9$), in 10^3 DPM. Platelets were incubated with [^{14}C]-arachidonic acid as a tracer substrate for 10 min at 37°C. The eicosanoids were extracted, separated by overpressure thin-layer chromatography and quantitated with a liquid scintillation counter. Statistically significant ($P < 0.05$) differences are indicated: [a]compared to the corresponding control value.

sure synthesized significantly less prostaglandin $F_{2\alpha}$, thromboxane B_2, prostaglandin D_2 and 12-L-hydroxy-5,8,10-heptadecatrienoic acid than did those of Wistar rats. The synthesis of 12-L-hydroxy-5,8,10-heptadecatrienoic acid in the platelets of SHR with higher blood pressure was also significantly attenuated as compared with SHR with lower blood pressure (Table 1).

Captopril in the concentration range applied did not modify significantly the total amount of eicosanoids synthesized by platelets of the SHR with lower blood pressure (Table 2). The lipoxygenase pathway of the arachidonate cascade did not change, while cyclooxygenase activity was significantly decreased in the presence of 10^{-10} and 10^{-8} M captopril. Therefore, the ratio of lipoxygenase to cyclooxygenase metabolites was significantly increased from 4.35 ± 0.30 to 5.26 ± 0.35 in the presence of 10^{-8} M captopril. Captopril significantly reduced the formation of vasoconstrictor cyclooxygenase metabolites (i.e. the sum of prostaglandin $F_{2\alpha}$ and thromboxane B_2), but the synthesis of vasodilator cyclooxygenase metabolites (i.e. the sum of prostaglandin $F_{1\alpha}$, prostaglandin E_2, prostaglandin D_2 and 12-L-hydroxy-5,8,10-heptadecatrienoic acid) was not changed (Table 2). As a consequence, the ratio of vasoconstrictor cyclooxygenase metabolites to vasodilator cyclooxygenase products was significantly attenuated by 10^{-10} M, 10^{-9} M and 10^{-8} M captopril in platelets of the SHR with lower blood pressure, from 1.37 ± 0.06 in the control to 1.13 ± 0.06, 1.00 ± 0.05 and 1.11 ± 0.06, respectively.

Captopril at the concentration of 10^{-9} M stimulated the arachidonate cascade in thrombocytes of the SHR with higher blood pressure (Table 3). This modification was due to the increase in both lipoxygenase and cyclooxygenase activity (Table 3). The ratio of lipoxygenase to cyclooxygenase metabolites was significantly decreased by 10^{-12} M captopril from 4.47 ± 0.14 to 3.67 ± 0.27. The vasodilator metabolites in the cyclooxygenase pathway of the arachidonate cascade were synthesized in significantly higher quantity in the presence of captopril, while the production of vasoconstrictor metabolites did not change (Table 3).

The production of 12-L-hydroxy-5,8,10-heptadecatrienoic acid was significantly attenuated by captopril in platelets of the SHR with lower blood pressure (Table 4). On the other hand, captopril significantly stimulated the formation of prostaglandin D_2 and prostaglandin E_2 (Table 4). The synthesis of prostaglandin $F_{2\alpha}$ was significantly elevated only by 10^{-11} M and was diminished by 10^{-8} M captopril in these platelets (Table 4). The synthesis of thromboxane B_2 was significantly reduced by all doses of captopril (Table 4). The production of 6-keto-prostaglandin $F_{1\alpha}$ was also decreased, but only by 10^{-10}–10^{-8} M captopril (Table 4).

Table 4
The effect of captopril on the cyclooxygenase metabolites of the platelets of Okamoto spontaneously hypertensive rats (SHR), with lower blood pressure

Products	Control	Captopril (M)				
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}
12-Hydroxyheptadecatrienoic acid	4.55 ± 0.24	4.28 ± 0.25	3.75 ± 0.19[a]	3.59 ± 0.17[a]	4.04 ± 0.19	3.13 ± 0.11[a]
Prostaglandin D_2	2.53 ± 0.18	2.89 ± 0.19	2.62 ± 0.13	2.67 ± 0.23	3.27 ± 0.24[a]	3.47 ± 0.26[a]
Thromboxane B_2	11.37 ± 0.88	9.45 ± 0.23[a]	9.39 ± 0.45[a]	8.32 ± 0.46[a]	8.93 ± 0.63[a]	8.64 ± 0.49[a]
Prostaglandin E_2	1.37 ± 0.09	1.41 ± 0.12	1.53 ± 0.12	2.02 ± 0.21[a]	2.20 ± 0.18[a]	1.86 ± 0.22
Prostaglandin $F_{2\alpha}$	1.88 ± 0.17	2.08 ± 0.19	2.53 ± 0.11[a]	1.79 ± 0.12	1.62 ± 0.11	1.38 ± 0.11[a]
6-keto prostaglandin $F_{1\alpha}$	1.05 ± 0.05	0.96 ± 0.07	1.03 ± 0.07	0.74 ± 0.03[a]	0.78 ± 0.04[a]	0.65 ± 0.04[a]

Results are reported as means ± standard error of the mean ($n = 9$), in 10^3 DPM. Platelets were incubated with [^{14}C]-arachidonic acid as a tracer substrate for 10 min at 37°C. The eicosanoids were extracted, separated by overpressure thin-layer chromatography and quantitated with a liquid scintillation counter. Statistically significant ($P < 0.05$) differences are indicated: [a]compared to the corresponding control value.

Table 5
The effect of captopril on the cyclooxygenase metabolites of the platelets of Okamoto spontaneously hypertensive rats (SHR), with higher blood pressure

Products	Control	Captopril (M)				
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}
12-hydroxyheptadecatrienoic acid	3.43 ± 0.15	4.32 ± 0.19[a]	4.57 ± 0.38[a]	4.46 ± 0.26[a]	4.43 ± 0.24[a]	3.57 ± 0.12
Prostaglandin D_2	2.29 ± 0.12	3.22 ± 0.19[a]	3.26 ± 0.13[a]	2.49 ± 0.10	3.44 ± 0.32[a]	3.39 ± 0.20[a]
Thromboxane B_2	10.08 ± 0.24	10.81 ± 0.36	11.69 ± 0.96	10.27 ± 0.97	11.58 ± 0.76	11.59 ± 0.66
Prostaglandin E_2	1.87 ± 0.11	1.48 ± 0.11	1.91 ± 0.12	1.79 ± 0.16	1.99 ± 0.18	1.83 ± 0.17
Prostaglandin $F_{2\alpha}$	1.60 ± 0.16	2.50 ± 0.20[a]	2.50 ± 0.23[a]	2.29 ± 0.38	2.20 ± 0.17	1.85 ± 0.15
6-keto prostaglandin $F_{1\alpha}$	1.03 ± 0.07	1.16 ± 0.06	1.23 ± 0.10	1.13 ± 0.12	0.96 ± 0.06	0.92 ± 0.08

Results are reported as means ± standard error of the mean ($n = 9$), in 10^3 DPM. Platelets were incubated with [^{14}C]-arachidonic acid as a tracer substrate for 10 min at 37°C. The eicosanoids were extracted, separated by overpressure thin-layer chromatography and quantitated determined with a liquid scintillation counter. Statistically significant ($P < 0.05$) differences are indicated: [a]compared to the corresponding control value.

The generation of the 12-L-hydroxy-5,8,10-heptadecatrienoic acid and prostaglandin D_2 was significantly stimulated by captopril in SHR with higher blood pressure (Table 5). On the other hand, the formation of thromboxane B_2, prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ was not influenced significantly by captopril (Table 5). Low concentrations (10^{-12}–10^{-11} M) of captopril stimulated the formation of prostaglandin $F_{2\alpha}$, while higher concentrations did not evoke a significant change (Table 5).

4. Discussion

Research on SHR, first described by Okamoto and Aoki (1963), has greatly advanced our understanding of blood pressure control mechanisms and the pathophysiology of hypertension. SHR have been shown to differ in many ways (eg. structural and functional alterations of the cell membrane, hematological differences, including platelet number and function) from their normotensive progenitor control strain (David-Dufilho et al., 1986; Huzoor et al., 1993; Ishida-Kainouchi et al., 1993). Schirner and Taube (1993) reported different effects of acetyl salicylic acid (aspirin) on the blood pressure of SHR with high and low blood pressure levels. The action of aspirin points to an important role of endogenous prostanoids in the regulation of the blood pressure of SHR.

We found major differences in the eicosanoid synthesis of platelets isolated from SHR and that of platelets from Wistar rats. In the present study, lipoxygenase activity was significantly elevated in SHR platelets, while the formation of cyclooxygenase products was significantly reduced in the higher blood pressure group of SHR, compared to normotensive Wistar rats. This shift in the arachidonate cascade in the direction of the lipoxygenase pathway might play an important role in the pathomechanism of hypertension in genetically hypertensive rats. In SHR the increased synthesis of 12-hydroxy-5,8,10,14-eicosatetraenoic acid, a lipoxygenase metabolite that has a vasoconstrictor effect (Schrör, 1993), might play a role in the development of high blood pressure in these animals. Stern et al. (1996) also reported an increased 12-lipoxygenase activity in SHR, which may contribute to the maintenance of elevated arterial blood pressure in this strain. The decreased synthesis of 12-L-hydroxy-5,8,10-heptadecatrienoic acid, a cyclooxygenase metabolite which is a potent endogenous inducer of prostacyclin synthesis in the vascular endothelial cells (Sadowitz et al., 1987), may also contribute to the elevation of blood pressure. The synthesis of vasoconstrictor thromboxane of the SHR platelets was, however, not elevated. Lemne et al. (1992) also reported a normal thromboxane level in the blood of patients with essential hypertension. These data suggest that platelet thromboxane synthesis does not play a role in the pathogenesis of hypertension either in SHR animals or in human beings.

12-hydroxy-5,8,10,14-eicosatetraenoic acid and 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid, besides the stimulation of their own synthesis, can inhibit cyclooxygenase activity (Bernard and Volker, 1991). These autoregulatory processes of the arachidonate cascade may also contribute to the reduction of the synthesis of cyclooxygenase metabolites in platelets of the SHR with higher blood pressure.

Conflicting results have been reported concerning the effect of captopril on platelet function. In essential hypertension, the reduction of the platelet intracellular calcium ion concentration by captopril was casually related to the decrease in blood pressure (Krzesinski et al., 1993). On the other hand, Wang et al. (1993) did not find any change in the basal calcium ion level in the platelets in the presence of captopril, whereas the thrombin-stimulated elevation of intracellular calcium concentration in the platelets was significantly reduced. James et al. (1988) reported a reduction in in vitro platelet aggregation in response to adrenaline, but not to ADP, in the presence of captopril. Smith et al. (1993) demonstrated that captopril increases the thromboxane production of platelets. Inhibition of platelet thromboxane synthesis with low doses of aspirin, however, does not enhance the antihypertensive effect of captopril. Previous findings from our laboratory (Gecse and Telegdy, 1992) showed that captopril elevated the synthesis of 12-hydroxy-5,8,10,14-eicosatetraenoic acid and 12-L-hydroxy-5,8,10-heptadecatrienoic acid of platelets isolated from normotensive Wistar rats.

In our present in vitro experiments captopril induced different changes in the arachidonate metabolism of the platelets, depending on the blood pressure of the SHR. Captopril significantly reduced the synthesis of thromboxane in platelets of the SHR with lower blood pressure, while there was no difference in thromboxane generation in platelets of the SHR with higher blood pressure. Captopril, however increased the production of other cyclooxygenase metabolites, e.g. prostaglandin D_2, a potent vasodilator, and 12-L-hydroxy-5,8,10-heptadecatrienoic acid, an endogenous stimulator of endothelial prostacyclin synthesis (Sadowitz et al., 1987). The increased amount of prostacyclin synthesized in this way might inhibit platelet aggregation and induce vasodilation, thereby countering the elevation of blood pressure (Chapleau et al., 1991). The synthesis of cyclooxygenase metabolites of platelets isolated from SHR with higher blood pressure was altered by captopril in a way such that it resembled that of normotensive Wistar rats. This effect of captopril in our experiments was presumably a direct one on platelets because the incubation mixture did not contain proteins, angiotensins, bradykinin or other cells which could have contained angiotensin converting enzyme.

The results of these in vitro experiments lead us to conclude that the arachidonate cascade of SHR animals differs from that of genetically healthy Wistar rats, and that the eicosanoid synthesis in SHR may contribute to the development of hypertension in this strain. There are also differences in arachidonic acid metabolism between SHR animals with lower or higher blood pressure. The synthesis of 12-L-hydroxy-5,8,10-heptadecatrienoic acid was significantly decreased, while that of prostaglandin E_2 was increased in the platelets of SHR with higher blood pressure, compared to SHR with lower blood pressure. Captopril-elicited changes in the eicosanoid synthesis of SHR platelets can be an additional mechanism involved in the antihypertensive and antiaggregating effect of the drug. We can assume that these effects of captopril on the arachidonate cascade of platelets are not related to the angiotensin converting enzyme inhibitory effect, but that the free sulfhydryl group of the drug is perhaps implicated in this process.

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