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STIMULATION OF PROSTACYCLIN SYNTHESIS BY NIZOFENONE

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Abstract—The effect of nizofenone on prostacyclin synthesis was investigated using rat arterial walls. Incubation of arterial walls with [^{14}C] arachidonic acid resulted in a time-dependent formation of prostacyclin, which was radiochromatographically detected as the stable breakdown product, 6-keto prostaglandin $F_{1\alpha}$. The addition of nizofenone dose-dependently stimulated the prostacyclin formation, and significant increases of 47 and 106% were observed at 0.1 and 0.3 mM, respectively. No stimulation of prostaglandin E_2 and thromboxane A_2 synthesis was observed in the experiments with ram seminal vesicle microsomes and human platelet microsomes. These findings suggest that nizofenone has a selective stimulatory action on prostacyclin synthesis.

It is well accepted that prostacyclin is a potent vasodilator and also the most potent endogenous inhibitor of platelet aggregation [1]. Prostacyclin is thus thought to participate in vascular control mechanism and to be an important line of defence against thrombus formation, vasospasm and other vascular disorders [2, 3].

Recently, a new cerebroprotective agent, nizofenone [4–7], was found to alleviate the delayed ischemic deficits due to cerebral vasospasm following subarachnoid hemorrhage [8]. To evaluate the mechanism of this alleviative effect, the influence of nizofenone on arachidonic acid metabolism was investigated in rat arterial walls, ram seminal vesicle and human platelet microsomes. The present paper describes a selective stimulative action of nizofenone on prostacyclin synthesis.

MATERIALS AND METHODS

Prostacyclin synthesis. Prostacyclin formation in rat arterial walls was measured by the method reported by Salzman *et al.* [9]. About 10 mg of arterial wall rings were preincubated in 0.5 ml of 50 mM Tris–HCl buffer (pH 8.0) at 4° for 5 min with drugs. After the addition of [^{14}C]arachidonic acid (50 nCi; final concentration 1.9 μM), the mixtures were incubated at 37° for 20 min; the incubation was terminated by the addition of 0.1 ml of 0.2 M citric acid. The incubation mixture was extracted with ethyl acetate and the extract was evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of chloroform–methanol (2:1) and applied to a thin-layer chromatographic plate (Merck, Silica Gel 60 F_{254}). The plate was developed in the organic phase of ethyl acetate/isooctane/acetic acid/water (11:5:2:10). Authentic standards (prostaglandin E_2, $F_{2\alpha}$ and 6-keto $PGF_{1\alpha}$) co-chromatographed with the sample were visualized with iodine vapour. The radioactive products were detected by a radiochromatogram scanner (Packard model 7201). The areas corresponding to the products were scraped off, and then the radioactivity was measured by a liquid scintillation counter (Packard Model 3380).

Prostaglandin E_2 synthesis. Ram seminal vesicle microsomes (1 mg/0.2 ml) were incubated with 4.3 μM [^{14}C]arachidonic acid (50 nCi) in 0.1 M potassium phosphate buffer (pH 7.4) for 1 min at 25°. Products were extracted and determined by radio thin-layer chromatography, according to the procedure reported by Kuehl *et al.* [10].

Thromboxane A_2 synthesis. Human platelet microsomes (0.3 mg/0.2 ml) were incubated with 4.3 μM [^{14}C]arachidonic acid 50 nCi), 5 mM L-tryptophan and 2 μM hemin in 0.1 M Tris–HCl buffer (pH 7.4) at 25° for 5 min. Thromboxane B_2 and prostaglandins (D_2, E_2 and $F_{2\alpha}$) were extracted and measured by radio chromatography, as described by Yoshimoto *et al.* [11].

RESULTS

When [^{14}C]arachidonic acid was incubated with rat aorta wall rings, the formation of prostacyclin was detected as the stable breakdown product, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$). The amount of prostacyclin formed was dependent on the incubation time up to 60 min. This prostacyclin formation by arterial walls was almost completely inhibited by the addition of indomethacin (10 μM) and tranylcypromine (3 mM).

As shown in Fig. 1, the addition of nizofenone (0.3 mM) remarkably increased the arterial prostacyclin formation and significant increases of 47 and 106% were observed at 0.1 and 0.3 mM, respectively (Table 1). The stimulatory effect of nizofenone on

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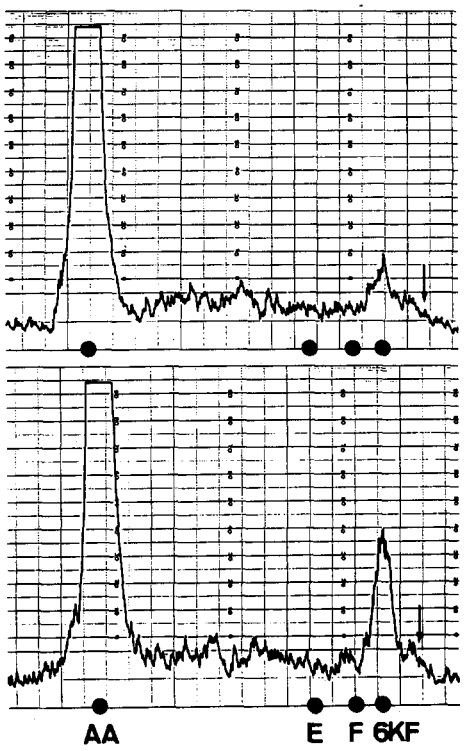

Fig. 1. Radiochromatographs of prostacyclin formation in rat arterial walls. Arterial wall rings were incubated with 1.9 μM [^{14}C]arachidonic acid for 20 min in the absence (upper panel) and presence (lower panel) of 0.3 mM nizofenone. Abbreviations; E, F, 6KF and AA are prostaglandin E_2, $F_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and arachidonic acid, respectively. The arrows designate the positions of the origins.

Table 1. Effects of drugs on prostacyclin synthesis in rat arterial walls

	Conc (mM)	6-Keto $PGF_{1\alpha}$ formed (pmole/10 mg/20 min)	Increase (%)
Control		38.2 ± 3.3	
Nizofenone	0.1	56.3 ± 1.1*	47
	0.3	78.8 ± 6.8**	106
Dipyridamole	0.1	57.3 ± 7.3**	50
	0.3	103.8 ± 16.4**	172
Papeverine	0.3	52.2 ± 2.6	37

Values represent the mean ± S.E. for 3 or 6 (for control) determinations.
* $P < 0.05$, ** $P < 0.01$.

prostacyclin synthesis was approximately comparable to that of dipyridamole. This result for dipyridamole is consistent with that reported with rat stomach fundus homogenate preparation [12].

In order to distinguish whether nizofenone stimulates the cyclooxygenase step or the prostacyclin synthetase step, we studied the conversion of arachidonic acid to prostaglandin G_2, H_2 and E_2 by ram seminal vesicle microsomes (Table 2). Nizofenone did not stimulate the conversion to these prostaglandins in the dose range of 0.1–0.5 mM, as it had stimulated arterial wall prostacyclin synthesis. On the thromboxane A_2 synthesis in human platelet microsomes, no stimulation at all, but rather distinct inhibition was observed at the concentrations of 0.3 mM or more (Table 3). There was concomitant increment of prostaglandin D_2, E_2 and $F_{2\alpha}$ formation,

Table 2. Effect of nizofenone on prostaglandin E_2 synthesis in ram seminal vesicle microsomes

	Conc. (mM)	PGG_2	PGH_2 (pmole/mg/min)	PGE_2
Control		83.4 ± 5.2	71.4 ± 4.3	30.1 ± 8.6
Nizofenone	0.1	74.0 ± 3.4	64.5 ± 2.6	34.4 ± 7.7
	0.3	72.2 ± 1.7	67.1 ± 1.7	31.8 ± 6.9
	0.5	67.9 ± 8.6	59.3 ± 0.9*	36.1 ± 9.5
Tryptophan	5	62.3 ± 3.0*	186.6 ± 4.9**	99.7 ± 2.5**

Values represent the mean ± S.E. for 3 determinations.
* $P < 0.05$, ** $P < 0.01$.

Table 3. Effect of nizofenone on thromboxane A_2 synthesis in human platelet microsomes

	Conc. (mM)	TXB_2	PGD_2	PGE_2 (pmole/0.3 mg/5 min)	$PGF_{2\alpha}$
Control		62.3 ± 0.6	5.1 ± 0.3	13.5 ± 0.6	6.5 ± 0.6
Nizofenone	0.1	63.0 ± 1.3	6.6 ± 0.8	17.1 ± 1.5	7.1 ± 0.8
	0.3	49.1 ± 2.3**	9.9 ± 0.9**	31.2 ± 1.5**	14.3 ± 1.5**
	1	24.3 ± 2.8**	17.7 ± 0.4**	52.2 ± 2.2**	24.9 ± 2.5**
Imidazole	1	20.0 ± 2.2**	14.5 ± 2.3**	31.0 ± 1.7**	33.6 ± 5.9**

Values represent the mean ± S.E. (N = 3).
** $P < 0.01$.

as well as with imidazole, an inhibitor of thromboxane synthetase.

These findings suggest that nizofenone has a selective stimulative action on prostacyclin synthesis, which seems to be mediated through the acceleration of the prostacyclin synthetase step in arachidonic acid cascade.

DISCUSSION

The present *in vitro* study exhibited a selective stimulative effect of nizofenone on prostacyclin synthesis, with no influence on cyclooxygenase reaction. Prostacyclin synthetase is more sensitive than cyclooxygenase or thromboxane synthetase to inactivation by various oxidizing species, such as fatty acid hydroperoxides, free radicals or oxidants [13, 14]. Therefore, the removal of such highly reactive peroxides or free radicals could prevent inactivation of prostacyclin synthetase. Thus, the stimulation of prostacyclin synthesis by nizofenone may be partly explained by the protective effect on prostacyclin synthetase through its oxygen radical-scavenging action [7]. Further studies to clarify the exact mechanism of this stimulatory action are in progress.

Boullin *et al.* [15] have reported that cerebral vasospasm following subarachnoid hemorrhage in humans may be due to disordered physiological control of the calibre of cerebral arteries caused by diminished synthesis of prostacyclin. Sasaki *et al.* [16] have also shown that prostacyclin synthetic capacity of canine basilar arteries is progressively diminished following experimental subarachnoid hemorrhage. Furthermore, prostacyclin administered intravenously in cats reverses vasospasm induced by application of oxyhemoglobin [17], and the intracarotid infusion in baboon also reverses the vasoconstriction effect of indomethacin during hypercapnia [18]. Thus, the therapeutic effects of nizofenone against cerebral vasospasm [8] might be related to this ability to stimulate prostacyclin synthesis.

Nizofenone, as well as imidazole, inhibited thromboxane B_2 formation, while the formation of prostaglandin D_2, E_2 and $F_{2\alpha}$ were increased (Table 3). This finding indicates that nizofenone like imidazole inhibits thromboxane synthetase, resulting in shunting towards other prostaglandins.

Thromboxane A_2 is a more potent vasoconstrictor than serotonin [19] and appears to trigger the majority of transient cerebral ischemic attacks and episodes of infarction [3, 20]. Thus, the thromboxane synthetase-inhibitory action of nizofenone may also partly participate in its cerebral protective effects.

In conclusion, the present study indicates that nizofenone selectivity stimulates prostacyclin synthesis with inhibition of thromboxane formation. This action of nizofenone on arachidonic acid metabolism may contribute to its cerebroprotective effectiveness against cerebral vasospasm and ischemia.

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