Long-lasting inhibition of platelet prostaglandin but normal vascular prostacyclin generation following sulphinpyrazone administration to rats

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Buchanan et al (1978) have reported that the inhibitory effect of sulphinpyrazone on collagen-induced platelet aggregation in vivo persisted for up to 18 h after drug administration. Since unchanged sulphinpyrazone could not be detected in plasma of animals within 4 h of administration, these authors suggested that the inhibitory effect might be ascribed to some metabolite(s). Similar findings have been presented by Butler et al (1979) who found the inhibitory effect on arachidonic acid-induced platelet aggregation in guinea-pigs was greater 7 h than 1 h after oral administration of sulphinpyrazone. Kirstein Pedersen & Jacobsen (1979) have recently identified two active metabolites of sulphin-pyrazone after intravenous administration of this drug to rabbits.

We report here a dose-related inhibition of platelet prostaglandin generation in rats given sulphinpyrazone orally. This effect was maximal between 3 and 18 h after drug administration and disappeared within 36 h.

These results extend to another animal species the concept that the metabolism of sulphinpyrazone may play a major role in the drug's platelet inhibitory activity. The observation that vascular prostacyclin activity was not inhibited at any time after sulphinpyrazone could also be relevant for the antithrombotic activity of this compound.

Male CD-COBS rats (Charles-River, Italy), 250–350 g, were given a single oral dose of either sulphinpyrazone (Ciba-Geigy, Italy) or its suspending vehicle (0·5% carboxymethylcellulose). Animals were killed from 90 min to 36 h thereafter by ether anaesthesia. Blood was collected and the abdominal aorta and inferior vena cava removed and processed as previously described (Villa et al 1979). Malondialdehyde (MDA) formation was measured by a modification (Villa et al 1979) of the spectrophotometric assay described by Smith et al (1976) after stimulation of platelet-rich plasma with 25 NIH units ml^{-1} thrombin (Topostasin, Roche, Switzerland), final concentration. Prostacyclin activity was determined as platelet aggregation inhibitory potency and characterized as previously described (Villa et al 1979; Remuzzi et al 1979).

Table 1. Time course of vascular prostacyclin activity after a single oral dose of sulphinpyrazone (200 mg kg^{-1}). For each interval, means ± s.e.m. from 3 to 6 animals are reported.

Time after treatment (h)	Prostacyclin activity (ng mg^{-1} wet tissue)	
	Abdominal aorta	Inferior vena cava
0	3·20 ± 0·22	0·65 ± 0·08
3	3·05 ± 0·28	0·62 ± 0·12
6	3·25 ± 0·32	0·69 ± 0·10
18	3·12 ± 0·30	0·59 ± 0·12

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Fig. 1 shows that platelet MDA generation was progressively inhibited during the first 3 h after 200 mg kg^{-1} of sulphinpyrazone. Between 80 and 90% inhibition persisted for the next 15 h, declined to about 50% after 24 h and had disappeared at 36 h. The time course of the inhibitory effect of 100 mg kg^{-1} sulphinpyrazone was similar although less marked. At a dose of 50 mg kg^{-1} sulphinpyrazone appeared to be completely ineffective.

Prostacyclin activity released from the abdominal aorta and inferior vena cava was not affected by sulphinpyrazone at any dose and interval tested (data obtained after 200 mg kg^{-1} sulphinpyrazone are reported in Table 1). Likewise, no inhibition was found when vessel rings from either control or treated rats were incubated in platelet-free plasma from animals given 200 mg kg^{-1} sulphinpyrazone 6 h before (data not shown). This experiment rules out the possibility that the lack of effect of sulphinpyrazone on prostacyclin generation could be due to an artifact related to incubation of the vascular specimens in buffer.

Gordon & Pearson (1978) also found that synthesis of prostacyclin by cultured porcine aortic endothelial cells was not inhibited in vitro by concentrations of sulphinpyrazone which can be reached in plasma after therapeutic doses.

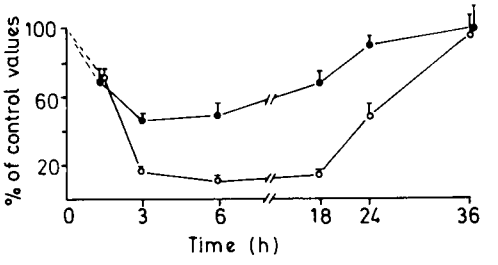

FIG. 1. Time course of platelet malondialdehyde (MDA) formation after single oral doses of sulphinpyrazone (100 mg kg^{-1}, upper curve or 200 mg kg^{-1}, lower curve). Each point represents mean (± s.e.m.) from 3 to 6 animals. Control values for platelet MDA (mean ± s.e.m. of 18 individual experiments) were 0·258 ± 0·014 nmol/1·4 × 10^9 platelets min^{-1}.

In conclusion the present study supports the suggestion that metabolites of sulphinpyrazone may be responsible for this drug's platelet inhibitory activity. The lack of effect of these metabolites on vascular prostacyclin generation could help clarify the mechanism of action of sulphinpyrazone as an antithrombotic agent.

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REFERENCES

Buchanan, M. R., Rosenfeld, J., Hirsh, J. (1978) Thromb. Res. 13: 883–892

Butler, K. D., Pay, G. F., Wallis, R. B., White, A. M. (1979) Thromb. Haemost. 42: 101

Gordon, J. L., Pearson, J. D. (1978) Br. J. Pharmacol. 64: 481–483

Kirstein Pedersen, A., Jakobsen, P. (1979) Thromb. Res. 16: 871–876

Remuzzi, G., Marchesi, D., Misiani, R., Mecca, G., de Gaetano, G., Donati, M. B. (1979) Ibid. 16: 517–525

Smith, J. B., Ingerman, C. M., Silver, M. J. (1976) J. Lab. Clin. Med. 88: 167–172.

Villa, S., Livio, M., de Gaetano, G. (1979) Br. J. Haematol. 42: 425–431

Dopamine acts peripherally on rat tail arteries

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There is much evidence supporting the concept that dopamine (DA) is a centrally acting amine. The central dopaminergic system may be involved in Parkinson's disease and probably in schizophrenia (Hornykiewicz 1977), in thermoregulation (Cox & Lee 1977), in intraocular pressure (Shannon et al 1976), in Huntington's disease (Reinse et al 1977). DA also exerts peripheral effects, e.g. in the cardiovascular as well as in the renal system, in shock (Goldberg 1977), and in the vas deferens (Simon & Van Maanen 1976). However, although the pharmacological basis for the clinical use of DA is increasing, the action of DA on peripheral arteries is still controversial. We have examined the activity of DA on peripheral arteries and compared it with the activity of noradrenaline (NA).

Male Wistar rats (250–300 g) were anaesthetized with urethane i.p. The rat tail artery was separated and perfused according to Nicholas (1969). The length of artery was standardized at 4–5 cm. The perfusion at a constant rate of 2 ml min^{-1} with oxygenated Krebs solution (37 °C) was maintained by a peristaltic roller pump. In all the arteries the perfusion was stabilized at a pressure between 20–30 mm Hg within 20–30 min. Vasoconstriction produced a rise in perfusion pressure which directly related to the intensity of the drug effect. Tested drugs were added to the external bath solution (10 ml) by discrete injection. Cumulative dose-response curves were constructed by increasing the dose in geometrical sequence according to van Rossum (1963). The maximum effect was reached for each dose within about 30 s. When a single response to a drug was observed, and maximum effect reached, the bath solution was replaced with Krebs solution (3 times). Cocaine HCl (3 mg $litre^{-1}$) was added to the Krebs solution (pH 7·4) to prevent the neuronal uptake of catecholamines (Pennefather 1976; Marshall 1977). Results are expressed as means with s.d. Student's *t*-test was used to evaluate differences between control and experimental groups.

Drugs used were: noradrenaline (Levonor, Polfa); dopamine (3-hydroxytyramine HCl, Serva Feinbiochemika); phentolamine (Regitine, Ciba-Geigy); dihydroergotamine methanosulphonate (Galenica); propranolol hydrochloride (Galenica); INPEA (*N*-isopropyl-*p*-nitrophenyletanolamine, Selvi); apomorphine HCl (Polfa); haloperidol (Gedeon Richter); imidazole (Flucka).

Experiments were carried out with both α- (phentolamine 5×10^{-7} M) and β-adrenergic (propranolol 10^{-6} M or INPEA 10^{-6} M) blocking drugs in the medium. DA constricted arteries in standard Krebs solution as well as in the presence of the adrenergic blocking drugs (Fig. 1). The cumulative dose-response curve indicates

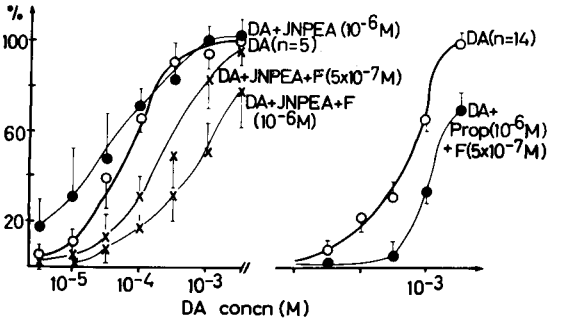

FIG. 1. The vasconstrictor response of rat isolated tail arteries to cumulative doses of dopamine (DA). Experiments were carried out according to van Rossum (1963). INPEA (10^{-6}M), Propr = propranolol (10^{-6}M). Fent = phentolamine (5×10^{-7} M).

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