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Salicylic acid prevents inhibition by aspirin of arachidonic acid-induced hypotension, bronchoconstriction and thrombocytopenia

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Bronchoconstriction, thrombocytopenia and hypotension by arachidonic acid (AA) are inhibited in aspirin-treated animals (Berry, 1966; Larsson & Änggård, 1973; Lefort & Vargaftig, 1975). Since salicylic acid and aspirin compete for albumin binding (Farr, 1971) and may antagonize each other (Ezer, Palosi, Hajos & Szporny, 1976), we investigated whether salicylic acid prevents inhibition by aspirin of the effects of AA. Pentobarbitone (30 mg/kg) anaesthetized guinea-pigs (i.p.) and rabbits (i.v.) were prepared for recording of blood pressure and, in case of guinea-pigs, of bronchoconstriction by the Konzett-Rössler method. Arterial blood was sampled for automatic platelet determinations and for *in vitro* aggregation (Born, 1962), using AA (0.01–0.1 mM) and ADP (0.01–1 μM). Platelet-rich plasma (PRP) prepared from citrated blood (3.8%, 0.1 vol), was incubated for 10–30 min with the drug solvent (polyethyleneglycol 300), with salicylic acid (1–4 mM) or with aspirin (0.05–0.1 mM), washed (Vargaftig, Tranier & Chignard, 1974), and resuspended in drug-free plasma or Tyrode solution. The aggregation behaviour was determined and assays for generation of thromboxane A2 activity on strips of superfused rabbit mesenteric artery and aorta carried out (Piper & Vane, 1969; Vargaftig & Dao, 1971; Bunting, Moncada & Vane, 1976). Hypotension in rabbits and bronchoconstriction and thrombocytopenia in guinea-pigs due to AA were unaffected by prior administration of salicylic acid (100–200 mg/kg i.v.) and were suppressed by aspirin (5 mg/kg i.v.). When salicylic acid was injected before aspirin at a 40 : 1 weight ratio, the latter failed to suppress the effects of AA. Platelet-rich plasma prepared from aspirin-treated animals was not aggregated by AA, and the ADP-induced second wave of aggregation in guinea-pigs was inhibited. Aggregation and generation of thromboxane A2 activity were obtained in AA-PRP incubates from animals pretreated with salicylic acid before aspirin. Incubation of PRP or of platelets resuspended in Tyrode solution containing bovine serum albumin or gelatin (0.35%) with aspirin, followed by resuspension in drug-free medium, resulted in suppression of aggregation and of generation of Thromboxane A2 due to AA and to bovine thrombin (2.5 μml^{-1}). Incubation with salicylic acid before adding aspirin prevented inhibition of the effects of AA and of thrombin. In contrast, incubation of platelets with amounts of warfarin (0.01–1 mM), expected to occupy most of the albumin binding sites (Koch-Weser & Sellers, 1976), failed to prevent aspirin-induced inhibition of platelet aggregation and generation of thromboxane A_2 due to AA or to thrombin. Salicylic acid antagonizes aspirin-induced inhibition of the *in vivo* effects of AA attributable to generation of prostaglandin intermediates and by-products. Since this antagonism occurs *in vitro* in absence of albumin, it does not result from competition for binding, and probably involves interaction at the cyclooxygenase level.

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Selective inhibition of platelet responses to bisenoic prostaglandins

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Prostaglandins (PGs) and prostaglandin analogues exert a variety of effects on human platelets. PGD_2 and PGE_1 are potent inhibitors of aggregation (Ki = 20 nM), and PGE_2 is a weaker inhibitor (Ki = 20 μM) (MacIntyre & Gordon, 1975).

In contrast, methylated derivatives of PGE_2 and stable synthetic derivatives of PGH_2 are potent inducers of platelet aggregation, and appear to mimic the native prostaglandin endoperoxides (Gordon & MacIntyre, 1976; Corey, Gordon, MacIntyre & Salzman, 1977). In the present study, sodium-*p*-benzyl-4-(1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl) phenyl phosphonate (N-0164), a prostaglandin antagonist and inhibitor of thromboxane synthase (Eakins, Rajadhyaksha & Schroer, 1976; Kulkarni & Eakins, 1976) was examined for its effect on human platelet responses to stimulatory and inhibitory prostaglandins.

Platelet aggregation and secretion of radio-labelled serotonin induced by arachidonic acid, 15(S)hydroxy-11α,9α-(epoxymethano) prostadienoic acid (U46619), 11-deoxy 15(S)-16(RS)-methyl-PGE_2 (Wy 19, 110) and ADP, were measured as described previously (Corey *et al.*, 1977). To investigate its inhibiting effect, N-0164 was preincubated in platelet-rich plasma (PRP) at 37°C before the addition of stimulatory prostaglandins (U46619 or Wy 19,110). In studies with inhibitory prostaglandins (PGE_1, PGE_2, PGD_2), N-0164 was preincubated in PRP for 1 min at 37°C before the addition of the prostaglandin, and 1 min later ADP was added, to induce platelet aggregation.

Both stimulatory prostaglandin analogues induced primary (reversible) aggregation at low concentrations (U46619 $\leqslant$ 0.2 μM; Wy 19,110 $\leqslant$ 0.3 μM) and secondary (irreversible) aggregation, accompanied by up to 60% release of radio-labelled serotonin, at higher concentrations. N-0164 (5–250 μM) caused a dose-dependent inhibition of aggregation and serotonin release induced by U46619 and Wy 19,110. Its inhibitory effect was not altered by preincubation, but was readily overcome by increasing the concentration of the agonist. In contrast, even after prolonged pre-incubation in PRP, N-0164 (250 μM) had little effect (< 20% inhibition) on aggregation and serotonin release induced by arachidonic acid (0.5–2 mM), and had no effect on responses to ADP (0.5–3 μM). Inhibition by PGD_2 (60 nM) and PGE_2 (60 μM) of platelet aggregation induced by ADP (0.3–1 μM) was abolished by N-0164 (100–250 μM), but the inhibitory effect of PGE_1 (60 nM) was unaltered.

These results indicate that N-0164 antagonizes the effects of bisenoic prostaglandins. Inhibition of platelet aggregation by PGE_1, PGE_2, and PGD_2 is attributed to their stimulation of adenylate cyclase (for review, see Mills & Macfarlane, 1977); the failure of N-0164 to affect the action of PGE_1 while blocking PGD_2 and PGE_2 is the clearest demonstration so far that PGE_1 exerts its effect on a different receptor. Since arachidonic acid stimulates platelets through its endoperoxide and thromboxane metabolites, it is curious that N-0164 abolished the effect of endoperoxide analogues yet did not inhibit the response to arachidonic acid. However, the relative importance of these metabolites is not known, and since N-0164 does not inhibit the effects of thromboxane A_2 on rabbit aorta (Kulkarni & Eakins, 1976), the ineffectiveness of N-0164 against arachidonic acid could be because thromboxane A_2 is mainly responsible for arachidonic acid's effect on platelets. If so, this would imply that N-0164 does not inhibit thromboxane synthase in intact platelets. It is also possible that the arachidonic acid metabolites stimulate platelets via intracellular sites inaccessible to N-0164, and further work is necessary to characterize more fully the actions of this interesting compound.

This study was supported by a grant from the MRC. U46619 was kindly donated by Dr G.L. Bundy, Upjohn Co. and Wy 19,110 by Dr R.L. Renichel, Wyeth. N-0164 was kindly donated by Dr E. Nelson, Nelson Research and Development Co.