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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.

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Some characteristics of the metabolism of β-phenylethylamine in rat isolated lung

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β-Phenylethylamine (PEN) is metabolized on a single passage through the pulmonary circulation of rats and rabbits (Bakhle & Youdim, 1976; Roth & Gillis, 1975) and the metabolism of this amine involves monoamine oxidase of the B type (MAO-B; Yang & Neff, 1973) in contrast to that of 5-hydroxytryptamine (5-HT) which involves MAO-A. Both these enzymes are intracellular and amine metabolism must therefore be preceded by transfer of substrate across the cell membrane. Either step, uptake or enzymic reaction, could be rate-limiting.

We have studied the kinetics of the metabolism of PEN and of 5-HT in isolated rat lungs, by measuring the amine metabolites over a range of concentrations. Amine labelled with ^{14}C was infused through isolated lungs, perfused with Krebs solution via the pulmonary artery, for 3 min and the effluent collected during, and after, the infusion for a total of 30 minutes. By this time the radioactivity in the effluent had fallen to background levels. Aliquots of the collected effluent were chromatographed on ion exchange columns (Amberlite CG-50; Southgate & Collins, 1969) and the radioactivity eluted from the columns with water (non-basic metabolites) measured by liquid scintillation methods.

For PEN over a concentration range of 0.1–150 μM, metabolism seems to be governed by a single rate-limiting step with an apparent K_m of 55 μM and V_{max} of 880 nmol $lung^{-1}$ 30 min^{-1}. In contrast, the metabolism of 5-HT over a similar range (0.1–50 μM) seems best described by two processes; one with K_m 2 μM and V_{max} 34 nmol and the other with a K_m value at least 25 times higher. The K_m values for metabolism in the perfused organ should be compared with the K_ms for 5-HT, 187 μM, and PEN, 20 μM, obtained with MAO isolated from liver (Houslay & Tipton, 1974).

Metabolism of PEN in lung is not inhibited by 5-HT (Bakhle & Youdim, 1976) and in these experiments, tryptamine and benzylamine (0.2 and 2.0 μM) did not inhibit the metabolism of ^{14}C-PEN (0.15 μM). Furthermore, the inactivation of noradrenaline (0.5–2 ng/ml) in isolated lungs, measured by bioassay, was not affected by PEN infused through the lung in concentrations up to 500 ng/ml. Tricyclic antidepressant drugs such as amitriptyline and imipramine inhibit uptake and hence metabolism of 5-HT and noradrenaline in lung (for refs. see Bakhle & Vane, 1974) but PEN metabolism was not inhibited by desmethylimipramine (10^{-5} M). However, in lungs from rats pretreated (3 h; 5 mg/kg i.p.) with deprenil, an inhibitor of MAO-B (Knoll & Magyar, 1972), metabolism of PEN was inhibited by 34%.

From these results, the rat lung has a greater capacity to metabolize PEN compared with 5-HT and the process by which PEN enters the cell may be different from those uptake systems already described for 5-HT and noradrenaline in the lung (Gillis, 1976).

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