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ANTI-AGGREGATORY EFFECT OF PROSTACYCLIN (PGI_2) *in vivo*

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Prostacyclin (PGI_2) when infused intravenously reduced the mortality of rabbits given high intravenous doses of arachidonic acid (AA). Prostaglandins E_1 and D_2 were ineffective. Indomethacin pretreatment abolished the toxic AA effect. Since the lethal effect of AA is partly due to the formation of platelet aggregates it is concluded that PGI_2 is a most potent anti-aggregatory prostaglandin *in vivo*.

Introduction Prostacyclin (PGI_2) was found to be the most potent anti-aggregatory prostaglandin in all platelet preparations so far investigated (Gryglewski, Bunting, Moncada, Flower & Vane, 1976; Moncada, Gryglewski, Bunting & Vane, 1976a, b; Gorman, Bunting & Miller, 1977). Prostaglandin E_1 (PGE_1) is about 7 to 50 times less potent than PGI_2 whereas the potency of PGD_2 varies within wide limits (about 1/2 to 1/10,000 that of PGI_2) according to the species investigated (Whittle, Moncada & Vane, 1978). The inhibition of platelet aggregation by PGE_1 *in vitro* (Kloeze, 1967) has been confirmed *in vivo* (Emmons, Hampton, Harrison, Honour & Mitchell, 1967; Kinlough-Rathbone, Packham & Mustard, 1970). To date, there is only one paper (using the everted hamster cheek pouch) demonstrating an anti-aggregatory effect of PGI_2 *in vivo* (Higgs, Moncada & Vane, 1977).

Arachidonic acid (AA), when injected into the ear vein of rabbits, results in sudden death. This is due to the formation of occlusive thrombi in the microvascular bed of the lung (Silver, Hoch, Kocsis, Ingerman & Smith, 1974). On the basis of these results, a model for testing anti-aggregatory active agents *in vivo* was proposed by Kohler, Wooding & Ellenbogen (1976). These authors were able to demonstrate that the occurrence of death is a specific effect of AA (other fatty acids were ineffective) which could be prevented by some agents known to inhibit platelet aggregation. The aim of our investigations was to examine the protective action of PGI_2, to compare it with the effects of PGE_1 and PGD_2 and to examine the influence of the cyclo-oxygenase inhibitor, indomethacin, on the lethal effect of an overdose of AA.

Methods Rabbits of either sex, weighing 1.8 to 3.0 kg, were anaesthetized with ethyl urethane (0.5 g/kg i.p.). Blood pressure was measured via a catheter in the femoral artery and a Statham pressure transducer (P 23dB) and the ECG (lead II) was also recorded. AA 1.5 mg/kg (as the sodium salt in a total volume of 1.0 ml) was injected into the ear vein over a 3 s period. In additional animals, 20 mg/kg oleic acid (OA) and linoleic acid (LA) were given in the same way. The prostaglandins were infused at a constant flow rate of 0.2 ml/min into the femoral vein for 8 min. Stock solutions of the prostaglandins (1 mg/ml PGE_1 and PGD_2 in absolute ethanol and 1 mg/ml PGI_2 in 1 N NaOH) were diluted to the required final volume with 0.9% w/v NaCl solution (PGE_1 and PGD_2) or with 0.005 N NaOH (PGI_2). Three minutes after starting the infusion, AA was given as described above. In 7 animals indomethacin (10 mg/kg i.p.) was administered 20 h before the injection of AA. Significance levels were deduced from the tabulated data of the 'exact Fisher test'.

The following substances were used: ethyl urethane (VEB Philopharm), arachidonic acid (Unilever), linoleic acid (Karl Roth OHG), oleic acid (Merck), PGI_2 (Wellcome), PGE_1 and PGD_2 (Upjohn), indomethacin (Chinoin).

Results The intravenous administration of 20 mg/kg OA ($n = 15$) and LA ($n = 15$), respectively, were without any toxic effects. In contrast, the injection of AA (1.5 mg/kg) was followed by severe respiratory distress, a drastic fall in blood pressure and severe arrhythmias. Of 45 animals, 34 died within 1 to 4 min (75.6% mortality). Among all prostaglandins investigated, only PGI_2 was able to reduce the AA-conditioned mortality in a dose-dependent manner (Figure 1). As the mortality was decreased the incidence of arrhythmias was also diminished. All prostaglandins except PGD_2 lowered the blood pressure significantly ($P < 0.001$). The decrease of blood pressure averaged $37 \pm 2\%$ (0.25 $\mu g\ kg^{-1}\ min^{-1}$ PGI_2), $49 \pm 3\%$ (0.5 $\mu g\ kg^{-1}\ min^{-1}$ PGI_2), $57 \pm 4\%$ (1 $\mu g\ kg^{-1}\ min^{-1}$ PGI_2), $67 \pm 5\%$ (2 $\mu g\ kg^{-1}\ min^{-1}$ PGI_2), $56 \pm 4\%$ (10 $\mu g\ kg^{-1}\ min^{-1}$ PGE_1) and $12 \pm 4\%$ (30 $\mu g\ kg^{-1}\ min^{-1}$ PGD_2).

Pretreatment of the rabbits with 10 mg/kg indomethacin abolished the toxic effects of AA. No animal died following AA injection ($n = 7$). The fall in blood pressure was less drastic and no arrhythmias occurred.

Discussion An *in vivo* model for testing anti-aggregatory drugs (Kohler *et al.*, 1976) has been established

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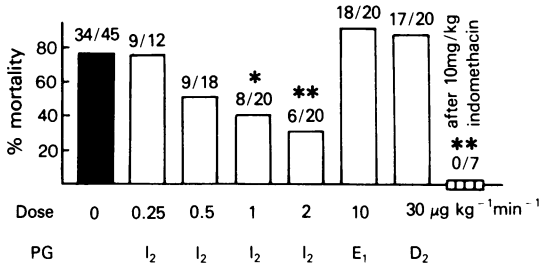

Figure 1 Influence of intravenous infusions of prostaglandins I_2, E_1 and D_2 on the mortality of rabbits resulting from single doses (i.v.) of 1.5 mg/kg arachidonic acid (open columns) in comparison with control animals (solid column) and indomethacin pretreated rabbits (hatched column). The results are expressed in % mortality after arachidonic acid. The first number above each column represents the number of animals that died and the second the total number of animals investigated. Significance of differences between prostaglandin-treated or indomethacin-pretreated groups vs control: * $P < 0.05$; ** $P < 0.01$.

by the metabolic conversion of AA into cyclic endoperoxides followed by the formation of either prostaglandins or thromboxane A_2 (TXA_2). The balance between TXA_2 and PGI_2 formation is thought to be responsible for the control of platelet aggregation under physiological conditions (for references see Srivastava, 1978).

An overdose of AA disturbs this balance. Thus our finding that pretreatment with indomethacin (which inhibits the increased formation of endoperoxides) abolished the toxic effects of AA is not surprising. Neither is the fact that OA and LA, which are not direct precursors for cyclic endoperoxide synthesis, were without any toxic effects. These results are in accordance with those of Kohler *et al.* (1976). PGI_2, which is known to be the most potent inhibitor of platelet aggregation *in vitro*, also protected against the toxic AA effect *in vivo*. On the other hand, PGE_1 and PGD_2 were ineffective in the dose used. The relative potencies ($PGI_2 = 1$), for complete inhibition of ADP-induced aggregation in rabbit platelet-rich plasma, are 0.06 for PGE_1 and 0.002 for PGD_2 (Whittle *et al.*, 1978). If these ratios are valid under *in vivo* conditions the PGD_2 dose used was too small. However, one might have expected a slight effect with 10 $\mu g\ kg^{-1}\ min^{-1}$ PGE_1. The ineffectiveness of PGE_1 in comparison with PGI_2 can be attributed to the rapid metabolism of this prostaglandin by the lungs and to the failure of the lungs to metabolize PGI_2 significantly (Armstrong, Lattimer, Moncada & Vane, 1978). The incomplete protective action of PGI_2 may be explained in two ways: (1) PGI_2 does not prevent the metabolic conversion of AA into cyclic endoperoxides and thus the formation of both prostaglandins (e.g. PGE_2) and TXA_2. PGE_2 for example inhibits the anti-aggregatory potency of PGI_2 *in vitro* (unpublished results) and possibly also *in vivo*. (2) PGI_2 and AA act additively in lowering the systemic arterial blood pressure.

Our results suggest that PGI_2 is the most potent anti-aggregatory prostaglandin *in vivo*.

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