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Phospholipase A_2 activity of guinea-pig perfused lungs: stimulation and inhibition by anti-inflammatory steroids

G.J. BLACKWELL, R.J. FLOWER, F.P. NIJKAMP & J.R. VANE

Dept. of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Guinea-pig isolated lungs release prostaglandin (PG) endoperoxides and thromboxane A_2 (TXA_2) into the perfusion fluid in response to stimuli including antigen challenge (Piper & Vane, 1969), mechanical trauma (Palmer, Piper & Vane, 1973), bradykinin or arachidonic acid (Vargaftig & Dao Hai, 1972) and rabbit aorta contracting substance – releasing factor (RCS-RF; Piper & Vane, 1969; Nijkamp, Flower, Moncada & Vane, 1976). Nijkamp *et al.* (1976) demonstrated that in relation to their anti-inflammatory potency, corticosteroids inhibited the generation of TXA_2 induced by RCS-RF, but not that due to the precursor arachidonic acid. Thus, inhibition of the release of arachidonic acid could be related to the therapeutic action of these steroids. Flower & Blackwell (1976) demonstrated that arachidonic acid was released from cellular phosphatides in response to similar stimuli, and this led us to speculate that agents which release TXA_2 from lungs do so by "activating" phospholipase A_2.

For these experiments, the guinea-pig perfused lungs and cascade superfusion apparatus were prepared and TXA_2 generation was measured as previously described (Nijkamp *et al.*, 1976). For assay of phospholipase A_2 activity, a mixture of 18.0 nmoles 2-acyl ([^{3}H]-oleoyl) phosphatidylcholine and 1.8 nmoles [^{14}C] oleic acid was injected into the pulmonary artery. The perfusate was collected for 7 min and the labelled fatty acids selectively extracted at pH 8.0 with 10 ml *n*-hexane. The solvent was evaporated to dryness and the $^3H/^{14}C$ ratio estimated by conventional liquid scintillation counting techniques.

When injected into the pulmonary artery, histamine (2–5 μg), RCS-RF (5–10 u), bradykinin (1–5 μg) and arachidonic acid (1–5 μg) caused a release of PG endoperoxides and TXA_2. Release of TXA_2 was blocked by indomethacin (1 μg/ml). The release induced by histamine or RCS-RF was also blocked by dexamethasone (ID_{50} 1.5 μg/ml) and hydrocortisone (ID_{50} 33 μg/ml).

There was a small (1–3%) basal hydrolysis of the labelled phosphatide by the perfused lung, which increased gradually with time. This hydrolysis was inhibited by mepacrine (20 μg/ml), procaine (40 μg/ml), betamethasone (2 μg/ml), dexamethasone (2 μg/ml) and hydrocortisone (50 μg/ml). The steroids exhibited a time-dependent inhibition, the maximum effect occurring after 30 min infusion. Histamine (2 μg), RCS-RF (5 u) and bradykinin (1 μg) stimulated phospholipid hydrolysis by 150–300%. Steroids and mepacrine blocked (60–90%) the stimulation due to histamine and RCS-RF but had only a small effect (10–20%) on the bradykinin stimulation.

In homogenates of guinea-pig lung, phospholipase A_2 activity was inhibited by mepacrine and procaine. Steroids were without effect, indicating that these agents require intact cells to function effectively.

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