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Effect of cromoglycate and eicosatetraynoic acid on the release of prostaglandins and SRS-A from immunologically challenged guinea pig lungs

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Guinea pigs were sensitized with ovalbumin (100 mg i.p. and s.c.) 3 weeks before the lungs were removed and perfused free of blood with Tyrode solution (Brocklehurst, 1960). Dextran (6%) was added to all the Tyrode used in these experiments. Tyrode solution containing [1-^{14}C]-arachidonic acid (10 μC, 58 μg) was then perfused at 2 ml/min in a closed circuit for 90 minutes. [^{14}C]-arachidonate which had not been taken up by the lungs was removed by 20 min perfusion with Tyrode solution. Ovalbumin (5 mg) injected into the inflow cannula caused the release of ^{14}C materials which were collected in 5 min fractions during 20 minutes. Aliquots of these samples were bioassayed on guinea pig ileum, in the presence of mepyramine (5×10^{-7} g/ml) and the remainder extracted with two volumes of diethyl ether at pH 3. The organic extracts were examined in various thin layer chromatographic systems using parent prostaglandins (PGs) as standards.

Six radio-labelled peaks were seen on scanning the TLC plates, two major and four minor. Two peaks had similar R_F values to PGE_2 and $PGF_{2\alpha}$ and a minor peak had the same R_F as arachidonate. The other peaks are as yet unidentified but probably include 13-14 dihydro PGE_2 and 13-14 dihydro 15-keto PGE_2 (Änggård and Samuelsson, 1965) and possibly similar metabolic products of $PGF_{2\alpha}$.

Eicosatetraynoic acid (TYA, 10 μg/ml), a competitive inhibitor of PG synthesis, or disodium cromoglycate (10 μg/ml) were added to the closed circuit perfusion system 60 min after the [^{14}C]-arachidonate, thus reducing the possibility that these compounds may interfere with uptake rather than PG synthesis. The lungs were then challenged and samples collected and processed as before. Each experiment was repeated at least three times and similar results were obtained on each occasion.

TYA reduced all the radio-labelled peaks whilst cromoglycate had no effect.

Bioactivity in the perfusates, a large proportion of which is assumed to be slow-reacting substance in anaphylaxis (SRS-A) was modified minimally by TYA (<10%) but was considerably reduced by cromoglycate (>50%).

These results indicate that it is unlikely that SRS-A is formed from arachidonate, that SRS-A is not a prostaglandin of the PG_2 series and that it is probably not related to the PG_1 or PG_3 series. Cromoglycate reduced the release of SRS-A but did not modify PG release.

References

ÄNGGÅRD, E. & SAMUELSSON, B. (1965). Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. *J. Biol. Chem.*, **240**, 3518-3521.

BROCKLEHURST, W.E. (1960). The release of histamine and formation of a slow-reacting substance (SRS-A) during anaphylactic shock. *J. Physiol., Lond.*, **151**, 416-435.

The concomitant release of bradykinin and prostaglandin in the inflammatory response to carrageenin

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Bradykinin (van Arman, Begany, Miller & Pless, 1965; Garcia Leme, Schapoval & Roche e Silva, 1967) and prostaglandins (Willis, 1969; Di Rosa, Papadimitriou & Willoughby, 1971) are both implicated in inflammation induced by carrageenin in the rat. Di Rosa *et al.* (1971) suggested that bradykinin was released transiently, after the initial release of histamine and that prostaglandins were responsible for the later phase of inflammation.

Prostaglandins sensitize pain receptors to mechanical or to chemical stimulation (Ferreira, 1972; Ferreira, Moncada & Vane, 1973). Inhibition of prostaglandin biosynthesis by aspirin-like drugs (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971) abolishes this sensitization and thereby produces analgesia.

We and others have now shown that the oedema caused by bradykinin and various inflammatory stimuli is also potentiated by prostaglandins (Moncada, Ferreira & Vane, 1973; Thomas & West, 1973; Williams & Morley, 1973; Lewis, Nelson & Sugrue, 1974). Thus the anti-oedema properties of aspirin-like drugs, like the analgesia, can be explained by removal of the potentiation, through inhibition of prostaglandin biosynthesis.

We have re-investigated the time course of the release of bradykinin using the enhancement of oedema induced by a synthetic bradykinin potentiator (BPP_{9a}) as an indicator of the presence of bradykinin. The nonapeptide affects vascular permeability induced by bradykinin, but not that produced by other inflammatory mediators (Ferreira, 1965; Greene, Camargo, Krieger, Stewart & Ferreira, 1972).

Groups of eight to ten rats were used and the increase in hind paw volume caused by carrageenin (0.1 ml of 0.5% w/v) was estimated over 6 h by subtracting the volume of the contralateral paw which received an equal volume of saline. BPP_{9a} (5 μg in 0.03 ml) was injected into both paws; it had no effect on normal paw volume but significantly increased the oedema when given at 0, 0.5, 1, 4 and 6 h after carrageenin. This enhancement was also seen in animals treated with indomethacin (10 mg/kg i.p.). Soya bean trypsin inhibitor (500 μg), which prevents bradykinin formation, abolished potentiation.

Potentiation of carrageenin oedema by prostaglandin E_1 was also studied in rats treated or not treated with indomethacin (10 mg/kg). A significant increase in paw oedema was observed in both groups of animals when prostaglandin E_1 (0.5 μg) was given, either 0.25 h before carrageenin or 0, 0.5, 1, 4 and 6 h afterwards. The increase was much greater in those animals in which oedema had been reduced by indomethacin.

These results indicate (a) that bradykinin is being formed during all of the first 6 h of carrageenin oedema and (b) that there is no tachyphylaxis to the potentiating action of prostaglandins on the effects of the other mediators.

These results re-inforce our previous conclusion that the late phase of carrageenin oedema results mainly from the potentiating action of prostaglandins on the effects of other mediators, especially bradykinin.