Potentiation of bradykinin-induced exudation following intradermal injection of particulate or colloidal materials in the rabbit: evidence for prostaglandin release and action in inflammation

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Prostaglandins, which have little effect on vascular permeability when injected alone into rabbit or guinea-pig skin, potentiate the local exudation produced by bradykinin and histamine (Williams & Morley, 1973). This potentiating activity of prostaglandins correlates with their vasodilator activity (Williams, 1976a). The present study is concerned with the source and nature of possible endogenous potentiating substances in inflammatory reactions.

Plasma exudation and blood flow changes were measured in rabbit skin using ^{131}I-albumin and ^{133}Xe, as previously described (Williams, 1976b). It was observed that an intradermal injection of *B. pertussis* (2.5×10^8 organisms/site) greatly potentiated the exudation produced by a following injection of bradykinin (500 ng/site) into the same site. Similar potentiation of responses to bradykinin (or histamine) was observed using zymosan, glycogen, antigen-antibody complexes, and carrageenin. No potentiation was observed with bradykinin/*B. pertussis* mixtures. Potentiation increased as the interval between the two injections was increased up to 1 h, but decreased with longer intervals (Figure 1). The small exudation produced by *B. pertussis* alone, followed a similar time-course.

The potentiation time-course suggested that an accumulation of haematogenous cells was a prerequisite. This was investigated in polymorphonuclear (PMN-) leucocyte-depleted rabbits using nitrogen mustard (1.75 mg/kg i.v., 4 days before experiment). A marked reduction in *B. pertussis* potentiation (1 h interval) was observed in these animals with no significant reduction in PGE_2 potentiation; (exudation, nitrogen mustard treated: bradykinin = 26.8 ± 5.4 µl, *B. pertussis* = 0.0 ± 1.1 µl, *B. pertussis*/bradykinin = 46.7 ± 8.9 µl, bradykinin + PGE_2 (100 ng) = 81.4 ± 17.7 µl; controls: bradykinin = 33.5 ± 7.8 µl, *B. pertussis* = 20.0 ± 4.9 µl, *B. pertussis*/bradykinin = 126.2 ± 19.5 µl, bradykinin + PGE_2 = 90.9 ± 14.4 µl; $n=7$ rabbits/group). This, and the observation that responses to bradykinin are potentiated by intradermal injection of PMN-leucocytes (Firrell, Peck & Williams, 1976), suggests that the source of the potentiating substance is the PMN-leucocyte.

Potentiation was suppressed by mixing the *B. pertussis* with indomethacin or dexamethasone before injection; (bradykinin = 35.1 ± 6.5 µl, *B. pertussis* = 13.1 ± 2.7 µl, *B. pertussis*/bradykinin = 95.3 = 6.8 µl, *B. pertussis* + indomethacin (1 µg/site)/bradykinin = 47.2 ± 4.2 µl, *B. pertussis* + dexamethasone (1 µg/site)/bradykinin = 50.0 ± 2.9 µl, $n=6$ sites).

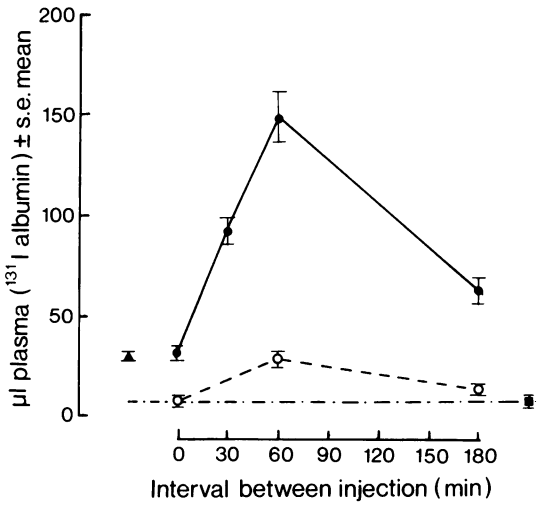

Figure 1 Time-course of potentiation of bradykinin (500 ng)-induced exudation by a preceding intradermal injection of *B. pertussis* (2.5×10^8 organisms) in rabbit skin. ● 1st injection of *B. pertussis*/2nd injection of bradykinin, ▲ saline/bradykinin, ○ *B. pertussis*/saline, ■ saline/saline. Abscissa is interval between 1st and 2nd injections. Exudation was measured for 30 min following 2nd injection. $n=6$.

These results suggest that in response to the introduction of particulate materials into tissues, PMN-leucocytes infiltrate and then release a vasodilator, probably a prostaglandin, which is able to potentiate the exudation produced by other inflammatory mediators.

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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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