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A POSSIBLE MODULATORY ROLE FOR PROSTACYCLIN (PGI_2) IN IgG_a-INDUCED RELEASE OF SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS IN RATS

J.F. BURKA & L.G. GARLAND

Department of Pharmacology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3 BS

Antigen challenge *in vivo* of rat peritoneal cells (enriched with monocytes and polymorphonuclear leucocytes) passively sensitized 2 h previously with homologous antibody of the IgG_a class released large amounts of slow-reacting substance of anaphylaxis (SRS-A, 1739 ± 59 u/ml) into the peritoneal fluid. This reaction was strongly inhibited by prostacyclin (PGI_2, $ED_{50} = 0.5$ μg/kg i.p.) and by isoprenaline ($ED_{50} = 0.2$ μg/kg i.p.) but prostaglandins E_1, E_2 and 6-oxo-prostaglandin $F_{1\alpha}$ were only weak inhibitors. Indomethacin (10 mg/kg, orally) augmented by 30% the release of SRS-A, whereas thromboxane B_2 (50 μg/kg i.p.) had no effect. Lowering the antigen (ovalbumin) dosage from 400 μg/ml to 10 μg/ml reduced the control release of SRS-A by 70% and increased the inhibitory effect of prostaglandins I_2, E_1 and isoprenaline. Augmentation of release by indomethacin remained unchanged. These preliminary data suggested that endogenous prostacyclin may modulate the anaphylactic release of SRS-A from rat peritoneal cells.

Introduction The immunological release of slow-reacting substance of anaphylaxis (SRS-A) provoked by IgG_a in the rat peritoneum seems to be a complement-dependent reaction involving polymorphonuclear leucocytes and monocytes, the yield of SRS-A being greater in rats primed with glycogen to increase the incidence of these cell types in the peritoneal cavity (Orange, Valentine & Austen, 1968). In contrast, the release of SRS-A mediated by IgE-like antibody is complement-independent and involves mast cells (Orange, Stechschulte & Austen, 1970). The release of SRS-A from rat peritoneal cells sensitized by IgE-like antibody is inhibited by agents that raise intracellular levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) such as prostaglandins E_1, E_2 and isoprenaline (Koopman, Orange & Austen, 1971). The present study examined the role of prostaglandins in modulating the release of SRS-A provoked by IgG_a.

Methods Male Wistar rats (200–250 g) were injected intraperitoneally with 20 ml of 0.1% oyster glycogen 18–22 h before 5 ml antiserum containing IgG_a antibody to ovalbumin (Orange *et al.*, 1968). Two hours after passive sensitization, 5 ml antigen (400 μg/ml) was injected intraperitoneally into groups of at least 4 rats. Modulatory agents were injected (i.p.) 30 s before antigen; at the concentrations used, none interfered with the bioassay of SRS-A. The animals were killed 5 min later by cervical dislocation while under CO_2 narcosis. The peritoneal fluid was aspirated and kept on ice until assayed against an internal standard on two guinea-pig ilea superfused in series with oxygenated (95% O_2 and 5% CO_2). Tyrode solution of the following composition (mmol/l): NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 0.3, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.8 and dextrose 5.6, containing the following antagonists (μmol/l): hyoscine hydrobromide 0.23, mepyramine maleate 0.24, methysergide bimaleate 0.43, phenoxybenzamine hydrochloride 0.29 and propranolol hydrochloride 0.68. This method allowed for a minimum amount of SRS-A to be assayed in duplicate. Samples of SRS-A were further characterized and found during silicic acid chromatography to have the same elution characteristics as those described by Orange, Murphy, Karnovsky & Austen (1973) and its guinea-pig ileum contracting activity was destroyed by arylsulphatase (Orange, Murphy & Austen, 1974) and inhibited by FPL 55712 (Augstein, Farmer, Lee, Sheard & Tattersall, 1973).

All agents for injection, with the exceptions of prostacyclin and indomethacin, were dissolved in isotonic saline. Prostacyclin was dissolved in 1 M Tris buffer (pH 8.4) to 1 mg/ml and diluted in 50 mM Tris buffer (pH 7.5). Indomethacin was dissolved in 1 M Tris buffer (pH 8.4) to 10 mg/ml and diluted with distilled water. Control groups of rats were treated at the same time as the test groups with the same vehicle. The vehicles used did not affected the antigenic release of SRS-A, nor did they release SRS-A *per se*.

Results Prostacyclin (PGI_2) potently inhibited the release of SRS-A ($ED_{50} = 0.5$ μg/kg i.p.), being more active than either prostaglandins E_1 or E_2 which significantly inhibited release, but by only $30 \pm 12\%$ (mean ± s.e., $n = 5$ for both prostaglandins E_1 and E_2) at a dose of 5 μg/kg i.p. (Figure 1a). The dose-response curve for prostacyclin was shallower than that for isoprenaline ($ED_{50} = 0.2$ μg/kg i.p.), which was included for comparison, and reached a maximum of $73 \pm 10\%$ (mean ± s.e., $n = 5$) inhibition at a dose of 5 μg/kg intraperitoneally. The stable metabolite of prostacyclin, 6-oxo-prostaglandin $F_{1\alpha}$, was only

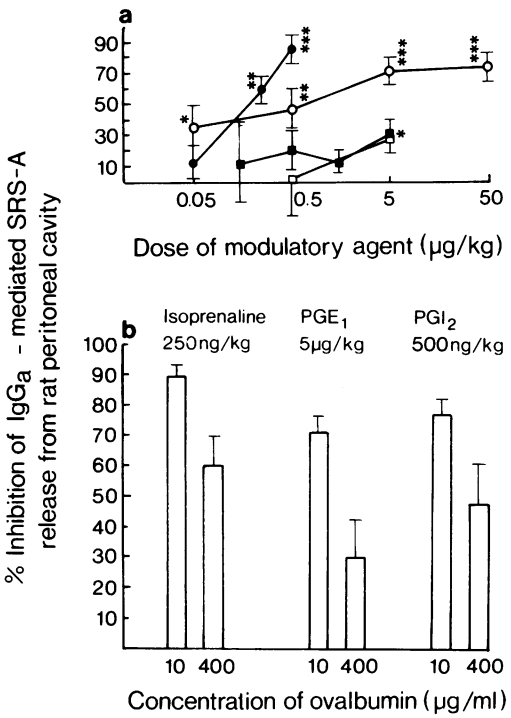

Figure 1 Inhibition of the anaphylactic release of slow-reacting substance of anaphylaxis (SRS-A) from rat peritoneal cells *in vivo* passively sensitized with homologous IgG_a 2 h before challenge with antigen. (a) The effect of various concentrations of inhibitors using antigen at 400 μg/ml. Isoprenaline (●); prostacyclin (PGI_2) (○); prostaglandin E_1 (□); prostaglandin E_2 (■). (b) The effect of inhibitors, each at a single concentration, with antigen concentrations of either 10 μg/ml or 400 μg/ml. Each value is the mean result from at least 4 rats; vertical lines show s.e. mean. Significance of the difference from control release of SRSA was calculated by Student's *t* test: $*P<0.05$; $**P<0.01$; $***P<0.0025$.

weakly active, inhibiting SRS-A release by $14\pm5\%$ (mean ± s.e., $n=4$) at a dose of 5 μg/kg. Thromboxane B_2 had no effect on SRS-A release in this system when given intraperitoneally in doses up to 50 μg/kg. Indomethacin (10 mg/kg), administered orally 2 h before challenge, augmented SRS-A release by $30\pm11\%$ (mean ± s.e., $n=19$).

The effect of modulating agents was influenced by the degree of antigenic stimulus. The maximum release of SRS-A (1739 ± 59 u/ml; mean ± s.e., $n=65$) was obtained with an antigen concentration of 400 μg/ml, whereas only 30% of maximum release (i.e. 500 ± 105 u/ml; mean ± s.e., $n=5$) was obtained with a concentration of 10 μg/ml. When the activities of isoprenaline, prostaglandins I_2 and E_1 were compared using these two levels of antigenic stimulus, inhibition of SRS-A release by each agent was significantly greater when the lower concentration of ovalbumin was used (Figure 1b). In contrast, enhancement of SRS-A release by indomethacin remained unchanged.

Discussion If the augmentation of SRS-A release in the presence of indomethacin is caused by removal of a prostaglandin negative-feedback mechanism (Walker, 1973), the present evidence would suggest that prostacyclin is the prostaglandin primarily responsible for modulating the IgG_a-provoked reaction in rat peritoneal cells. The relatively weak effects of prostaglandins E_1 and E_2 in this system are in marked contrast to their potent inhibition of the release of SRS-A from rat peritoneal cells sensitized by IgE-like antibody (Koopman *et al.*, 1971).

The mechanism of inhibition of SRS-A release by prostacyclin is not known. However, it has been reported to increase levels of cyclic AMP in platelets while preventing aggregation (Tateson, Moncada & Vane, 1977). Therefore, it is feasible that prostacyclin acts by increasing intracellular levels of cyclic AMP in rat peritoneal cells.

Boot, Brockwell, Dawson & Sweatman (1977) reported that thromboxane B_2 augments the immunological release of SRS-A from guinea-pig perfused lung. However, thromboxane B_2 does not appear to be a modulator of IgG_a-mediated SRS-A release from polymorphonuclear leucocytes or monocytes.

Modulation of SRS-A release appeared to depend on the degree of antigenic stimulus, greater inhibition being observed with a submaximal antigen concentration. This agrees with previous observations in guinea-pig lung and human leucocyte preparations where inhibition of histamine release by isoprenaline was greater when sub-optimal antigen concentrations were used (Assem & Schild, 1971). This inter-relationship should, perhaps, be anticipated for inhibitors such as isoprenaline and prostacyclin that may act by increasing intracellular levels of cyclic AMP since, in mast cells at least, the immune stimulus is associated with a transient fall in the resting level of cyclic AMP (Kaliner & Austen, 1974).

J.F.B. is a research fellow of the Canadian Heart Foundation.

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