Prostaglandin Inhibition of the Immunologic Release of Slow Reacting Substance of Anaphylaxis in the Rat[1] (35512)

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Slow reacting substance of anaphylaxis (SRS-A) has been implicated as a potential mediator of human anaphylactic bronchospasm on the basis of its observed release from human asthmatic lung tissue challenged *in vitro* with specific allergen and its ability to contract isolated human bronchial smooth muscle (1). Previous studies (2, 3) have demonstrated that the antigen-induced intraperitoneal (ip) release of SRS-A in the rat is mediated by two distinct homologous immunoglobulins, IgE and IgGa. The IgE-mediated release of SRS-A and histamine requires the presence of the peritoneal mast cell but not the circulating lymphocyte, polymorphonuclear (PMN) leukocyte, platelet, or an intact complement system. In contrast, the release of SRS-A mediated by the IgGa antibodies in whole hyperimmune serum requires both the PMN leukocyte and an intact complement system, but not the peritoneal mast cell (4). Recent studies (5, 6) have demonstrated that IgE- and IgGa-mediated release of SRS-A are inhibited by agents capable of increasing intracellular levels of adenosine 3′,5′-cyclic monophosphate (cyclic AMP). In view of the demonstrated ability of the prostaglandins to influence the function of the cyclic AMP system in several tissues (7–10), the capacity of these agents to modulate the immunologic release of SRS-A in the rat mediated by IgE and IgGa antibody was studied.

Materials and Methods. Propranolol (Inderal, Ayerst Laboratory, N. Y., N. Y.), isoproterenol (Isuprel, Winthrop Laboratories, N. Y., N. Y.), and aminophylline (G. D. Searle and Co., Chicago, Ill.) were purchased from the manufacturers. The prostaglandins (The Upjohn Co., Kalamazoo, Michigan), diethylcarbamazine citrate (Hetrazan, Lederle Laboratories, Pearl River, N.Y.) and disodium cromoglycate (Intal, Fison's Pharmaceuticals, Loughborough, Leicestershire, England) were gifts of the manufacturers.

Male Sprague-Dawley rats (200–300 g) were prepared by the ip injection of 0.5 ml of IgE-rich antiserum directed against dinitrophenyl (DNP)-keyhole limpet hemocyanin (DNP-KLH) or 0.5 ml of hyperimmune antiserum rich in IgGa antibody directed against DNP-bovine gamma globulin (DNP-BGG) (2). Two hr later, the rats were challenged by the ip injection of 2.0 mg of DNP-bovine serum albumin (DNP-BSA) in 5.0 ml of Tyrode's containing heparin, 50 μg/ml. Five min later, the rats were stunned and exsanguinated; the peritoneal fluid was harvested, centrifuged, and the supernatants were collected and bioassayed for SRS-A (4). Pharmacologic agents were diluted in 1.0 ml of 0.15 *M* saline and injected ip. 30 sec before challenge with specific antigen. All agents used in these studies were administered in concentrations which did not interfere with the bioassay of SRS-A. The results for each dose of each agent tested represent the mean inhibition of SRS-A release observed in 8–12 animals in at least 2–3 separate experiments.

Results. As shown in Fig. 1, pretreatment of rats with varying doses of PGE_1 and PGE_2 supressed the subsequent antigen-induced release of SRS-A mediated by IgE antibody. These two agents were comparable in their

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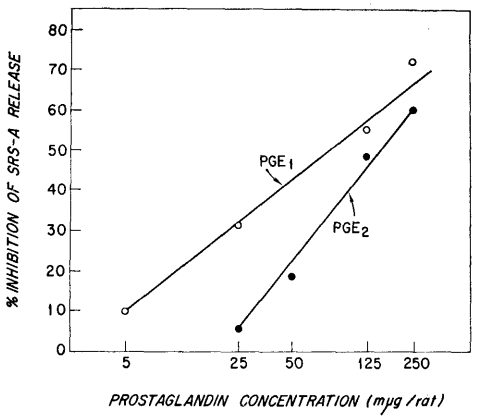


FIG. 1. Inhibition of the IgE-mediated release of SRS-A in the rat by PGE_1 and PGE_2.

inhibitory potency: PGE_1 produced 50% inhibition (ID_{50}) at a dose of 78 mμg/rat and PGE_2 at 160 mμg/rat.

Several additional prostaglandins were investigated for their ability to inhibit the IgE-mediated release of SRS-A to elucidate possible structure–function relationships. Table I summarizes data from several experiments which indicated that PGA_1 and PGA_2 were capable of inhibiting IgE-mediated SRS-A release and that these agents were approximately one-tenth the potency of PGE_1 and PGE_2. PGB_1, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and $PGF_{2\beta}$ were inactive when used at 50 to 100 times the ID_{50} of PGE_1 and PGE_2.

In experiments not shown, the inhibitory potency of the prostaglandins towards the IgGa-mediated release of SRS-A had the same rank order as that observed for the IgE-mediated release of SRS-A. In regard to comparative potency, PGE_1, PGE_2, PGA_1, and PGA_2 were the same as for the IgE-mediated reaction. The activity of PGB_1 was approximately one-tenth that of PGE_1 and PGE_2. $PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGF_{2\beta}$ again possessed no inhibitory activity.

In an effort to elucidate the site of action of PGE_1 in inhibiting the immunologic release of SRS-A, experiments were undertaken in which PGE_1 was combined with agents known to act within the cyclic AMP system. As shown in Table II (Expt. A), the β-adrenergic blocking agent, propranolol, prevented the 61% inhibition of SRS-A release achieved with isoproterenol but had no effect on the 73% inhibition achieved with a high dose of PGE_1 (250 mμg/rat.) As shown in Table II (Expts. B and C), threshold concentrations of aminophylline and isoproterenol yielded synergistic inhibition when employed together; in contrast, these threshold doses of aminophylline and isoproterenol did not increase the inhibition achieved with a threshold dose of 25 mμg/rat of PGE_1.

Previous investigations (2) have established that both diethylcarbamazine and disodium cromoglycate are capable of inhibiting IgE-mediated SRS-A release in the rat. Diethylcarbamazine has been shown to exhibit marked synergism with isoproterenol but not with aminophylline (5). Disodium cromoglycate exhibited no synergism with either agent. PGE_1 failed to act synergistically with either diethylcarbamazine or disodium cromoglycate (Table III).

Discussion. The potency of PGE_1 and PGE_2 in inhibiting the IgE-mediated release of SRS-A in the rat is about one-tenth that previously described for the β-adrenergic agent isoproterenol (5). About a twofold variation in the ID_{50} of PGE_1 was observed

TABLE I. The Capacity of Various Prostaglandins to Inhibit the Immunologic Release of SRS-A in the Rat.

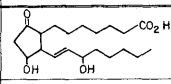
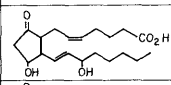
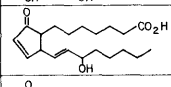
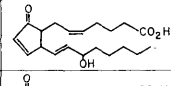
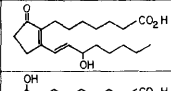
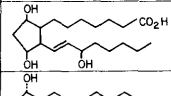
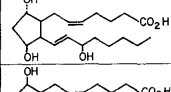
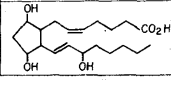
PROSTAGLANDIN	STRUCTURE	DOSE (mμg/rat)	% INHIBITION SRS-A RELEASE
PGE_1		25 250	38 62
PGE_2		25 250	13 63
PGA_1		250 2500	16 67
PGA_2		250 2500	10 61
PGB_1		250 2500	14 17
$PGF_{1\alpha}$		250 2500	5 0
$PGF_{2\alpha}$		250 2500	9 8
$PGF_{2\beta}$		250 2500	14 17

TABLE II. The Effects of Propranolol, Isoproterenol, and Aminophylline on the Inhibition of SRS-A Release Produced by PGE_1.

Expt.	PGE_1 (mμg/rat)	Propranolol (mμg/rat)	Aminophylline (μg/rat)	Isoproterenol (mμg/rat)	Inhibition SRS-A release (%)
A	250	—	—	—	73
	—	42	—	—	13
	—	—	—	8.9	61
	250	42	—	—	71
	—	42	—	8.9	13
B	25	—	—	—	22
	—	—	2.8	—	26
	—	—	—	4.8	0
	25	—	2.8	—	15
	—	—	2.8	4.8	56
C	25	—	—	—	35
	—	—	2.8	—	0
	—	—	—	4.8	16
	25	—	—	4.8	28
	—	—	2.8	4.8	73

in different experiments. The observations presented in Table I indicate that the carbonyl group in the 9 position, the position of a double bond in the cyclopentane ring and either the degree of saturation of the cyclopentane ring or the presence of a hydroxyl group in the 11 position, or both, can influence the inhibitory activity of the prostaglandins.

Recent studies have revealed that agents capable of increasing intracellular levels of cyclic AMP are capable of inhibiting the IgE-mediated release of histamine from human peripheral leukocytes (11, 12) and human lung (13), the IgE- and IgGa-mediated release of SRS-A in the rat (5, 6) and the IgE-mediated release of both histamine and SRS-A from monkey lung fragments (14). PGE_1 has been reported to increase tissue cyclic AMP levels in human platelets (7), rat bone and kidney tissue (8, 10) and the thyroid tissue of the dog (9); PGE_1-induced activation of adenyl cyclase has been demonstrated in platelet (7) and thyroid (9) preparations. In contrast, the decrease in tissue cyclic AMP observed in isolated fat cells incubated with PGE_1 has been attributed to both inhibition of adenyl cyclase and activation of phosphodiesterase (15). Prostaglandin inhibition of *in vitro* biologic reactions such as adenosine diphosphate (ADP)-induced platelet aggregation (7) and phagocytosis by macrophages (16) has been described, and in both systems synergism between agents acting at different sites within the cyclic AMP system was observed. In contrast, in the present *in vivo* studies synergism between PGE_1 and a methylxanthine was not observed (Table II, Expt. B). Further, the failure of the β-adrenergic blocking agent, propranolol, to reverse the inhibition of SRS-A release by PGE_1 (Table II, Expt. A) suggests a site of action of PGE_1 apart from the β-adrenergic receptor. The absence of synergism between PGE_1 and isoproterenol indicates that prostaglandins are not acting by inhibition of cytoplasmic phosphodiesterase.

TABLE III. The Effects of Diethylcarbamazine and Disodium Cromoglycate on the Inhibition of SRS-A Release Produced by PGE_1.

PGE_1 (mμg/rat)	Diethylcarbamazine (mg/rat)	Disodium cromoglycate (mg/rat)	Inhibition of SRS-A release (%)
25	—	—	48
—	0.5	—	18
—	—	1.0	12
25	0.5	—	35
25	0.5	1.0	10

Thus, indirect evidence did not establish that prostaglandin inhibition of SRS-A release is mediated through an increase in tissue cyclic AMP, but the concentrations used were much lower than those described for *in vitro* systems (7, 16). In interpreting these findings, it is well to note that Chase and Aurbach (8) were unable to demonstrate PGE_1-induced activation of adenyl cyclase or inhibition of phosphodiesterase in isolated rat calvaria although the tissue levels of cyclic AMP were increased following incubation with PGE_1. As shown in Table III, PGE_1 does not demonstrate synergistic inhibition of SRS-A release when used in combination with either diethylcarbamazine or disodium cromoglycate. Whether either of these agents significantly antagonizes the inhibitory activity of PGE_1 requires further investigation.

PGE_1 effectively inhibits the immunologic release of SRS-A in the rat and appears to be 10 times more potent than isoproterenol in relaxing the tracheal smooth muscle of the guinea pig *in vivo* (17) and the isolated human bronchial smooth muscle *in vitro* (18). The preliminary studies of Cuthbert (19) have demonstrated that PGE_1 produced comparable improvement in the pulmonary function of asthmatic patients to that observed with 10 times the dose of isoproterenol; whether this effect of PGE_1 is attributable to an action on smooth muscle, the immunologic release of SRS-A, or both, requires further investigation.

Summary. The prostaglandins, PGE_1 and PGE_2, effectively inhibit the IgE- and IgGa-mediated release of SRS-A in the rat in a dose–response fashion. Structure–function studies suggest that the carbonyl group in the 9 position, the position of a double bond in the cyclopentane ring, and either the degree of saturation of the cyclopentane ring or the presence of the hydroxl group in the 11 position, or both, are required for optimal inhibitory activity. Indirect evidence did not establish that prostaglandin inhibition of SRS-A release is mediated through an increase in tissue cyclic AMP levels and no synergism between PGE_1 and diethylcarbamazine or disodium cromoglycate was demonstrable.

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