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EFFECTS OF MODULATORS OF ARACHIDONIC ACID METABOLISM ON THE SYNTHESIS AND RELEASE OF SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS

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1 Slow-reacting substance of anaphylaxis (SRS-A) was released in the peritoneum of passively sensitized rats challenged with ovalbumin and from rat isolated peritoneal cells stimulated with calcium ionophore A23187.

2 Both monocytes (macrophages) and mast cells appear to be involved in the synthesis and release of SRS-A.

3 The immunological release of SRS-A *in vivo* is enhanced by indomethacin and inhibited by dexamethasone, mepacrine, 1-phenyl-3-pyrazolidone (1-P-3-P), and methylimidazole.

4 SRS-A release induced by A23187 *in vitro* is inhibited by dexamethasone, indomethacin, 1-P-3-P, eicosatetraynoic acid (ETA) and 15-hydroperoxy arachidonic acid. The inhibition induced by dexamethasone, indomethacin and 1-P-3-P is reduced by an increase in the calcium concentration from 1 mM to 5 mM, whereas the inhibition induced by ETA is increased.

5 The results suggest that a lipoxygenase is important in the synthesis and release by SRS-A.

Introduction

Slow-reacting substance of anaphylaxis (SRS-A) is released immunologically in the peritoneum of passively sensitized rats (Orange, Valentine & Austen, 1968) and a slow-reacting substance (SRS) by the calcium ionophore A23187 from isolated peritoneal cells (Bach and Brashler, 1974) and cultured basophilic leukaemia cells of the rat (Jakschik, Kulczycki, MacDonald & Parker, 1977). Inhibitors of the cyclo-oxygenase pathway of arachidonic acid metabolism enhance immunological SRS-A release from the lungs of man (Walker, 1972), ox (Burka & Eyre, 1975), and guinea-pig (Liebig, Bernauer & Peskar, 1976). Eicosatetraynoic acid (ETA) a cyclo-oxygenase and lipoxygenase inhibitor, does not modify the immunological release of histamine or SRS-A from guinea-pig lung (Engineer, Niederhauser, Piper & Sirois, 1978). In contrast, ionophore-induced release of SRS from rat cells is prevented by inhibitors of both the cyclo-oxygenase and lipoxygenase pathways and an inhibitor of thromboxane synthesis, azo analogue I, (Bach, Brashler & Gorman, 1977; Jakschik, Falkenheim & Parker, 1977).

Incubation of rat mononuclear or basophilic leukaemia cells with either [^{3}H]- or [^{14}C]-arachidonic acid before challenge with ionophore leads to incorporation of radioactivity into the partially purified SRS (Bach *et al.*, 1977; Jakschik *et al.*, 1977). These workers have postulated on this evidence that ionophore-induced SRS and possibly immunologically-induced SRS-A are metabolites of arachidonic acid. Recent studies in our laboratory have indicated that the slow-reacting substances induced by either stimulus are chromatographically indistinguishable (Blackwell, Burka & Flower, 1978). Therefore, we shall use the term SRS-A so that our results can relate to the classical substance described by Brocklehurst (1960).

The present study defines the effects of various inhibitors of arachidonic acid metabolism on both immunological and ionophore-induced release of SRS-A.

Methods

In vitro *experiments and bioassay*

Male Wistar rats (200 to 250 g) were injected intraperitoneally with 20 ml 0.1% oyster glycogen in isotonic saline 18 to 22 h before being killed with CO_2. Cells were obtained by peritoneal lavage with 5 ml isotonic saline containing heparin (2 u/ml). The peritoneal exudate was centrifuged at 150 *g* for 4 min

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at 4°C and the supernatant discarded. The cells were resuspended in modified Tyrode solution of the following composition (mM): NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 0.3, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.0 and dextrose 5.6. Aliquots (1 ml) of approximately 1–2 × 10^7 cells were prepared and placed in a shaking incubator at 37°C. Drugs were added 15 min before challenge with calcium ionophore A23187, after which incubation was carried out for a further 30 min. Prostaglandin I_2 (PGI_2, prostacyclin) and isoprenaline were added to the cells only 5 min before challenge. After incubation the cells were centrifuged at 300 *g* for 10 min at 4°C and the supernatant assayed for SRS-A activity on a superfused guinea-pig ileum as previously described (Burka & Garland, 1977). All SRS-A assays were referred to an internal standard. For purposes of comparison 10 u SRS-A gave a contraction equivalent to that induced by 2 ng histamine on atropinized guinea-pig ileum. Also, 1 of our units corresponds to 2 units from the laboratory of the late Dr R.P. Orange (Hospital for Sick Children, Toronto, Ontario, Canada). Prostaglandins were measured as PGE equivalents on the superfused rat stomach strip (Vane, 1957). The tissues were superfused with oxygenated (95% O_2 and 5% CO_2) Tyrode solution of the following composition (mM): 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 (μM): hyoscine hydrobromide 0.23, mepyramine maleate 0.24, methysergide bimaleate 0.43, phenoxybenzamine hydrochloride 0.29 and propranolol hydrochloride 0.68.

Other agents tested for SRS-A release in place of A23187 but found to be ineffective were gramicidin (1, 10 and 100 μg/ml), thrombin (20 u/ml), trypsin (10 μg/ml), concanavalin A (5 and 50 μg/ml) the formylated tripeptide, formyl-Met-Leu-Phe (5 and 50 μg/ml) and compound 48/80 (1 and 10 μg/ml).

In vivo *experiments*

The protocol of Burka & Garland (1977) was followed. Briefly, rats pretreated with glycogen were injected with 5 ml antiserum containing IgG_a antibody to ovalbumin 2 h before challenge. Drugs were administered orally simultaneously. Two hours after passive sensitization, 5 ml ovalbumin (400 μg/ml) was injected intraperitoneally into groups of at least four rats. The animals were killed with CO_2 5 min later, the peritoneal fluid aspirated, centrifuged and kept on ice until assayed.

Solubility and sources of drugs

The following drugs and chemicals were used: dexamethasone sodium phosphate (Decadron) and indomethacin (Merck, Sharpe & Dohme); mepacrine hydrochloride and mepyramine maleate (May & Baker); 1-phenyl-3-pyrazolidone, (±)-propranolol hydrochloride, oyster glycogen type II and concanavalin A (Sigma); calcium ionophore A23187 (Lilly); ovalbumin, 5× crystallized (Koch Light); hyoscine hydrobromide (BDH); methysergide bimaleate (Sandoz) and phenoxybenzamine hydrochloride (Smith, Kline & French). Other drugs used were produced by Wellcome. All drugs were dissolved in either isotonic saline or Tyrode solution, except those mentioned below. 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 or 20 mg/ml and diluted with distilled water or buffered salt solutions. Control groups of rats or cells were treated at the same time as the test groups with the same vehicle. The vehicles used did not affect the release and activity of SRS-A, nor did they release SRS-A *per se*. Only mepacrine (100 μg/ml) tended to antagonize the activity of SRS-A. A23187 was dissolved in dimethylsulphoxide (DMSO) to a concentration of 1 mg/ml. The final concentration of DMSO was never more than 1% and had no effect on SRS-A release or activity.

Table 1 SRS-A release under varying conditions: (A) number of cells; (B) concentration of A23187, (C) concentration of Ca^{2+}

A (A23187: 10 μg/ml; Ca^{2+}: 1 mM)

No. of cells	*SRS-A* (*u*) (mean ± s.e.).	n
1 × 10^6	0 ± 0	4
3 × 10^6	228 ± 20	4
1 × 10^7	753 ± 38	4
3 × 10^7	1250 ± 155	4
7 × 10^7	2125 ± 144	4
2 × 10^8	1250	1

B (Ca^{2+}: 1 mM; 2 × 10^7 cells)

A23187 (*μg/ml*)	*SRS-A* (*u*) (mean ± s.e.).	n
1	600 ± 100	3
3	783 ± 93	3
5	1400 ± 115	3
10	1355 ± 152	3

C (A23187: 5 μg/ml; 10^7 cells)

Ca^{2+} (*mM*)	*SRS-A* (*u*) (mean ± s.e.).	n
0	40 ± 33	4
0.2	550 ± 33	4
0.5	620 ± 23	4
1.0	780 ± 69	4
2.0	610 ± 53	4
5.0	580 ± 12	4

Results

In vitro *release of SRS-A*

Optimal release of SRS-A from rat peritoneal cells with calcium ionophore A23187 is achieved at an ionophore concentration of 5 μg/ml and a calcium concentration of 1 mM (Table 1B and C). Pretreatment of rats with 0.1% glycogen increased the number of cells recoverable from the rat peritoneum from 1.08 ($\pm$ 0.10) $\times$ 10^7 cells/rat to 3.63 ($\pm$ 0.48) $\times$ 10^7 cells/rat. SRS-A release is dependent on the number of cells present, but decreases when more than 7 $\times$ 10^7 cells are present (Table 1A). The differential cell count (Table 2) indicates an increase in the proportion of neutrophils and a decrease in mast cells. Treatment of rats with only vehicle (20 ml sterile isotonic saline) also increased the number of cells to 2.43 ($\pm$ 0.30) $\times$ 10^7/rat. The proportion of neutrophils was increased and that of monocytes decreased.

However, when a constant number of cells (2 $\times$ 10^7) from each group was challenged with A23187 (5 μg/ml), twice as much SRS-A was released from the cells of non-treated rats than from those treated with glycogen or saline alone.

Attempts were made to induce SRS-A release with the histamine releasers, compound 48/80 (Uvnas & Thon, 1961), concanavalin A (Siraganian & Siraganian, 1974) and formyl-Met-Leu-Phe (Hook, Schiffman, Aswanikumar & Siraganian, 1976) thrombin and trypsin (two substances that activate cellular release processes) and gramicidin, an antibiotic which changes the structure of cell membranes and allows cation transport. Only compound 48/80 released some SRS-A, but the amount was less than 10% of that released by A23187 (5 μg/ml) under the same conditions. None of the other substances induced any SRS-A release.

Under optimum conditions for SRS-A release (i.e. A23187 5 μg/ml; 1 mM $CaCl_2$), dose-response relationships were examined for the following drugs: dexamethasone (1 to 100 μg/ml) and mepacrine, (1 to 100 μg/ml), both inhibitors of phospholipase A_2 activity (Blackwell, Flower, Nijkamp & Vane, 1978); indomethacin (1 to 100 μg/ml), a cyclo-oxygenase inhibitor (Vane, 1971); 5,8,11,14-eicosatetraynoic acid (ETA) (0.1 to 100 μg/ml), and 1-phenyl-3-pyrazolidone (1-P-3-P) (1 to 100 μg/ml), inhibitors of both cyclo-oxygenase and lipoxygenase (Hamberg and Samuelsson, 1974; Blackwell & Flower, 1978); methylimidazole (100 μg/ml), a thromboxane synthetase inhibitor (Moncada, Bunting, Mullane, Thorogood, Vane, Raz & Needleman, 1977); 15-hydroperoxy arachidonic acid (15-HPAA) (0.1 to 40 μg/ml), a PGI_2 synthetase (I_{50} 0.37 μg/ml) and lipoxygenase inhibitor (I_{50} 0.5 μg/ml) (Gryglewski, Bunting, Moncada, Flower & Vane, 1976; Salmon, unpublished results); 13-hydroperoxy linoleic acid (13-HPLA) (0.1 to 40 μg/ml), a PGI_2 synthetase inhibitor which does not inhibit lipoxygenase in concentrations up to 10 μg/ml (Salmon, unpublished results) (Table 3). PGI_2 and isoprenaline were examined as agents that increase intracellular levels of cyclic AMP, but did not affect SRS-A release at concentrations up to 3 μg/ml.

Dexamethasone, indomethacin, ETA, 1-P-3-P and 15-HPAA all inhibited SRS-A release (IC_{50}s: 85, 15, 10, 3 and 25 μg/ml respectively). An IC_{50} for mepacrine could not be obtained as mepacrine (100 μg/ml) antagonized SRS-A on the guinea-pig ileum. 13-HPLA only inhibited SRS-A release by 21 $\pm$ 5% (mean $\pm$ s.e., $n = 5$, $P < 0.01$) at the highest concentration used (40 μg/ml). Methylimidazole (100 μg/ml)

Table 2 (A) Differential counts of peritoneal cells and (B) total numbers of cells recovered from the peritoneum and A23187-induced SRS-A release 18 h after injection of saline or glycogen intraperitoneally.

A

Treatment	*Neutrophils*	*Lymphocytes*	*Monocytes*	*Eosinophils*	*Mast Cells*	n
Control	3 ± 1	9 ± 4	65 ± 5	15 ± 3	8 ± 3	4
20 ml saline	23 ± 4**	13 ± 4	47 ± 3**	15 ± 4	4 ± 1	4
20 ml 0.1% glycogen	25 ± 6**	10 ± 4	51 ± 6	13 ± 1	0.3 ± 0.3*	4

B

Treatment	*Total cells/rat*	n	*SRS-A released* (u/2 $\times$ 10^7 cells)	n
Control	10.8 (±1.0) $\times$ 10^6	8	3500 ± 611	3
20 ml saline	24.3 (±3.0) $\times$ 10^6***	9	1640 ± 147***	5
20 ml 0.1% glycogen	36.3 (±4.8) $\times$ 10^6***	5	1740 ± 73***	5

All values are mean ± s.e. mean.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

slightly enhanced SRS-A release by 22 ± 3% (mean ± s.e., $n = 4$, $P = 0.05$). 15-HPAA (0.1 and 1.0 μg/ml) also enhanced SRS-A release by 15 ± 4% and 16 ± 3% (mean ± s.e., $n = 4$, $P < 0.05$) respectively, although higher concentrations effectively inhibited release. None of the other drugs enhanced SRS-A release at the concentrations examined.

Prostaglandin release by A23187 (5 μg/ml) was approximately 65 ng/ml (measured as PGE_2 equivalents). When prostaglandin release was examined in the presence of the drugs at their IC_{50} concentrations for inhibition of SRS-A release, indomethacin and TYA inhibited release completely, whereas 1-P-3-P and 15-HPAA had no effect. Dexamethasone and mepacrine (100 μg/ml) inhibited prostaglandin release by 51 and 59% respectively.

The inhibition of SRS-A release induced by indomethacin, 1-P-3-P and dexamethasone could be reduced by increasing the calcium concentration and that by dexamethasone (100 μg/ml) was significantly ($P < 0.005$) reduced from 55 ± 4% (mean ± s.e., $n = 8$) to 11 ± 18% (mean ± s.e., $n = 4$) (Figure 1). In contrast, the inhibition induced by ETA was significantly ($P < 0.0025$) increased from 50 ± 5% (mean ± s.e., $n = 8$) to 81 ± 4% (mean ± s.e., $n = 4$) on increasing the calcium concentration from 1 mM to 5 mM.

Changing the calcium concentration did not alter the effects of the drugs on prostaglandin release. Indomethacin and ETA (10 μg/ml) totally inhibited the production of PGE_2-like activity. In contrast 1-P-3-P (3 μg/ml) did not inhibit PGE_2-like production.

In vivo *experiments*

Indomethacin (1 to 100 mg/kg), 1-P-3-P (20 to 100 mg/kg), dexamethasone (0.01 to 40 mg/kg), mepacrine (10 to 100 mg/kg), methylimidazole (10 to 100 mg/kg) were all administered orally to the rats 2 h before challenge with antigen. Indomethacin enhanced SRS-A release, but the dose-response curve was bell-shaped with an optimum at 3 mg/kg (Figure 2). In contrast, 1-P-3-P, dexamethasone, mepacrine and methylimidazole all inhibited SRS-A release (Figure 3). Release of PGE-like activity was also inhibited by these agents.

Table 3 Effect of some inhibitors of arachidonic acid release and metabolism on the immunological *in vivo* and A23187-induced *in vitro* release of SRS-A

Drug	ID_{50} in vivo (*mg/kg*)	IC_{50} in vitro (*μg/ml*)
Dexamethasone	10	85
Mepacrine	60	not obtainable
Indomethacin	enhancement	15
1-P-3-P	55	3
ETA	ND*	10
15-HPAA	ND	25
13-HPLA	ND	>40
Methylimidazole	20	>100

* ND = not done; 1-P-3-P = 1-phenyl-3-pyrazolidine; ETA = eicosatetraynoic acid; 15-HPAA = 15-hydroperoxy arachidonic acid; 13-HPLA = 13-hydroperoxy linoleic acid.

Discussion

SRS-A can be released from rat peritoneal cells both immunologically and with the calcium ionophore A23187. The particular cell type is still not known and more than one cell type may be involved in the mechanisms of stimulus, synthesis and release of SRS-A (Bach & Brashler, 1974; Orange, 1977). Although pretreatment of rats with glycogen increases the incidence of neutrophils, SRS-A release on a constant cell basis does not rise, but rather falls by approximately 50%. The fall coincides with a fall in the incidence of monocytes (macrophages) and mast cells. The present evidence suggests that the monocytes account for the production of 50% of the SRS-A in non-treated rats and mast cells account for the other 50%, which disappears when the rats are pretreated with glycogen. The advantage of using glycogen is the 3 fold increase in the number of cells per rat that it induces. For this reason, Orange *et al.* (1968) used glycogen-treated rats to produce SRS-A *in vivo*. The higher yield in rats pretreated with glycogen appears to be due to the increased number of cells present in the peritoneum, rather than to the proliferation of a particular cell type. An interaction between different cell types may also be an explanation (Bach & Brashler, 1974). Therefore it appears that Orange's (1977) proposition that SRS-A release from mast cells is mediated by IgE, from neutrophils by IgG_a, and from macrophages by calcium ionophore is an oversimplification.

The immunological release of SRS-A from rat peritoneal cells passively sensitized with rat IgG_a antibody occurs only *in vivo*. Attempts to induce SRS-A release from these same cells with antigen *in vitro* were unsuccessful (Bach & Brashler, 1974). However, *in vitro* release from SRS-A can be obtained by means of the calcium ionophore A23187 (Bach & Brashler, 1974; these results). It was not possible to induce SRS-A release with a number of other chemical agents that are known to induce histamine release from isolated mast cells.

We do not understand why A23187 is unique in being able to induce SRS-A release *in vitro* and the mechanisms of release are also incompletely understood. Foreman, Mongar & Gomperts (1973) showed

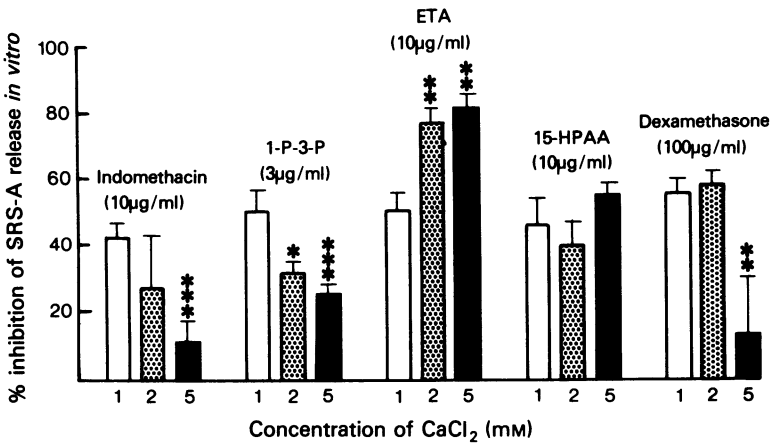

Figure 1 Effects of calcium concentrations (open columns: 1 mM; stippled columns: 2 mM; solid columns: 5 mM) on the inhibition of A23187-induced SRS-A release by indomethacin, 1-phenyl-3-pyrazolidine (1-P-3-P), eicosatetraynoic acid (ETA), 15-hydroperoxy arachidonic acid (15-HPAA) and dexamethasone. Results are the mean of at least 4 experiments in each group. Vertical lines indicate s.e. means. $*P < 0.05$; $**P < 0.005$; $***P < 0.0005$.

that histamine release from rat mast cells by A23187 was accompanied by an influx of calcium ions, which is identical to what occurs during immunological release (Lichtenstein, 1975). Both release processes are also energy- and temperature-dependent (Lichtenstein, 1975). The major difference between the two mechanisms is that ionophore-induced release of histamine is independent of modulation by changes in the intracellular levels of cyclic adenosine 3'5'-monophosphate (cyclic AMP). The present results confirm these findings, for isoprenaline or PGI_2, both of which increase intracellular levels of cyclic AMP (Lichtenstein & Margolis, 1968; Tateson, Moncada & Vane, 1977), are incapable of modulation of A23187-induced SRS-A release, but do inhibit the immunological release of SRS-A (Burka & Garland, 1977).

The results also indicate that modulation by some inhibitors of arachidonic acid metabolism differ between two types of mediator release. The most conspicuous difference was observed with the cyclo-oxygenase inhibitor, indomethacin. Indomethacin enhanced the immunological release of SRS-A. However the enhancement did not exceed 55% and the variance was high. This is in contrast to enhancement as great as 300 to 400% observed with indomethacin in perfused guinea-pig lung (Engineer *et al.*, 1978). In contrast to the enhancement observed *in vivo*, Indomethacin inhibited A23187-induced SRS-A release *in vitro*. ETA and 1-P-3-P, both cyclo-oxygenase and lipoxygenase inhibitors (Blackwell & Flower, 1978), also inhibited SRS-A release. These results suggest that either SRS-A is a product of arachidonic metabolism via the cyclo-oxygenase or lipoxygenase pathways, or that the above inhibitors are also capable of inhibiting other enzymes responsible for the synthesis of SRS-A. That a product of radioactive arachidonic acid co-chromatographs with partially purified SRS-A (Bach *et al.*, 1977; Jakschik *et al.*, 1977) is not formal proof that SRS-A is an arachidonate metabolite, but it does lend some support to the hypothesis. If SRS-A is a product of the cyclo-oxygenase it does not follow the PGI_2 pathway, as 13-HPLA an inhibitor of PGI_2 synthesis (Salmon, unpublished results), does not modulate SRS-A release. 15-HPAA, also an inhibitor of PGI_2 synthesis (Gryglewski *et al.*, 1976) inhibited SRS-A release, but 15-HPAA also inhibits lipoxygenase (Salmon, unpublished results). Methylimidazole, an inhibitor of thromboxane synthesis (Moncada *et al.*, 1977) does modulate SRS-A release. This is in contrast to the inhibition of SRS-A release by the thromboxane synthesis inhibitor, azo analogue I (Bach *et al.*, 1977). However, it is not known if azo analogue I also inhibits lipoxygenase. Methylimidazole (IC_{50}: 20 mg/kg) inhibits SRS-A release *in vivo*. However, it is unlikely that SRS-A is produced by thromboxane synthetase *in vivo* as the release of PGE-like material is also inhibited at the same concentration as SRS-A is inhibited. The present evidence suggests that a lipoxygenase is important in the synthesis and release of SRS-A.

Free arachidonic acid is liberated from the bound form by the action of phospholipase A_2. If SRS-A is indeed derived from arachidonic acid, one would expect that substances that inhibit the action of phospholipase A_2, such as steroids and mepacrine (Blackwell *et al.*, 1978), would inhibit SRS-A release. The present results indicated that both these agents inhibit

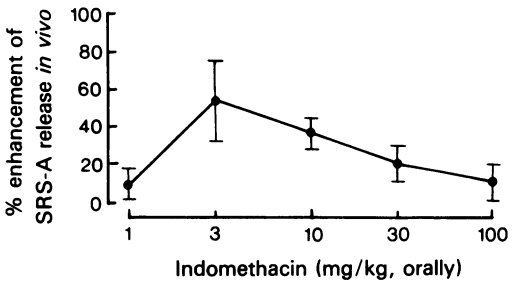

Figure 2 Enhancement (%) of SRS-A release into the peritoneal fluid following challenge with ovalbumin (400 μg/ml) 2 h after oral administration of indomethacin. Results are the means of at least 4 rats. Vertical lines indicate s.e. means.

SRS-A release *in vitro* at concentrations similar to those required to inhibit phospholipase A_2 activity (Blackwell *et al.*, 1978). Dexamethasone and mepacrine also inhibit SRS-A and prostaglandin release *in vivo*.

Walker (1972), Engineer *et al.* (1978), and others have attempted to explain the enhancement of SRS-A release obtained with aspirin-like drugs by the inhibition of the endogenous prostaglandins that are released secondary to the primary mediators of anaphylaxis. This enhancement would not be observed *in vitro* with A23187 as this model is independent of modulation by cyclic AMP. However, if this explanation is true, then one would expect that 1-P-3-P, dexamethasone and mepacrine would all enhance SRS-A release *in vivo*, as they all inhibit prostaglandin release. In fact, they all inhibit SRS-A release. It is possible that a lipoxygenase product is enhancing SRS-A release. Engineer *et al.* (1978) showed that ETA, while decreasing the levels of $PGF_{2\alpha}$-like activity, did not alter the release of SRS-A or histamine from perfused guinea-pig lung. To confirm that 1-P-3-P was not acting as a metabolic inhibitor in rat peritoneal cells, histamine release was also monitored *in vivo*. 1-P-3-P did not alter histamine release (unpublished data). It is interesting that *in vitro*, at the IC_{50} for 1-P-3-P for SRS-A release (3 μg/ml), prostaglandin release is not inhibited. Therefore, the enhancement observed with aspirin-like drugs is still not completely explained.

Lewis & Whittle (1977) demonstrated that the inhibition of the A23187-induced histamine release by aspirin-like drugs could be reversed by increasing the calcium concentration of the incubation medium.

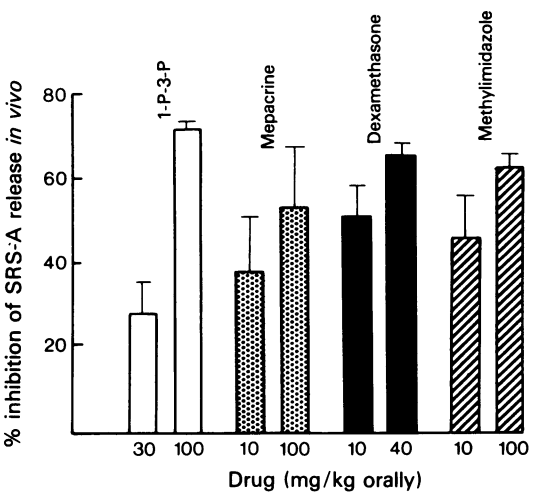

Figure 3 Inhibition (%) of SRS-A release into the peritoneal fluid following challenge with ovalbumin (400 μg/ml) 2 h after oral administration of 1-phenyl-3-pyrazolidine (1-P-3-P), mepacrine, dexamethasone and methylimidazole. Results are the means of at least 4 rats. Vertical lines indicate s.e. means.

Similar results were obtained with some of the drugs used in these experiments: indomethacin, 1-P-3-P, and dexamethasone. In contrast, the inhibition by ETA was increased when the calcium concentration was raised. The inhibition by 15-HPAA was not changed by alteration of the calcium concentration. Mepacrine was not tested, as concentrations greater than 30 μg/ml interfered with SRS-A-induced contractions of the guinea-pig ileum. Lewis & Whittle (1977) proposed that aspirin-like drugs may inhibit histamine release by either inhibiting calcium uptake by the cells, or inhibiting the mobilization of bound calcium within the cell. The first proposal is unlikely for A23187-induced release, as the ionophore itself is capable of moving calcium into the cell (Foreman *et al.*, 1973). The second proposal seems a strong possibility as SRS-A synthesis, both immunologically or ionophore-induced, is calcium-dependent (Kaliner & Austen, 1973; Bach & Brashler, 1974; this study). The data suggest that some of the drugs that affect arachidonic acid metabolism are capable of controlling the levels of free and bound calcium within the cell. Further experiments must be carried out to confirm this hypothesis and determine its significance.

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