Br. J. Pharmac. (1977), **61**, 229–235

THE INHIBITION OF HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS BY NON-STEROID ANTI-INFLAMMATORY DRUGS AND ITS REVERSAL BY CALCIUM

G.P. LEWIS & B.J.R. WHITTLE[1]

Department of Pharmacology, Institute of Basic Medical Sciences,
Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN

1 The non-steroid anti-inflammatory drugs, indomethacin, flufenamate and meclofenamate, inhibited the release of histamine from rat peritoneal mast cells induced by pharmacological or immunological challenge *in vitro*.

2 Anti-inflammatory steroids had little effect on histamine release from the mast cells.

3 Th inhibition of histamine release by the aspirin-like drugs was not prevented by incubation with glucose, unlike the inhibition of 2,4,dinitrophenol or antimycin-A. This suggests that the non-steroid anti-inflammatory compounds do not act by preventing the energy production from oxidative metabolism, required for histamine release.

4 The inhibition of the calcium ionophore A23187-induced histamine release by the aspirin-like drugs was reversed by an increase in the calcium concentration of the incubation medium.

5 The results suggest that the non-steroid anti-inflammatory compounds inhibit histamine release by actions on calcium influx into the mast cell, or on calcium mobilization or utilization within the mast cell.

Introduction

Primary prostaglandins of the E series and their analogues can inhibit the release of histamine from rat peritoneal mast cells *in vitro* induced by pharmacological or immunological challenge (Loeffler, Lovenberg & Sjoerdsma, 1971; Kaliner & Austen, 1974; Thomas & Whittle, 1976). To define a possible role for endogenous mast cell prostaglandins in the histamine release process, the effects of non-steroid anti-inflammatory agents, known to inhibit prostaglandin biosynthesis (Vane, 1971), were investigated. However, these drugs, like the primary prostaglandins studied, were found to reduce histamine release from the mast cells (Thomas & Whittle, 1976). In the present paper, possible mechanisms by which these aspirin-like drugs inhibit histamine release have been investigated.

A preliminary account of this work has been presented to the British Pharmacological Society (Whittle, 1976).

[1] Present address: Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Methods

Mast cells were obtained by lavage of the rat peritoneal cavity with a modified buffered medium (pH7) containing bovine serum albumin (0.1% w/v). Unless otherwise stated, the lavage solution had the following composition (mM):NaCl 154, KCl 2.7, $CaCl_2$ 0.9, buffered with Na_2HPO_4 4.5 and KH_2PO_4 2.2. For experiments using higher calcium concentrations, the concentration of the phosphate buffers was reduced (by 50%) to avoid precipitation. In studies with 'calcium-free' medium, analar water (BDH; containing less than 1 part per million calcium) was used to dissolve the above salts.

Male Wistar rats (300–400 g, body weight) were lightly anaesthetized with ether, decapitated and exsanguinated, and the buffered-albumin medium (14 ml) slowly injected intraperitoneally. After a 2 min massage of the rat abdomen, the peritoneal cavity was opened by a mid-line incision and the fluid (10 ml) was transferred to a plastic tube and centrifuged (approximately 200 *g* for 3 minutes). The supernatant was discarded and the cell pellet resuspended in the buffer-albumin medium (2.5 ml). Aliquots (0.1 ml) were distributed to small plastic tubes containing albumin-

free buffered solution (0.1 ml) and the drug under investigation (0.1 ml). Following the pre-incubation (at 22°C or 37°C where appropriate), the histamine-liberating agent (0.1 ml) was added and the cells further incubated. The incubation was terminated by addition of the cold albumin-free buffered solution (0.5 ml at 4°C) and the tubes centrifuged (700 *g* for 5 minutes).

Aliquots (0.1 ml) of the supernatant were transferred to plastic vials (Sterilin Ltd.) containing distilled water (2ml) and the histamine content was assayed by alkaline condensation (0.4 ml 1 N NaOH) with O-phthaladehyde (10 mg/ml in methanol; 0.1 ml) followed by acidification (0.2 ml 3 HCl after 4 min) and measurement of the fluorescence (excitation 350 nm, emission 440 nm; Shore, Burkhalter & Cohn, 1959). Histamine concentrations determined by this fluorometric method were in good agreement with values obtained by bioassay on the superfused guinea-pig ileum (5 experiments). In the concentrations employed in the study, none of the drugs interfered with this fluorometric histamine assay.

In any experiment using the batch of mast cells from one rat, each experimental sequence and drug treatment was performed at least in duplicate, and each experiment was repeated with 5 or more batches of mast cells.

The number of mast cells in the resuspended cell pellet was counted in a haemocytometer, following staining of a sample of the cell suspension with a toluidine-blue solution (Bray & Van Arsdel, 1961).

Rats were sensitized by subcutaneous administration of the antigen, either egg albumin (50 mg) or inactivated horse serum (0.5 ml), together with administration of *B. pertussis* suspension (0.5 ml s.c.; 4×10^{10}/ml, Wellcome Reagents Ltd.). The rats were re-injected with the antigen alone on the second day, and the animals used 14–28 days later.

Drugs

The histamine liberator compound 48/80, Triton X-100 (Sigma Chemical Co.) and *n*-decylamine (Aldrich Chemical Co.) were stored in distilled water (4°C) and freshly diluted to the desired concentration with albumin-free buffered medium (at least a 100-fold dilution was used to avoid possible artifacts). Adenosine triphosphate (Sigma Chemical Co.), crude phospholipase-A (*Vipera russellii* venom, Koch-light Labs.) and the antigens, inactivated horse serum (Wellcome Reagents Ltd.) or egg albumin (fraction *V*, Sigma Chemical Co.) were dissolved directly in the buffered medium. The non-steroid anti-inflammatory agents indomethacin (Merck, Sharp & Dohme, Ltd.), meclofenamic acid and flufenamic acid (Parke Davis & Co.) were dissolved directly in the buffered medium; the use of sodium bicarbonate solution to increase solubility was avoided to obviate any possible interference. The anti-inflammatory steroids dexamethasone sodium phosphate (Merck, Sharp & Dohme, Ltd.) and betamethasone sodium phosphate (Glaxo) were dissolved in a similar fashion, as were the other compounds, 2,4,dinitrophenol and glucose (BDH).

Antimycin-A (Sigma Chemical Co.) and the ionophore-A23187 (Lilly Research Centre Ltd.) were stored in absolute ethanol or methanol and diluted (at least 100-fold) with the buffered solution immediately before use; the final alcohol content so obtained had no detectable effect on the mast cells.

Statistical analysis

Results are expressed as mean ± standard error of the mean, where (*n*) represents the number of values in the group. The significance of difference between the grouped data was evaluated by Student's *t* test for paired or unpaired values, where appropriate. $P < 0.05$ was taken as significant.

Results

Characteristics of mast cell suspensions

The number of mast cells in the resuspended mixed peritoneal cell pellet was $3.5-6 \times 10^{-6}$/2.5 ml ($n = 56$) with a total histamine content (following cell disruption with perchloric acid, 0.4 M) of 30–60 μg/2.5 ml, i.e. 8.3 ± 0.4 μg histamine/10^6 mast cells.

Histamine release from mast cells

The low resting release of histamine (1–3% of the total histamine content) from the mast cells could be readily increased in a dose-dependent manner by incubation with the histamine liberator, compound 48/80 (0.05–1 μg/ml, 5 min at 22°C or 37°C) or adenosine triphosphate (50–400 μg/ml; 5 min at 37°C for optimal release). The release induced by the crude phospholipase-A from *Vipera russellii* (2.5–20 μg/ml; 10 min at 37°C for optimal release) may be related to the presence of a lytic factor since phospholipase-A from three other sources had little histamine-releasing activity (Brain, Lewis & Whittle, 1977). The dose-release relationship with the calcium ionophore A23187 (0.125–0.5 μg/ml; 5 min at 22°C or 37°C) was relatively steep, as was that observed with non-specific liberators, Triton X-100 (50–200 μg/ml) and *n*-decylamine (20–80 μg/ml).

Inhibition of histamine release by non-steroid anti-inflammatory agents

Samples of cell suspension were pre-incubated at room temperature with indomethacin (0.5–80 μg/ml for

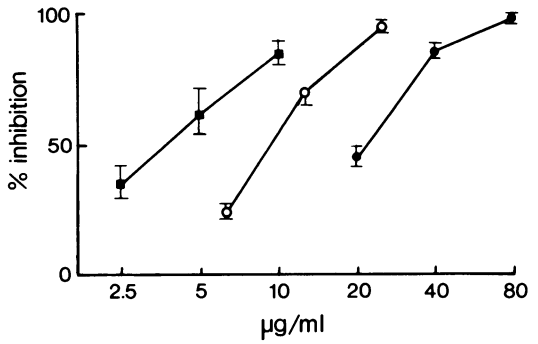

Figure 1 Inhibition of compound 48/80-induced histamine release from rat peritoneal mast cells by non-steroid anti-inflammatory drugs: (■) meclofenamate; (○) flufenamate; (●) indomethacin. Results are shown as the mean of at least 5 experiments for each group. Vertical lines show s.e. mean.

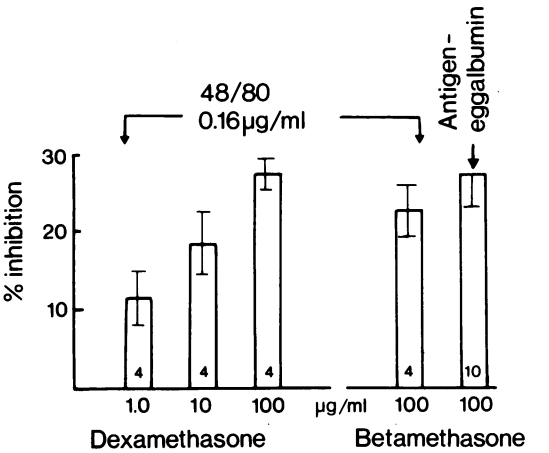

Figure 2 Effects of anti-inflammatory steroids on the release of mast cell histamine induced by compound 48/80 and the antigen, egg albumin. Results are shown as mean of at least 4 experiments in each group. Vertical lines show s.e. mean.

5 min), followed by addition of compound 48/80 (0.16 μg/ml) in a dose giving 30–50% release of the total histamine in the mast cells in the control experiments. The lower concentrations of indomethacin (0.6–5 μg/ml) had no effect on the histamine release, whereas higher concentrations (10–80 μg/ml) caused a dose-dependent inhibition of release (Figure 1). Indomethacin (0.6–80 μg/ml) had no significant effect on the low resting histamine release (5 experiments).

In a further 6 experiments, indomethacin (20 μg/ml) gave a parallel displacement of the dose-release curve with compound 48/80 (0.1–0.8 μg/ml). In other experiments, the effects of temperature and the duration of pre-incubation with indomethacin (10 μg/ml) were studied. Inhibition of 48/80-induced release was comparable at either 22°C or 37°C ($23 \pm 6\%$ and $21 \pm 7\%$ inhibition respectively, $n=5$) and increasing the period of pre-incubation from 5 min to 20 min had little effect on the degree of inhibition. For subsequent experiments, a 5 min pre-incubation period was therefore used.

The non-steroid anti-inflammatory agents meclofenamic acid and flufenamic acid also caused a similar dose-dependent inhibition of 48/80-induced histamine release (Figure 1); the doses causing 50% inhibition of submaximal histamine release (ID_{50}) were 3.5 and 9.0 μg/ml respectively, compared with an ID_{50} of 21.5 μg/ml for indomethacin. As shown in Table 1, pre-incubation with the aspirin-like drugs also inhibited submaximal histamine release induced by adenosine triphosphate (200 μg/ml), by crude phospholipase-A (5 μg/ml), by the calcium ionophore A23187 (0.3 μg/ml) and by antigenic challenge with egg albumin (1000 μg/ml), and also with inactivated horse serum (1 in 500 final dilution with buffered medium). In these experiments, the aspirin-like drugs were more potent as inhibitors of ionophore A23187-induced histamine release (Table 1). In contrast, these drugs had no consistent effect on non-specific histamine liberation by the surface-active agents, Triton X-100 (0.01%) or *n*-decylamine (25 μg/ml).

Table 1 Inhibition of histamine release from rat peritoneal mast cells

Histamine liberator	*(μg/ml)*	*Indomethacin*	*Meclofenamate*
Compound 48/80	(0.16)	21.5	3.5
Ionophore-A23187	(0.3)	7.0	1.5
Phospholipase-A	(5.0)	13.5	2.0
Adenosine triphosphate	(200)	10.0	3.0
Antigen-egg albumin	(1000)	13.0	6.5

The concentration of inhibitor (μg/ml) giving 50% reduction of histamine release (ID_{50}), following 5 min pre-incubation with mast cells, was determined from at least 5 experiments. Control histamine release, with the concentration of each liberator shown, was 30–50% of total histamine content of the cells.

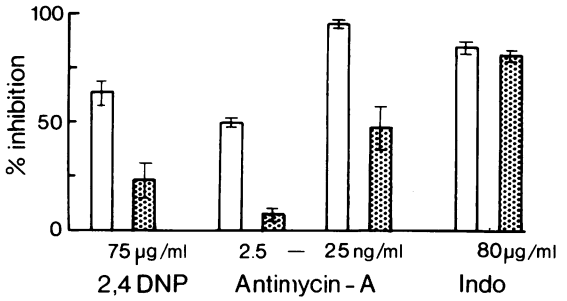

Figure 3 Effects of pre-incubation with glucose (5 mM for 10 min) on the inhibition of compound 48/80-induced histamine release by 2,4,dinitrophenol (2,4 DNP), antimycin-A and indomethacin (Indo). Stippled columns—preincubation with glucose; open columns—no preincubation with glucose. Results are the mean of 5 experiments in each group. Vertical lines show s.e. mean.

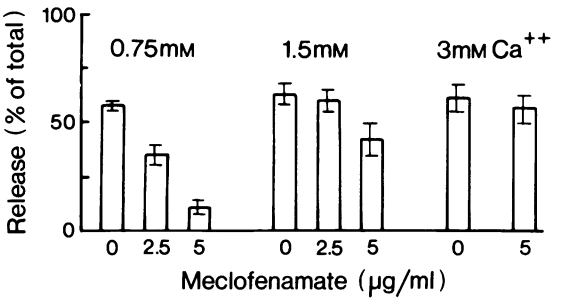

Figure 4 Effects of calcium concentration (0.75–3 mM) on the inhibition of ionophore A23187-induced histamine release by meclofenamate. Results, shown as histamine release, % of total histamine in the mast cells, are the mean of at least 4 experiments in each group. Vertical lines show s.e. mean.

Effects of anti-inflammatory steroids

As shown in Figure 2, the anti-inflammatory steroids dexamethasone (1–100 μg/ml) and betamethasone (1–100 μg/ml) in high concentrations, caused a small inhibition of submaximal histamine release induced by compound 48/80 and the antigen, egg albumin (4 experiments in each group). In other experiments, the histamine release induced by crude phospholipase-A was also inhibited by dexamethasone (by $39.6 \pm 4.3\%$, $n=7$; with 100 μg/ml).

Effects on oxidative metabolism

The inhibitory effects of the aspirin-like drugs on histamine release were compared with those of drugs known to interfere with oxidative metabolism. Submaximal histamine release induced by compound 48/80 (0.2 μg/ml) was inhibited in a dose-dependent manner by 2,4,dinitrophenol (50–100 μg/ml) or antimycin-A (2.5–25 ng/ml) (Figure 3). Pre-incubation of the mast cells with medium containing glucose (1–5 mM) for 10 min, significantly ($P<0.01$, in each case) reversed this inhibition (Figure 3). In contrast, glucose pre-incubation had no significant ($P<0.05$) effect on the inhibition of histamine release by indomethacin (20–80 μg/ml) or meclofenamate (5–20 μg/ml).

In a further 5 experiments, the inhibition of ionophore A23187 (0.3 μg/ml)-induced histamine release by antimycin-A (0.6 ng/ml) was reduced by pre-incubation with glucose-(1 mM)-containing medium (from $39 \pm 9\%$ to $15 \pm 10\%$ inhibition; $P<0.01$). The inhibition with a higher dose of antimycin-A (6 ng/ml) was likewise reduced by pre-incubation in medium containing glucose (from $90 \pm 3\%$ to $71 \pm 5\%$ inhibition; $n=5$). However, there was no marked effect on the inhibition by meclofenamate (5 μg/ml; $51 \pm 3\%$ and $43 \pm 5\%$ inhibition, with and without glucose respectively).

Another difference between the two classes of compound was that whereas the dose of the 'metabolic inhibitor' required to prevent histamine release could vary greatly (up to 10-fold) from month to month, the inhibitory concentrations of the aspirin-like drugs remained constant in experiments carried out over one year.

Effects of calcium concentration

Initial experiments confirmed previous observations (Foreman, Mongar & Gomperts, 1973) that the release of histamine by ionophore A23187 was dependent not only on the dose (0.1–0.5 μg/ml) but also on the calcium concentration (0.5–3 mM) of the incubation medium. Optimal histamine release, with a submaximal dose of the ionophore (0.3 μg/ml), was obtained with a calcium concentration of 0.75 mM; an increase in the calcium concentration up to 3 mM did not elevate the release further (Figure 4). The dose-dependent inhibition of histamine release by meclofenamate (2.5–5 μg/ml) or indomethacin (10–40 μg/ml) was related to the calcium concentration of the incubation medium (Figure 4). In four experiments, the inhibition of release by meclofenamate (5 μg/ml) was significantly reduced (from $81 \pm 3\%$ to $8 \pm 4\%$ inhibition, $P<0.001$) when the calcium concentration was increased from 0.75 mM to 3 mM (Figure 4). Similarly, the inhibition of ionophore-induced histamine release by indomethacin (15 μg/ml) was reduced (from 62 ± 4 to $21 \pm 4\%$, $P<0.01$) by increasing the calcium concentration from 0.75 mM to 1.5 mM.

This reversal by calcium was not a non-specific

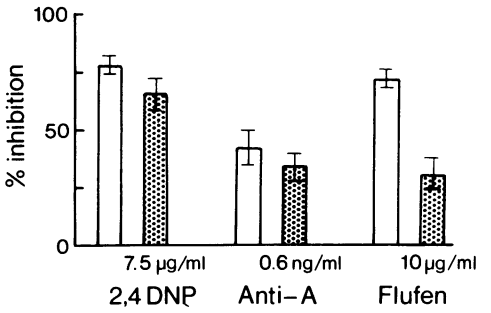

Figure 5 Effects of calcium concentration (open columns 1 mM; stippled columns 2 mM) on the inhibition of ionophore A23187-induced histamine release by 2,4,dinitrophenol (2,4 DNP), antimycin-A (Anti-A) and flufenamate (Flufen). Results are the mean of 5 experiments in each group. Vertical lines show s.e. mean.

action since in the 5 experiments shown in Figure 5, the inhibition of ionophore-induced histamine release by 2,4,dinitrophenol (7.5 µg/ml) or antimycin-A (0.6 ng/ml) was not significantly altered, whereas the inhibition by flufenamate (10 µg/ml) was considerably reduced by the increased calcium concentration (from 1 to 2 mM; $P < 0.001$).

In an initial series of experiments to investigate the effects of calcium lack on histamine liberation was compound 48/80, the histamine release induced in 'calcium-free' buffered medium was also inhibited by indomethacin ($45 \pm 8\%$, $n=7$; with 40 µg/ml) and meclofenamate ($41 \pm 4\%$, $n=4$; with 5 µg/ml).

Discussion

Prostaglandins of the E series inhibit anaphylactic histamine release *in vitro* from rat peritoneal mast cells (Kaliner & Austen, 1974; Thomas & Whittle, 1976) and from chopped human lung (Walker, 1973). This *in vitro* action which follows incubation with exogenous prostaglandins could reflect a pathophysiological modulator role for prostaglandins *in vivo* during anaphylaxis. The finding that low concentrations of non-steroid anti-inflammatory agents which inhibit prostaglandin formation (Vane, 1971), can augment the release of histamine and slow reacting substance of anaphylaxis (SRS-A) from isolated perfused lung of guinea-pig following challenge with antigen (Engineer, Piper & Sirois, 1976), again suggests that prostaglandins can modulate the anaphylactic release of histamine and SRS-A. In contrast, in the present study, low concentrations of the aspirin-like drugs had no effect on histamine release from rat mast cells, suggesting that endogenous mast-cell prostaglandins are not involved in the histamine release process. It is possible, therefore, that in the perfused lung, the prostaglandins are released from cells other than those which release histamine or SRS-A. These latter mediators, released during challenge, may lead to prostaglandin generation, which could then act through a 'negative feed-back mechanism' to modulate the further release of the mediators.

The findings that non-steroid anti-inflammatory drugs could inhibit the release of histamine from rat peritoneal mast cells induced by a variety of stimuli support the observations that indomethacin reduces rat mast cell degranulation (Taylor, Francis, Sheldon & Roitt, 1974) and histamine release from human lung *in vitro* (Walker, 1973). In earlier studies, several other aspirin-like compounds, such an phenylbutazone or sodium salicylate were found to inhibit anaphylactic histamine release *in vitro* from rat skin (Mota & Ishii, 1960), from rat mast cells (Norn, 1965) and from chopped guinea-pig lung (Mongar & Schild, 1957), although high concentrations were used. However, inhibition of compound 48/80-induced histamine release from rat mast cells by non-steroid anti-inflammatory drugs, in a similar concentration range as in the present study, has been reported (Yamasaki & Saeki, 1967).

In the present work, the doses of aspirin-like drugs required to inhibit histamine release were somewhat greater than those which should be required to inhibit prostaglandin biosynthesis, and thus these effects are probably not related to effects on prostaglandin formation. However, it should be pointed out that the presence of albumin in the incubation medium would be expected to reduce the concentration of free aspirin-like drugs which are known to bind avidly to albumin. The ability of these drugs to inhibit histamine release from mast cells following challenge with several pharmacological agents and antigen, but not that induced by non-specific surface-active agents, suggests an action on some common mechanism involved in the release process.

Since agents which can elevate cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in mast cells have been shown to inhibit histamine release (Loeffler *et al.*, 1971; Taylor *et al.*, 1974; Kaliner & Austen, 1974), it is possible that the non-steroid anti-inflammatory compounds, by virtue of their ability to inhibit phosphodiesterase (Newcombe, Thanassi & Ciosek, 1974) were acting by a similar mechanism. It is also of interest that in the present study, the steroid anti-inflammatory agents caused a small inhibition of histamine release induced by compound 48/80 and immunological challenge, as was found for antigen-evoked histamine release from human skin (Greaves & Plummer, 1974), since it has been suggested, from studies on human lymphocytes, that such compounds can likewise increase cyclic AMP levels by phosphodiesterase inhibition (Lavin, Rachelefsky & Kaplan, 1975). However, the aspirin-like drugs were very potent inhibitors of histamine release induced by the ionophore A23187, suggesting that other mechanisms

may also operate; an increase in cyclic AMP levels, either by phosphodiesterase inhibitors or dibutyryl analogue of cyclic AMP, is not thought to prevent ionophore-induced histamine release (Garland & Mongar, 1976).

It was possible that these non-steroid anti-inflammatory compounds could affect the processes of oxidative metabolism involved in histamine release. Several such compounds have been shown, for example, to uncouple oxidative phosphorylation *in vitro* in rat liver mitochondria (Whitehouse, 1964). However, in the present study, addition of glucose to the incubation medium reversed the inhibitory effects of 2,4,dinitrophenol (which uncouples oxidative phosphorylation) or antimycin-A (which acts on the cytochrome system) by promoting anaerobic glycolysis (Peterson, 1974; Diamant, Grosman, Stahl Skov & Thomle, 1974), but had no effect on the inhibition by meclofenamate or indomethacin. This agrees with previous observations that glucose had little effect on the inhibition of histamine release by aspirin-like drugs compared with its effects on the inhibition by several metabolic inhibitors (Yamasaki & Saeki, 1967). The present findings strongly suggest, therefore, that these non-steroid anti-inflammatory drugs, in the concentrations used, do not interfere with the energy production from oxidative metabolism required for the histamine release process.

The finding that increased calcium concentrations could overcome the inhibition of ionophore-stimulated histamine release by aspirin-like drugs suggests that they act on a calcium-dependent stage of the release process. The entry of calcium into the mast cell is known to be an important stage in the release process (Foreman *et al.*, 1973) and certain 'anti-allergic' drugs, such as disodium cromoglycate, which inhibit histamine release, may act by preventing this calcium movement (Foreman, Mongar, Gomperts & Garland, 1975). It is therefore pertinent that several aspirin-like drugs have been shown to interfere with the uptake and binding of calcium in various tissues (Northover, 1973). Thus, inhibition of histamine release, following challenge with a variety of agents, by the non-steroid anti-inflammatory drugs *in vitro* could reflect actions on calcium influx into the mast cell. However, the process of calcium entry into the mast cell following ionophore-stimulation appears to differ from that following stimulation with antigen, in that it is not altered by cromoglycate or phosphodiesterase inhibitors (Foreman, Hallett & Mongar, 1977); and histamine release by compound 48/80 may not depend on the movement of extracellular calcium (see Cochrane & Douglas, 1974). It is therefore possible that aspirin-like drugs affect calcium mobilization or utilization within the mast cell. This is further supported by the present observations that the histamine release induced by compound 48/80 in calcium-free medium, which is thought to involve movement of intracellular calcium (Cochrane & Douglas, 1974) could also be inhibited by the aspirin-like drugs.

It is not known whether such actions could occur *in vivo* with anti-inflammatory doses of the aspirin-like drugs. However, it may be expedient to be aware of such a potential interaction between non-steroid anti-inflammatory agents and calcium when interpreting the actions of such drugs *in vitro* on any calcium-sensitive system.

This work was supported by the M.R.C. The calcium ionophore A23187 was kindly supplied by the Lilly Research Centre. We thank R.U. Thomas for excellent technical assistance.

References

BRAIN, S., LEWIS, G.P. & WHITTLE, B.J.R. (1977). Actions of phospholipase-A on mast-cell histamine release and paw oedema in the rat. *Br. J. Pharmac.*, **59**, 440–441P.

BRAY, R.E. & VAN ARSDEL, P.P. (1961). *In vitro* histamine release from rat mast cells by chemical and physical agents. *Proc. Soc. exp. Biol. Med.*, **106**, 255–269.

COCHRANE, D.E. & DOUGLAS, W.W. (1974). Calcium-induced extrusion of secretory granules (exocytosis) in mast cells exposed to 48/80 or ionophores A-23187 or X-537A. *Proc. natn. Acad. Sci., U.S.A.*, **71**, 408–412.

DIAMANT, B., GROSMAN, N., STAHL SKOV, P. & THOMLE, S. (1974). Effect of divalent cautions and metabolic energy on the anaphylactic histamine release from rat peritoneal mast cells. *Int. Arch. Allergy exp. Immun.*, **47**, 412–424.

ENGINEER, D.M., PIPER, P.J. & SIROIS, P. (1976). Interaction between the release of SRS-A and of prostaglandins. *Br. J. Pharmac.*, **57**, 460–461P.

FOREMAN, J.C., HALLETT, M.B. & MONGAR, J.L. (1977). Site of action of the antiallergic drugs cromoglycate and doxantrazole. *Br. J. Pharmac.*, **59**, 473–474P.

FOREMAN, J.C., MONGAR, J.L. & GOMPERTS, B.D. (1973). Calcium ionophores and the movement of calcium ions following the physiological stimulus to a secretory process. *Nature, Lond.*, **245**, 249–251.

FOREMAN, J.C., MONGAR, J.L., GOMPERTS, B.D. & GARLAND, L.G. (1975). A possible role for cyclic AMP in the regulation of histamine secretion and the action of cromoglycate. *Biochem. Pharmac.*, **24**, 538–540.

GARLAND, L.G. & MONGAR, J.L. (1976). Differential histamine release by dextran and the ionophore A23187: The actions of inhibitors. *Int. Arch. Allergy appl. Immun.*, **50**, 27–42.

GREAVES, M.W. & PLUMMER, V.M. (1974). Glucocorticoid inhibition of antigen-evoked histamine release from human skin. *Immunology*, **27**, 359–364.

KALINER, M. & AUSTEN, K.F. (1974). Cyclic AMP, ATP, and reversed anaphylactic histamine release from rat mast cells. *J. Immun.*, **112**, 664–674.

LAVIN, N., RACHELEFSKY, G. & KAPLAN, S.A. (1975). Inhibition of cyclic-AMP phosphodiesterase in human lymphocytes by physiological concentrations by hydrocortisone. *Horm. Metab. Res.*, **7**, 253–258.

LOEFFLER, L.J., LOVENBERG, W. & SJOERDSMA, A. (1971). Effects of dibutyryl-3′,5′-cyclic adenosine monophosphate, phosphodiesterase inhibitors and prostaglandin E_1 on compound 48/80-induced histamine release from rat peritoneal mast cells *in vitro*. *Biochem. Pharmac.*, **20**, 2287–2297.

MONGAR, J.L. & SCHILD, H.O. (1957). Inhibition of the anaphylactic reaction. *J. Physiol., Lond.*, **135**, 301–319.

MOTA, I. & ISHII, T. (1960). Inhibition of mast cell disruption and histamine release in rat anaphylaxis in vitro. Comparison with compound 48/80. *Br. J. Pharmac.*, **15**, 82–87.

NEWCOMBE, D.S., THANASSI, N.M. & CIOSEK, C.P. (1974). Cartilage cyclic nucleotide phosphodiesterase: inhibition by anti-inflammatory agents. *Life Sci.*, **14**, 505–519.

NORN, S. (1965). Influence of antirheumatic agents on the release of histamine from rat peritoneal mast cells after an antigen-antibody reaction. *Acta pharmac. tox.*, **22**, 369–378.

NORTHOVER, B.J. (1973). Effect of anti-inflammatory drugs on the binding of calcium to cellular membrane in various human and guinea-pig tissues. *Br. J. Pharmac.*, **48**, 496–504.

PETERSON, C. (1974). Histamine release induced by compound 48/80 from isolated rat mast cells: Dependence on endogenous ATP. *Acta pharmac. tox.*, **34**, 356–367.

SHORE, P.A., BURKHALTER, A. & COHN, V.H. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmac. exp. Ther.*, **127**, 182–186.

TAYLOR, W.A., FRANCIS, D.H., SHELDON, D. & ROITT, I.M. (1974). Anti-allergic actions of disodium cromoglycate and other drugs known to inhibit cyclic 3′,5′-nucleotide phosphodiesterase. *Int. Archs Allergy appl. Immun.*, **47**, 175–193.

THOMAS, R.U. & WHITTLE, B.J.R. (1976). Prostaglandins and the release of histamine from rat peritoneal mast cells. *Br. J. Pharmac.*, **57**, 474–475P.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

WALKER, J.L. (1973). The regulatory role of prostaglandins in the release of histamine and SRS-A from passively sensitized human lung tissue. In *Advances in the Biosciences*, **9**, ed. Bergstrom, S. & Bernhard, S. pp. 235–240. Braunschweig: Pergamon Press.

WHITEHOUSE, M.W. (1964). Uncoupling of oxidative phosphorylation by some acrylactic acids (anti-inflammatory or hypocholesterolemic drugs). *Nature, Lond.*, **201**, 629–630.

WHITTLE, B.J.R. (1976). Calcium and the inhibition of histamine release from rat peritoneal mast cells by non-steroid anti-inflammatory agents. *Br. J. Pharmac.*, **58**, 446P.

YAMASAKI, H. & SAEKI, K. (1967). Inhibition of mast-cell degranulation by anti-inflammatory agents. *Arch. int. Pharmacodyn. Ther.*, **168**, 166–179.

(Received January 25, 1977.)