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# Effects of Isoproterenol on Histamine Release Induced from Monodispersed Guinea-Pig Lung Cells by Different Secretagogues[1,2]

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## ABSTRACT

The effects of isoproterenol on histamine release induced by specific antigen (ovalbumin), a calcium ionophore (A23187) and a phorbol ester 4β-phorbol-12β-myristate-13α-acetate (TPA) were examined using passively sensitized, monodispersed guinea-pig lung cells containing 2 to 5% mast cells. Ovalbumin evoked histamine release in a manner dependent on added calcium. Isoproterenol inhibited this release and the inhibition was not overcome by increasing the concentration of calcium. The small amount of histamine release above spontaneous release induced by ovalbumin in the absence of added calcium was not altered by isoproterenol. Incubating the cells with ovalbumin in the absence of added calcium resulted in a time-dependent desensitization of histamine release induced by subsequent exposure to calcium. Isoproterenol did not affect either the rate or the maximum magnitude of antigen desensitization. Antigen-induced histamine release at maximum desensitization was not inhibited by isoproterenol. A23187 induced histamine release in a manner dependent on added calcium. This dependency was inversely related to the concentration of A23187. Isoproterenol had no affect on either the rate or magnitude of histamine release by A23187 regardless of the concentration of added calcium. TPA evoked histamine release in a manner independent of added calcium. Isoproterenol did not alter the rate or magnitude of histamine release induced by TPA. Both forskolin ($10^{-5}$ M) and dibutyryl cyclic AMP ($10^{-3}$ M) inhibited ovalbumin-induced histamine release, but neither substance altered A23187-induced release. Forskolin also failed to alter TPA-induced histamine release. These results suggest that *beta* adrenergic-mediated inhibition of immunologic histamine release from guinea-pig lung cells is a result of a reduction of the selective increase in mast cell membrane calcium flux evoked by antigen-antibody interaction and that this may be a cyclic AMP-dependent effect.

*Beta* adrenergic receptor agonists are known to inhibit immunologic release of histamine from a variety of sources, including guinea-pig, human and monkey lung (Schild, 1936; Assem and Schild, 1969; Ishizaka *et al.*, 1971), isolated human lung mast cells (Schulman *et al.*, 1982) and human leukocyte preparations (Lichtenstein and Margolis, 1969). Although the inhibitory effect is suggested to be secondary to an increase in mast cell cyclic AMP, there is little known about subsequent events in the cascade leading to histamine release inhibition. That cyclic AMP modulates release is supported by the circumstantial evidence that many other substances that are known to increase cellular cyclic AMP content, including cyclic AMP (or its dibutyryl analog), phosphodiesterase inhibitors, prostaglandin $E_2$, histamine (*via* $H_2$ receptors), cholera toxin and forskolin, are also capable of inhibiting immunologic histamine release (Bourne *et al.*, 1972; Foreman, 1981; Undem and Buckner, 1984a).

Several observations implicate an important role of calcium flux across the mast cell membrane in immunologic histamine release as well as cyclic AMP-dependent inhibition of release. Lichtenstein and De Bernardo (1971) found that agents which increase cyclic AMP, including *beta* agonists, act to inhibit histamine release from leukocytes by inhibiting the initial signal formation step (which is calcium-independent) evoked by antigen-antibody interaction. At the same time, these agents had little effect on the calcium-dependent secretory step occurring subsequent to transduction of the antigen-antibody signal. Consistent with this observation, Foreman *et al.* (1977) presented evidence to suggest that cyclic AMP in rat peritoneal mast cells inhibits immunologic histamine release by impeding the selective increase in cell membrane permeability to calcium evoked by antigen-antibody interaction. This hypothesis has been challenged, however, by the findings that cyclic AMP

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**ABBREVIATIONS:** TPA, 4β-phorbol-12β-myristate-13α-acetate; DMSO, dimethyl sulfoxide.

derivatives, as well as antiallergic drugs, are effective inhibitors of histamine release from rat peritoneal mast cells evoked by antigen in the absence of added calcium and by a calcium ionophore (Pearce and Truneh, 1981; White and Pearce, 1982, 1983).

There have been relatively few studies on *beta* adrenergic-mediated inhibition of histamine release and the calcium dependence of that effect in guinea-pig lung mast cells although several studies concerned with characterization of the receptors have appeared (Assem and Schild, 1971; Malta and Raper, 1975; Sorenby, 1975; Barrett-Bee and Lees, 1978; Wong and Buckner, 1980; Undem and Buckner, 1984b). In this study, experiments were designed to obtain information on the potential mechanisms of the *beta* adrenergic response. For this purpose, a monodispersed guinea-pig lung cell preparation was used in order to reduce intraexperimental variation and to obtain more accurate kinetic information on histamine release.

## Methods

Female, albino guinea pigs (Bio-Lab, St. Paul, MN), weighing 250 to 350 g, were sacrificed by a sharp blow to the head and exsanguinated *via* the carotid artery. The lungs from one or two animals were removed and perfused *via* the trachea and pulmonary artery with 50 ml of a modified Tyrode's solution consisting of (millimolar): NaCl, 137; KCl, 2.6; $NaH_2PO_4$, 0.36; $MgCl_2$, 1; $CaCl_2$, 1; glucose, 5.5; $NaHCO_3$, 11.9; and containing gelatin, 1 g/l. The pH of the solution was adjusted to 7.4 using 1 N hydrochloric acid. The lungs were trimmed of large blood vessels, airways and extraneous tissue and minced finely with scissors. The lung fragments were digested using four consecutive 30-min exposures to collagenase (125 U/g of tissue) and elastase (10 U/g of tissue). After each enzyme treatment, freed cells were collected by filtering the lung preparation through a Nytex mesh of 100 $\mu$ pore size (Tetko, Elmsford, NY). After the fourth enzyme treatment, the residual tissue was expressed through a 20-ml plastic syringe to free the remaining loosely bound cells which were also collected by filtration. The harvested cells were analyzed for mast cell content by the alcian blue staining method of Gilbert and Ornstein (1975). The cells obtained from the last two enzyme treatments contained the largest number of mast cells (approximately $10^6$/g) at the highest purity (2–5%) and these fractions were combiend for use in the histamine release experiments.

**Histamine release studies.** The cells obtained from the enzymatic digestions were washed two times with 50 ml and resuspended in 1.5 ml of modified Tyrode's solution. For passive sensitization, 1.5 ml of serum obtained from guinea pigs actively sensitized to ovalbumin (Undem and Buckner, 1984b) were added to the cells which were then incubated for 60 min at 37°C in a Dubnoff metabolic incubator (with shaking). The cells were then washed once with 50 ml and resuspended in sufficient modified Tyrode's solution to make 40,000 to 70,000 mast cells per ml. When extracellular calcium was to be diminished, the cells were washed first with 50 ml of calcium-free modified Tyrode's containing 0.4 mM EDTA (Mongar and Schild, 1958) and then with 50 ml of calcium-free modified Tyrode's without EDTA before resuspension. One-milliliter aliquots of the cells suspensions were equilibrated for 5 min at 37°C (with shaking) in plastic test tubes before subsequent drug treatment. All drugs were added to the cell suspensions in 10 $\mu$l volumes.

In preliminary studies, it was found that isoproterenol, forskolin and dibutyryl cyclic AMP produced maximum inhibition of ovalbumin-induced histamine release when added to the cells 5 min before antigen. Therefore, this time period of pre-exposure with these substances was used for all subsequent experiments. When indomethacin was used, it was added to the modified Tyrode's solution after the cells were sensitized passively. A23187 produced maximum histamine release after 30 min of exposure and maximum histamine release induced by TPA usually occurred after 60 min ($n = 4$). These time periods were usually used as the time of exposure for the secretagogues. Maximum histamine release produced by ovalbumin usually occurred after 5 min of exposure, but because a small additional amount of histamine was occasionally seen in samples analyzed during the period between 5 and 30 min, the latter time was chosen for antigen exposure. The time course for histamine release by ovalbumin was not different at added calcium concentrations of $10^{-4}$ and $10^{-3}$ M. Preliminary studies also demonstrated that maximum inhibition of antigen-induced histamine release was obtained with isoproterenol concentrations of $10^{-6}$ or $10^{-5}$ M, consistent with previous findings using guinea-pig minced lung fragments (Undem and Buckner, 1984b).

When examining the effect of isoproterenol on antigen desensitization, the *beta* agonist was added to the cells suspended in calcium-free modified Tyrode's solution 5 min before ovalbumin challenge. At specified times after ovalbumin addition, $CaCl_2$ ($10^{-3}$ M) was added and the reactions allowed to continue for 30 min. In experiments examining the dependence of histamine release on added calcium, specified concentrations of $CaCl_2$ were added to each cell sample in a volume of 10 $\mu$l 5 min before challenge.

The histamine release reaction was terminated at specified times by adding 1 ml of ice-cold modified Tyrode's solution and placing the sample in an ice bath. The samples were then centrifuged and the supernatants analyzed for histamine content by automated fluorometry (Siraganian, 1974). In each experiment, at least two samples received no secretagogue and were used to obtain spontaneous release values. A separate spontaneous release determination was made for each treatment variation. Total histamine content was determined by measuring the amount of histamine released after exposure of two samples from each batch to 0.4 N perchloric acid. The percentage of histamine release in each sample was calculated by dividing that amount in the supernatant by the total content and correcting for spontaneous release. The spontaneous histamine release averaged 4.5 ± 0.5% (S.E.M., $n$ = 28) and was not altered by any treatment variation. The total histamine content ranged from 3.5 to 7 pg/mast cell. None of the drugs or reagents used in this study interfered with the histamine fluorometric assay at the concentrations utilized.

Cell viability was determined after most experiments by trypan blue exclusion. The number of cells which excluded trypan blue was always greater than 93% and was not affected by either ovalbumin or TPA. A23187 (1 $\mu$g/ml) resulted in a larger than 25% decrease in the number of cells which excluded trypan blue in two of nine experiments. Data from these experiments were not included in the analyses. At the lower concentrations of A23187, no decrease in trypan blue exclusion was observed.

**Analyses.** The percentage inhibition of histamine release was calculated as [1 − (percentage of histamine release in the presence of inhibitor/percentage of histamine release in the absence of inhibitor)] ×100. In desensitization experiments, percentage of desensitization was calculated as [1 − (percentage of histamine release after desensitization/percentage of histamine release without desensitization)] ×100. The concentration of added calcium required to evoke 50% of the maximum histamine release ($EC_{50}$) under each experimental condition was obtained visually from a plot of percentage of histamine release *vs.* log calcium concentration, converted to the negative logarithm and expressed as a −log molar $EC_{50}$ value. Means and S.E.M.s were calculated for data obtained in each experimental series. Differences between means were determined using analysis of variance or Student's $t$ test for paired data and $P < .05$ was considered statistically significant.

**Drugs, reagents and solutions.** The following were used in these studies: chicken egg albumin (ovalbumin, grade V), (−)-isoproterenol-(+)-bitartrate, collagenase (type I), elastase (type I), TPA, $N^6,O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP) and indomethacin (Sigma Chemical Co., St. Louis, MO); and A23187 and forskolin (CalBiochem, San Diego, CA).

Stock solutions of forskolin ($10^{-2}$ M), TPA ($10^{-2}$ M) and A23187 (10 mg/ml) were prepared in DMSO and stored at −20°C. Dilutions from these stock solutions were made with 0.9% sodium chloride on the day of each experiment. DMSO did not alter spontaneous or antigen-

induced histamine release at the concentrations used in these experiments. Dilutions of isoproterenol were made on the day of each experiment from $10^{-2}$ M refrigerated stock solutions prepared in 0.9% sodium chloride with 0.05% sodium metabisulfite. Indomethacin was dissolved in 95% ethanol such that the final concentration of ethanol in the physiological salt solution was 0.0095%. Other drugs were prepared in 0.9% sodium chloride and final concentrations are usually expressed in molar strengths.

## Results

**Ovalbumin-induced histamine release.** Ovalbumin released histamine from the lung cells in a manner dependent on added calcium. At the optimum added calcium concentration ($10^{-3}$ M), the ovalbumin $-\log EC_{50}$ (milligrams per milliliter) was 5.2 ± 0.1 and the maximum histamine release was 20 ± 2% of total ($n$ = 4). The concentration-response curve for ovalbumin in the monodispersed lung cell preparation is about 2 log U to the left of that obtained using minced lung (Wong and Buckner, 1980; Undem and Buckner, 1984b). With the monodispersed cells, maximum histamine release occurred with $10^{-3}$ mg/ml of ovalbumin and this concentration was used in all subsequent experiments.

The calcium dependence of ovalbumin-induced release of histamine in the absence and presence of isoproterenol is illustrated in figure 1. Isoproterenol depressed the calcium concentration-response curve in a manner similar to that seen against ovalbumin at a fixed calcium concentration (Wong and Buckner, 1980; Undem and Buckner, 1984b). The ability of isoproterenol to inhibit the calcium-dependent release of histamine by ovalbumin was not overcome by increasing the concentration of added calcium (fig. 1). In six of eight experiments, ovalbumin induced measurable histamine release above spontaneous release in the absence of added calcium. This histamine release was not altered by isoproterenol (fig. 1).

In two additional experiments, isoproterenol ($10^{-5}$ M) inhibited ovalbumin-induced histamine release an average of 46% (48 and 44%) and 42% (40 and 44%) in the absence and presence of indomethacin, $5 \times 10^{-6}$ M, respectively.

**A23187-induced histamine release.** The calcium ionophore, A23187, released histamine from lung cells in a manner dependent on added calcium. Concentration-response effects of calcium chloride obtained in the presence of three concentrations of A23187 can be found in figures 2 and 4. The −log

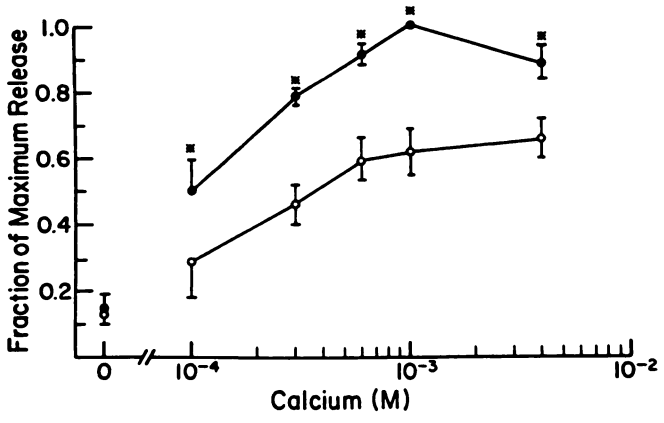


Fig. 1. Log concentration-response effects of calcium chloride in releasing histamine from monodispersed guinea-pig lung cells in the presence of ovalbumin ($10^{-3}$ mg/ml). Values are expressed as a fraction of the maximum release (16 ± 2%) obtained in each paired experiment. Histamine release at zero added calcium represents the average of six experiments and each point on the curves are the averages of five experiments. All values were corrected for spontaneous histamine release which was independent of calcium chloride concentration. An asterisk indicates a statistically significant difference between values obtained in the absence (●) vs. the presence (○) of isoproterenol ($10^{-5}$ M) and vertical lines represent S.E.M.

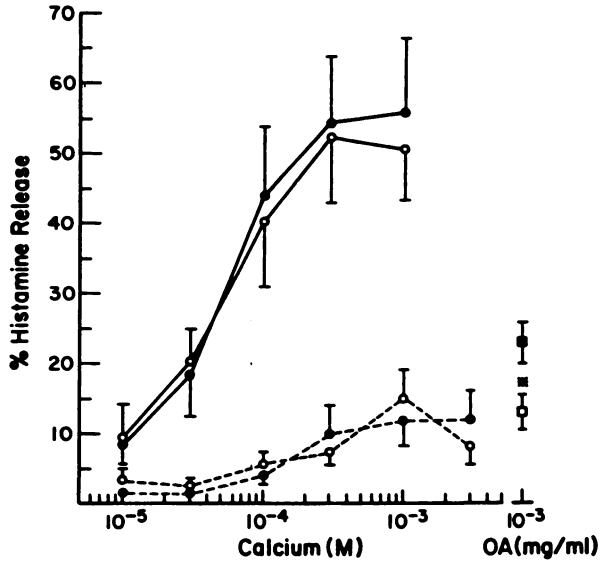


Fig. 2. Log concentration-response effects of calcium chloride in releasing histamine from monodispersed guinea-pig lung cells in the presence of A23187 [1 μg/ml (——) and 0.1 μg/ml (- - -)]. In the presence of A23187 (1 μg/ml) the −log molar $EC_{50}$ values for calcium chloride were 4.3 ± 0.1 and 4.2 ± 0.2 and the maximum histamine release values were 56 ± 10 and 57 ± 8% without (●) and with (○) isoproterenol ($10^{-5}$ M), respectively. In the presence of A23187, (0.1 μg/ml), the respective values without and with isoproterenol were 3.4 ± 0.2 and 3.5 ± 0.2 and 14 ± 3 and 10 ± 2%. The effect of isoproterenol ($10^{-5}$ M) (□) on ovalbumin (OA)-induced release (■) in the presence of $10^{-3}$ M calcium chloride in paired experiments is also illustrated. Each point represents the average of five experiments and vertical lines indicate S.E.M. An asterisk denotes a statistically significant difference between values obtained with and without isoproterenol.

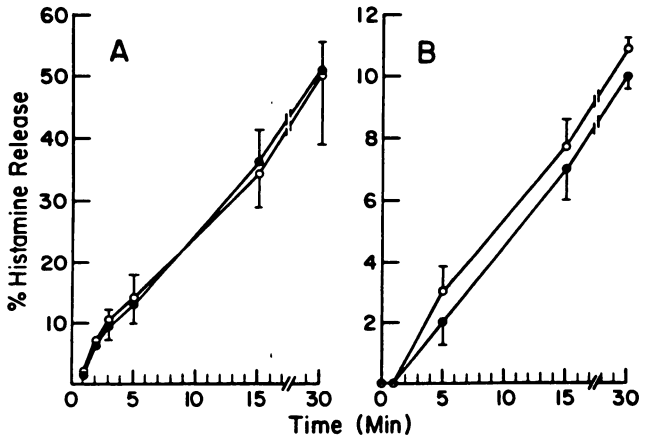


Fig. 3. Time-response effects of A23187 (1 μg/ml) (A) and TPA ($10^{-7}$ M) (B) for release of histamine from monodispersed guinea-pig lung cells in the absence (●) and presence (○) of isoproterenol ($10^{-5}$ M). The calcium chloride concentration in all experiments was $10^{-3}$ M. Each point represents the average of three (A) or four (B) experiments and vertical lines indicate S.E.M.

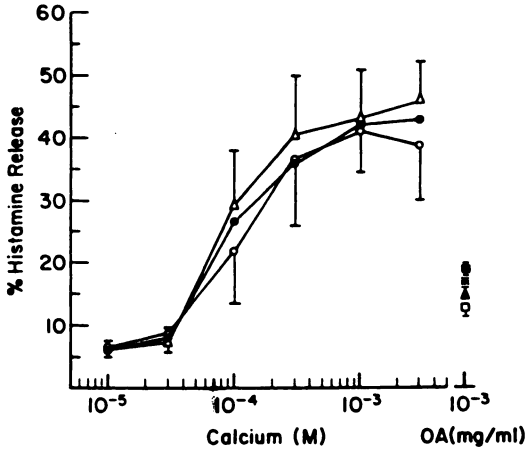


**Fig. 4.** Log concentration-response effects of calcium chloride in releasing histamine from monodispersed guinea-pig lung cells in the presence of A23187 (0.3 μg/ml). The respective −log molar $EC_{50}$ and maximum histamine release values for calcium chloride were 3.9 ± 0.2 and 44 ± 10% without (●), 3.9 ± 0.2 and 42 ± 12% with forskolin ($10^{-5}$ M) (○) and 4.0 ± 0.2 and 49 ± 15% with dibutyryl cyclic AMP ($10^{-3}$ M) (△). Also illustrated are the effects of the same concentrations of forskolin (□) or dibutyryl cyclic AMP (▲) on ovalbumin (OA)-induced histamine release (■) in the presence of $10^{-3}$ M calcium chloride in paired experiments. Each point represents the average of three experiments and vertical lines indicate S.E.M. The asterisk denotes that there was a statistically significant difference between values obtained in control *vs.* those obtained in the presence of forskolin and of dibutyryl cyclic AMP in OA experiments.

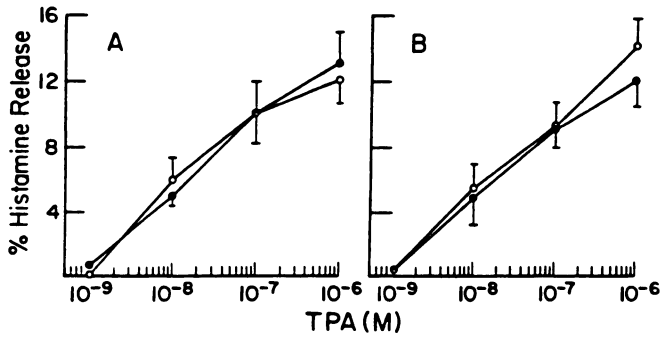


**Fig. 5.** Log concentration-response effects of TPA in releasing histamine from monodispersed guinea-pig lung cells in the presence (A) or absence (B) of added calcium chloride ($10^{-3}$ M) without (●) or with (○) isoproterenol ($10^{-5}$ M). Each point represents the average of four experiments and vertical lines indicate S.E.M. In three paired experiments with $10^{-3}$ M calcium chloride, ovalbumin ($10^{-3}$ mg/ml)-induced histamine release was 21 ± 9 and 8 ± 2% in the absence and presence of isoproterenol ($10^{-5}$ M), respectively ($P < .05$).

molar $EC_{50}$ and maximum histamine release values for calcium chloride were dependent on the concentration of A23187.

Isoproterenol did not alter A23187-induced histamine release regardless of the concentration or time of exposure to A23187 or the concentration of added calcium (figs. 2 and 3A). At concentrations which inhibited ovalbumin-induced histamine release, neither forskolin ($10^{-5}$ M) nor dibutyryl cyclic AMP ($10^{-3}$ M) altered histamine release induced by 0.3 μg/ml of A23187 regardless of the concentration of added calcium (fig. 4).

**TPA-induced histamine release.** Concentration-response effects of TPA in the absence and presence of added calcium are illustrated in figure 5. TPA caused histamine release from lung cells in a concentration-dependent manner between $10^{-9}$ and $10^{-6}$ M. Concentrations larger than $10^{-6}$ M were not examined. The histamine release induced by TPA was independent of added calcium. The apparent −log molar $EC_{50}$ values for TPA in the presence and absence of added calcium ($10^{-3}$ M) were 7.8 ± 0.2 and 7.8 ± 0.2, respectively. The responses to TPA ($10^{-6}$ M) were 12 ± 1 and 13 ± 2% histamine release without and with added calcium, respectively. The time course for TPA-induced histamine release was slow, requiring more than 1 min of exposure before measurable release was observed (fig. 3B).

Isoproterenol did not alter TPA-induced histamine release in the absence or presence of added calcium (figs. 3B and 5). In two additional experiments, forskolin ($10^{-5}$ M) had no effect on histamine release induced by $10^{-6}$ M TPA in the presence of $10^{-3}$ M calcium chloride. Histamine release averaged 12% (12 and 12%) and 12% (12 and 12%) in the absence and presence of forskolin, respectively. In paired samples, forskolin produced a 28% (15 and 40%) inhibition of ovalbumin-induced histamine release.

**Ovalbumin-induced desensitization.** Incubation of the lung cells with ovalbumin in the absence of added calcium resulted in a time-dependent decrease of histamine release induced by subsequent exposure to calcium chloride (fig. 6). The histamine releasing capacity of calcium was reduced approximately 90% by a 10-min exposure to ovalbumin ($10^{-3}$ mg/ml) in calcium-free solution. In two paired experiments, the magnitude of histamine release evoked by A23187 (1 μg/ml) in the presence of $10^{-3}$ M calcium chloride was the same without (49%) and with (49%) a 10-min pre-exposure to ovalbumin. Isoproterenol reduced the magnitude of antigen desensitization observed after 1 min, but had no effect on the maximum magnitude of desensitization or on the time required to reach 50% of the maximum degree of desensitization (fig. 6). The residual histamine release after maximum desensitization was not significantly different from that observed in the absence of added calcium (fig. 1) and was not inhibited by isoproterenol.

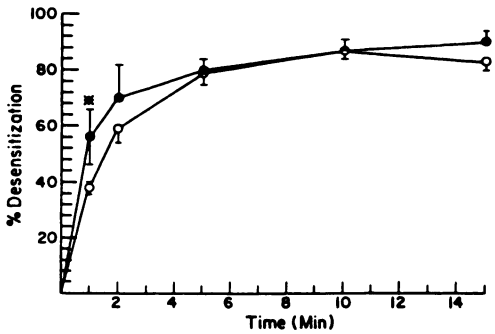


**Fig. 6.** Desensitization of histamine release from monodispersed guinea-pig lung cells by incubation with ovalbumin ($10^{-3}$ mg/ml) for fixed time periods in calcium-free modified Tyrode's solution before addition of calcium chloride ($10^{-3}$ M). Histamine released at zero time represents that obtained by addition of calcium chloride 5 min before ovalbumin under each experimental condition. In the respective absence (●) and presence (○) of isoproterenol ($10^{-5}$ M), control histamine release (no desensitization) values were 23 ± 3 and 10 ± 1% ($P < .01$), percentage of desensitization values at maximum desensitization were 92 ± 3 and 87 ± 6% ($P > .05$), histamine release values after maximum desensitization were 3 ± 1 and 2 ± 1% ($P > .05$) and the times required for half-maximum desensitization were 1.4 ± 0.5 and 1.8 ± 0.2 min ($P > .1$). Each point represents the average of six experiments and vertical lines indicate S.E.M. An asterisk denotes a statistically significant difference between values obtained with and without isoproterenol.

## Discussion

The results of this study suggest that the inhibition of antigen-induced histamine release from guinea-pig lung mast cells by activation of *beta* adrenergic receptors is a result of an impairment of the link between antigen-antibody interaction and an increase in calcium flux across the cell membrane. This conclusion is supported by the following observations.

1) The inhibition of antigen-induced histamine release by isoproterenol could not be overcome by increasing the extracellular calcium concentration. This implicates a noncompetitive type of inhibition of calcium flux and is consistent with earlier observations of functional antagonism between *beta* agonists and specific antigen (Undem and Buckner, 1984b).

2) Isoproterenol did not inhibit the small amount of antigen-induced histamine release above spontaneous release that was found in the absence of added calcium. Histamine release under these conditions is consistent with observations on other cells that the stimulus evoked by antigen-antibody interaction is not calcium-dependent (Lichtenstein and De Bernardo, 1971; Ishizaka *et al.*, 1983) and implicates a link between bridging of cell surface antibody receptors and mobilization of small stores of intracellular (or tightly bound cell membrane) calcium. The lack of effect of isoproterenol here suggests that *beta* receptor activation does not influence that link or whatever other process might be involved in the release mechanism in the absence of added calcium. The absence of added calcium does not alter the ability of isoproterenol to increase cyclic AMP levels in guinea-pig pulmonary tissue (Wong and Buckner, 1978), but a similar lack of calcium dependence in the lung mast cells remains to be demonstrated.

3) Isoproterenol did not inhibit the histamine released after maximum desensitization to antigen. Inasmuch as the concentration of ovalbumin used in these experiments was maximally effective, it is probable that the desensitization observed was of the "nonspecific" type (Sobotka *et al.*, 1979a) which is suggested to involve a depression of calcium flux across the cell membrane (Dembo and Goldstein, 1980). That desensitization does not involve the release mechanism beyond cell membrane events is supported by the observation that antigen desensitization did not alter the ability of A23187 to evoke histamine release from the guinea-pig lung cells. Therefore, if we assume that the histamine released at the time of maximum desensitization is not a result of the link between antigen activation and calcium flux across the cell membrane, lack of effect of isoproterenol on this release would be consistent with 2) above.

4) Isoproterenol did not alter A23187-induced histamine release. In acting as a calcium ionophore, A23187 releases histamine by directly increasing the intracellular concentration of free ionized calcium (Reed and Lardy, 1972; Foreman *et al.*, 1973). Therefore, the so-called antigen-activated calcium gating mechanism at the level of the cell membrane is not involved in the subsequent A23187-induced release of histamine. The observations that A23187-induced noncytolytic histamine release from guinea-pig lung cells was dependent on added calcium and that the requirement for added calcium was inversely related to the A23187 concentration are consistent with similar observations on rat peritoneal mast cells (Foreman *et al.*, 1973; Bennett *et al.*, 1979). Lack of effect of isoproterenol on A23187-induced histamine release is consistent with the view that intracellular events involved in the release reaction are not altered by activation of *beta* receptors.

5) Isoproterenol did not alter TPA-induced histamine release. The observation that histamine release from guinea-pig lung cells by TPA is independent of added calcium is consistent with the effect of this phorbol ester on several cell types (Rink *et al.*, 1983; Rasmussen and Barrett, 1984), including basophils (Schleimer *et al.*, 1981) and rat mast cells (White *et al.*, 1984), and could involve activation of protein kinase *C* (Castagna *et al.*, 1982; Niedel *et al.*, 1983). Nevertheless, because release by TPA appears to involve intracellular events, the lack of an effect of isoproterenol is consistent with previously discussed observations.

In contrast to other studies using guinea-pig minced lung (Barrett-Bee, 1981), our results do not support the contention that *beta* receptor activation inhibits histamine release by enhancing the rate of antigen desensitization. In fact, at 1 min of ovalbumin treatment before addition of calcium, the magnitude of desensitization was significantly diminished. Furthermore, the time required to achieve half-maximal desensitization to antigen was not altered by isoproterenol.

Sobotka *et al.* (1979b) reported that *beta* adrenergic (as well as other substances that increase cellular cyclic AMP levels)-mediated inhibition of histamine release from human leukocytes can be abolished by pretreatment of the cells with indomethacin, thereby implicating a role for cyclooxygenase products of arachidonic acid metabolism in the inhibitory action. In contrast, the *beta* adrenergic mediated inhibition of antigen-induced histamine release observed in the present study, as well as in the other studies with guinea-pig minced lung (Wong and Buckner, 1980), was not abolished by indomethacin. Therefore, these responses to *beta* agonists in guinea-pig lung are not secondary to formation of cyclooxygenase metabolites.

The findings that dibutyryl cyclic AMP and forskolin mimicked the effect of isoproterenol adds to the existing circumstantial evidence that cyclic AMP might be a second messenger in adrenergic mediated inhibition of histamine release from lung mast cells. In studies with other mast cell types, however, this is not so clear. Pearce *et al.* (1978) found dibutyryl cyclic AMP to be capable of inhibiting A23187-induced histamine release from guinea-pig mesenteric mast cells, whereas isoproterenol was without effect. It is possible that dibutyryl cyclic AMP has effects when added exogenously to cell suspensions which differ from those derived from intracellular synthesis of cyclic AMP. Inasmuch as forskolin activates adenylate cyclase independently of membrane bound receptors (Seamon and Daly, 1981) and mimicked the effects and lack of effects of isoproterenol in this study, a modulation by cyclic AMP of the antigen-induced calcium flux across the mast cell membrane may be involved in the abilities of these substances to inhibit histamine release.

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