IMMUNOLOGICAL RELEASE OF HISTAMINE AND SLOW REACTING SUBSTANCE OF ANAPHYLAXIS FROM HUMAN LUNG

IV. Enhancement by Cholinergic and Alpha Adrenergic Stimulation[*]

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Human lung tissue passively sensitized with homologous IgE antibody releases the chemical mediators histamine, slow reacting substance of anaphylaxis (SRS-A)[1] (1–3), and eosinophil chemotactic factor of anaphylaxis (ECF-A) (4) upon exposure to specific antigen. Previous studies (3, 5) have demonstrated that this reaction is modulated by agents capable of affecting tissue levels of cyclic adenosine $3',5'$-monophosphate (cyclic AMP). Beta adrenergic stimulation produces measurable increases in tissue levels of cyclic AMP and concomitant inhibition of mediator release; both effects are prevented by beta adrenergic blockage. In contrast, alpha adrenergic stimulation is associated with decreases in tissue levels of cyclic AMP and enhancement of mediator release. The inverse relationship between the tissue levels of cyclic AMP and the quantity of mediators released suggests that the changes occurring in the cyclic AMP levels of the total cell population of intact lung fragments reflect comparable changes in the subpopulation of target cells involved in mediator release and that these target cells possess adrenergic receptor sites of the beta and alpha prototype.

In order to define further the nature of the pharmacological receptors present on the target cells in human lung tissue, a study was initiated to determine if cholinergic receptors were also functionally present. The results obtained reveal a cholinergic mechanism of enhancement and confirm and extend the studies of alpha adrenergic enhancement of the IgE-mediated release of histamine and SRS-A from human lung tissue in vitro.

Materials and Methods

Acetylcholine, carbamylcholine chloride, DL-norepinephrine hydrochloride, L-phenylephrine hydrochloride, adenosine 3',5'-cyclic monophosphoric acid, and atropine sulfate were obtained from Sigma Chemical Co. (St. Louis, Mo.). Propranolol hydrochloride (Inderal, Ayerst Laboratories, New York), antigen E (Abbott Laboratories, North Chicago, Ill.), N^6,O^2-dibutyryl cyclic AMP (Calbiochem, San Diego, Calif.), cyclic (8-^{3}H) AMP (14.2 μCi/mmole) and (8-^{14}C) adenine (50 μCi/mmole) (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.), Dowex 50 (200–400 mesh) H+ form (Bio-Rad Laboratories, Richmond, Calif.), 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) and 2,5-diphenyloxazole (PPO) (Packard Instrument Co. Inc., Downers Grove, Ill.), and 24-mm cellulose ester filters (0.45 μ) (Millipore Corp., Bedford, Mass.) were all supplied as noted. Dr. Lionel Simon of ICN Nuclear Acid Research Institute (ICN Corp., Irvine, Calif.) kindly supplied 8-bromo-cyclic 3',5'-guanosine monophosphate (8-bromo-cyclic GMP).

Preparation of Human Lung Tissue for the Antigen-Induced Release of Mediators.—Human lung tissue obtained at the time of surgery was prepared for the antigen-induced release of mediators as previously described (3). The lung tissue was dissected free of pleura, bronchi, and large blood vessels; fragmented, washed, and replicated into 200-mg samples; sensitized by incubation in the serum of either patient E.W. (3) or S.A.G. (IgE level, 250 mμg/ml) for 2 hr at 37°C or for 18 hr at room temperature; and washed free of serum and placed in Tyrode's buffer containing the agent(s) under study. The sensitized fragments were challenged 3 min later with antigen E (0.2 μg/ml), unless specified otherwise in the text, and the histamine and SRS-A released into the diffusate were quantitated by bioassay on the isolated guinea pig ileum. Residual tissue histamine was extracted from the lung fragments by boiling for 8 min.

All drugs were prepared in Tyrode's buffer just before use, and at the concentrations studied did not interfere with the bioassay of either histamine or SRS-A and did not induce a nonspecific release of these mediators in the absence of antigen challenge. The concentrations of histamine or SRS-A released from the sensitized tissues by antigen challenge in the absence of any drugs were taken as 100% and the per cent inhibition or enhancement of the release of these mediators in the presence of each agent was calculated on that basis.

Cyclic AMP Assays.—Cyclic AMP was assayed by the isolation of radiolabeled cyclic AMP after incorporation of adenine-^{14}C into the tissues (6, 7) or by the cyclic AMP binding protein assay as described by Gilman (8). In the radioincorporation technique (7), 200-mg replicates of human lung tissue were incubated with adenine-^{14}C for 2 hr at 37°C, washed extensively to remove excess adenine-^{14}C, incubated with the agent(s) under study for 3 min (unless stated otherwise in the text), and rapidly transferred to iced distilled water and boiled in order to liberate intracellular ^{14}C-labeled nucleotides. The radiolabeled cyclic AMP was isolated by cation exchange chromatography and subsequent paper chromatography and quantitated by liquid scintillation counting.

For the assay utilizing the cyclic AMP binding protein isolated from bovine skeletal muscle (9), 200-mg replicates of lung tissue, after incubation in Tyrode's buffer with any agent(s) under study, were transferred to 1.0 ml iced 5% trichloroacetic acid (TCA) and boiled for 30 min. The lung fragments were then homogenized by hand in a Ten Broeck Homogenizer (Fisher Scientific Co., Pittsburgh, Pa.), centrifuged at 6000 g for 20 min, and the supernatant decanted. 0.1 ml of 1 N HCl was added to the supernatant and the TCA extracted three times with 5 volumes each of water-saturated ethyl ether (Fisher Scientific Co.). The supernatant was evaporated to dryness, resuspended in 0.5 ml acetate buffer (50 mM, pH 4.0), and a sample of 10–100 μl (depending upon the experimental design) was added to assay tubes containing 0.5 pmoles of ^{3}H-labeled cyclic AMP, 50 μg of bovine serum albumin in acetate buffer (50 mM, pH 4.0), and 50 μg of cyclic AMP binding protein in that order to a total final volume of 250 μl. After incubation of the mixture for 60 min at 0°C, the volume

was brought to 1 ml with iced potassium phosphate buffer (20 mM, pH 6.0), and 5 min later the mixture was filtered through cellulose ester filter previously soaked with the potassium phosphate buffer and subsequently washed with 10 ml of the same buffer (8). The filter was placed in chilled Bray's solution for 12 hr and counted in a Nuclear-Chicago Mark I liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill.). The blank in the absence of binding protein was 20–30 cpm. In each experiment the amount of cyclic AMP in the lung tissue replicate bound to the binding protein was determined by comparison with the per cent inhibition of binding of the ^{3}H-labeled cyclic AMP in a standard curve of 2–25 pmoles of cyclic AMP run simultaneously. Human lung tissue was found to contain from 167 to 825 pmoles cyclic AMP/g wet weight (ww) with a mean of 417 pmoles/g ww in eight experiments.
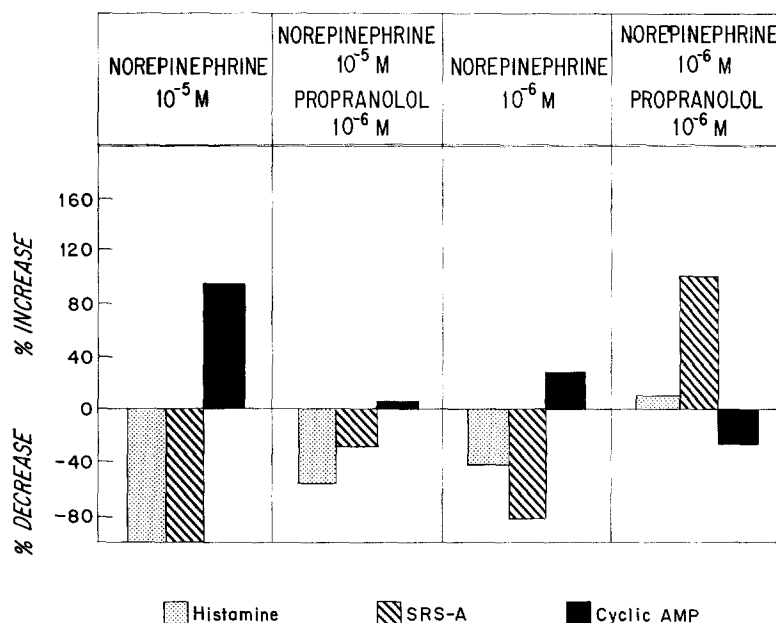

Fig. 1. The effect of norepinephrine with and without propranolol on the immunologic release of histamine and SRS-A from human lung tissue along with the concomitant changes in the tissue levels of cyclic AMP. Propranolol was added to the incubation medium 1 min before the introduction of norepinephrine and the replicate tissues were either challenged with antigen or assayed for cyclic AMP 2 min later. In this experiment, cyclic AMP was assayed by the cyclic AMP binding protein technique.

RESULTS

Alpha Adrenergic Stimulation.—The effect of the catecholamine norepinephrine with and without the beta adrenergic blocking agent proporanolol on the immunologic release of histamine and SRS-A as well as on the tissue levels of cyclic AMP is demonstrated in Fig. 1. Norepinephrine (10^{-5} M) produced an increase of 94% in the cyclic AMP levels, from 198 pmoles in the controls to 385 pmoles/g ww in the drug-treated samples, and total suppression of the

release of both mediators. Propranolol (10^{-6} M), which by itself had no effect on either cyclic AMP levels or mediator release, prevented the norepinephrine-induced increase in cyclic AMP levels and lowered the inhibition of mediator release to less than 50%. A lesser concentration of norepinephrine (10^{-6} M) increased cyclic AMP by 28% and significantly inhibited the antigen-induced release of mediators. The introduction of an equimolar concentration of propranolol (10^{-6} M) resulted in a 25% decrease in the level of cyclic AMP and an enhancement of histamine release of 11% and of SRS-A release of 100%. In eight additional experiments using equimolar concentrations of norepineph-
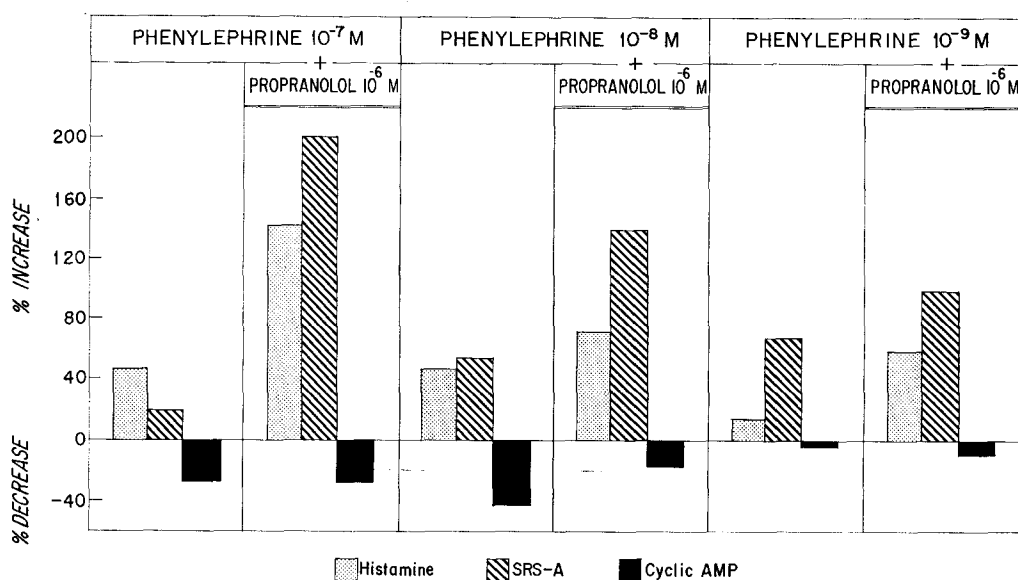

FIG. 2. The effect of phenylephrine with and without propranolol on the immunologic release of histamine and SRS-A from human lung tissue along with the concomitant changes in the tissue levels of cyclic AMP. The same protocol as outlined in Fig. 1 was used and cyclic AMP was assayed by the cyclic AMP binding protein technique.

rine and propranolol (10^{-6} M), the mean average decrease in cyclic AMP levels was 32% (range of decrease 17-68%) while the mean enhancement of the release of histamine and SRS-A was 30% (range of increase 7-40%) and 97% (range of increase 40-180%), respectively.

The capacity of the relatively specific alpha adrenergic agent phenylephrine to decrease cyclic AMP levels and enhance mediator release with and without the introduction of a beta adrenergic blocking agent is illustrated in Fig. 2. Phenylephrine alone, in concentrations ranging from 10^{-7} to 10^{-9} M, decreased the levels of cyclic AMP while enhancing the antigen-induced release of histamine and SRS-A in a nondose-related fashion. The introduction of pro-

pranolol (10^{-6} M) uncovered a dose-response effect of phenylephrine in enhancing mediator release without further depleting the levels of cyclic AMP. In five experiments, phenylephrine (10^{-8} M) produced a mean depletion of cyclic AMP of 32% (range of decrease 25–41%) while the mean enhancement in the release of histamine was 48% (range of increase 14–70%) and of SRS-A was 64% (range of increase 33–108%).

Cholinergic Stimulation.—The effect of the cholinergic agent acetylcholine (10^{-7}–10^{-11} M) on the immunologic release of mediators and the tissue levels
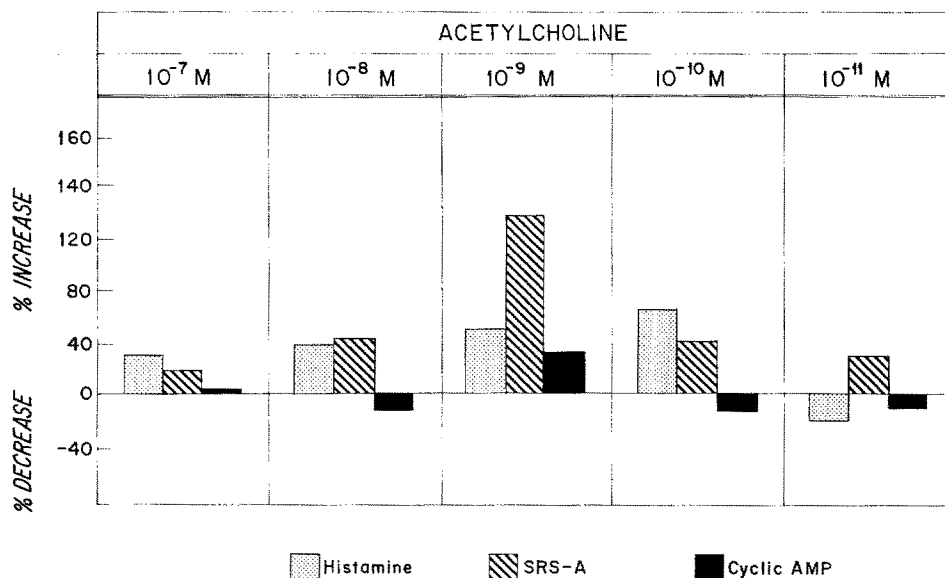

Fig. 3. The effect of acetylcholine on the immunologic release of histamine and SRS-A from human lung tissue along with the concomitant changes in the tissue levels of cyclic AMP. Lung tissue was incubated with varying concentrations of acetylcholine for 3 min before either antigen challenge or cyclic AMP assay by the cyclic AMP binding protein technique.

of cyclic AMP is presented in Fig. 3. Enhancement of histamine release was observed with acetylcholine in concentrations of 10^{-7}–10^{-10} M while enhancement of SRS-A release was evident at all concentrations of acetylcholine tested with a peak effect at 10^{-9} M. The levels of cyclic AMP showed no consistent pattern.

Because of its resistance to hydrolysis by acetylcholinesterase, carbamylcholine chloride (Carbachol) was employed in all subsequent experiments. Carbachol (10^{-9}–10^{-12} M) was found to result in enhancement of the IgE-mediated release of both histamine and SRS-A at all concentrations with the maximal effect seen at 10^{-10} M Carbachol (Fig. 4). Cyclic AMP levels were again found to bear no consistent relationship with mediator release. In 16

experiments utilizing 10^{-10} M Carbachol, the mean enhancement of the immunologic release of histamine was 44% (range of increase 8–181%) and of SRS-A, 110% (range of increase 23–270%), while the mean change in cyclic AMP levels was less than 1% from control (range of change +32 to −14%).

The antigen-induced release of chemical mediators at various times after cholinergic stimulation with Carbachol (10^{-10} M) is illustrated in Fig. 5. Enhancement of mediator release was evident at 1 min and reached a peak effect
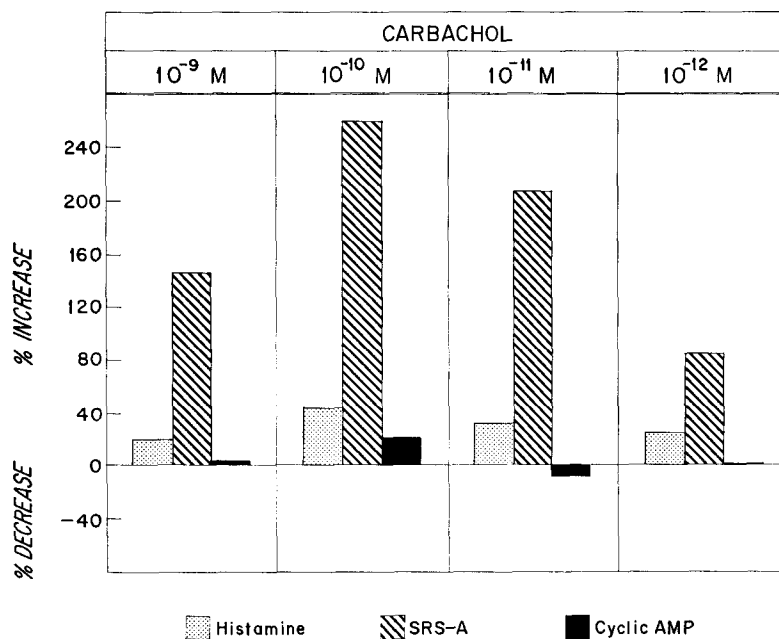

FIG. 4. The effect of Carbachol on the immunologic release of histamine and SRS-A from human lung tissue along with the concomitant changes in the tissue levels of cyclic AMP. The same protocol as outlined for Fig. 3 was used and cyclic AMP was assayed by the radio-incorporation and subsequent isolation of ^{14}C-labeled cyclic AMP technique.

within 5 min while the level of cyclic AMP was unchanged throughout the experiment.

Preincubating the tissues with the antimuscarinic agent atropine sulfate (10^{-7}–10^{-10} M) for 1 min before the addition of Carbachol (10^{-10} M) revealed that in concentrations of 10^{-8} M or more the agent completely prevented the Carbachol-induced enhancement of mediator release, while at concentrations of 10^{-9} M or less it had no effect (Table I). In the doses used, atropine itself had no influence upon mediator release.

The capacity of atropine (10^{-8} M) to inhibit cholinergic enhancement but not alpha adrenergic enhancement is illustrated in Fig. 6. Atropine (10^{-8} M) pre-

vented the Carbachol-stimulated enhancement, had no effect on the phenyl-
ephrine-induced enhancement, and when used in the presence of both agents
reduced the enhancement to that observed with phenylephrine alone.

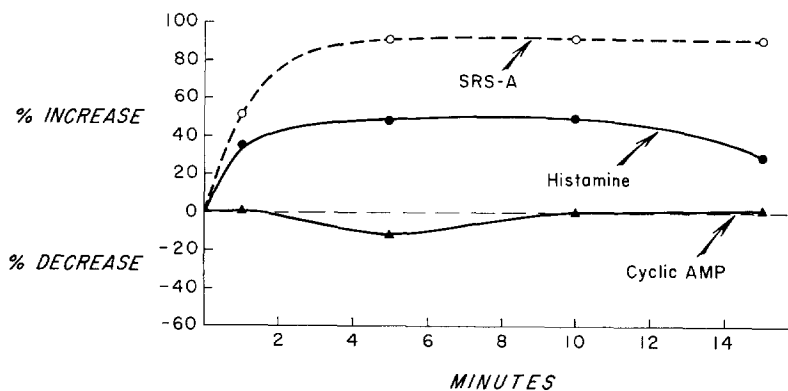*Effects of Cyclic GMP.*—The knowledge that cholinergic stimulation in-

Fig. 5. The effect of varying the time of preincubation with Carbachol (10^{-10} M) on the immunologic release of histamine and SRS-A from human lung tissue along with the concomitant changes in the tissue levels of cyclic AMP. Lung tissue was incubated with Carbachol for 1–15 min before either antigen challenge or cyclic AMP assay by the radioincorporation and subsequent isolation of ^{14}C-labeled cyclic AMP technique.

TABLE I

Prevention by Atropine of the Carbachol-Induced Enhancement of the Immunologic Release of Histamine and SRS-A from Human Lung Tissue

Agonist	Antagonist	Histamine (change)	SRS-A (change)
		%	%
Carbachol 10^{-10} M		+23	+54
Carbachol 10^{-10} M	Atropine 10^{-7} M	−11	−8
Carbachol 10^{-10} M	Atropine 10^{-8} M	−7	−20
Carbachol 10^{-10} M	Atropine 10^{-9} M	+40	+35
Carbachol 10^{-10} M	Atropine 10^{-10} M	+21	+54

creases the tissue levels of cyclic GMP (10–12) prompted a study of the direct
effect of cyclic GMP on the immunologic release of chemical mediators. 8-
bromo-cyclic GMP produced a dose-response enhancement of the immunologic
release of both histamine and SRS-A (Fig. 7). The enhancing effect of 8-bromo-

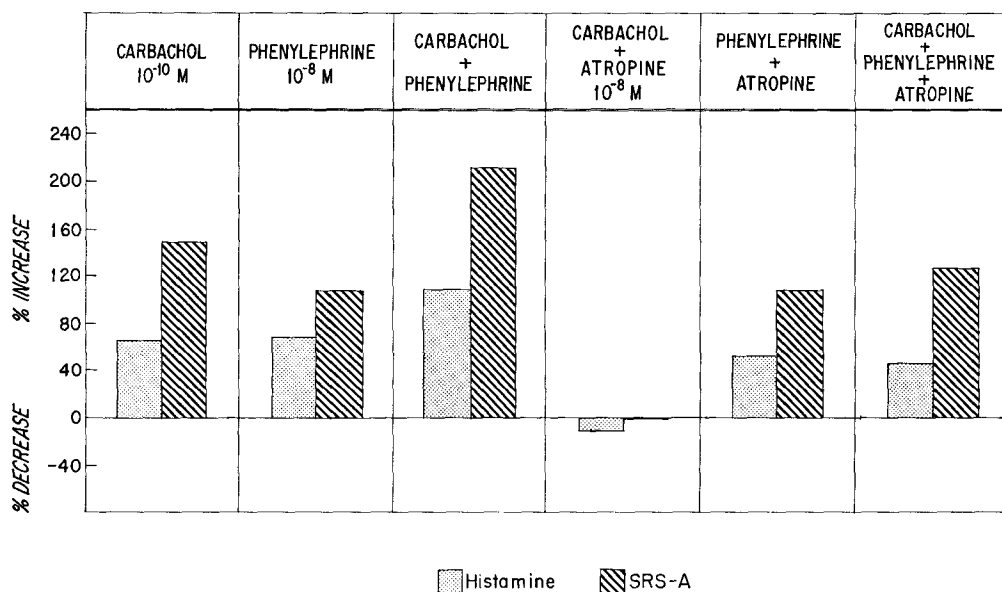| | CARBACHOL 10^{-10} M | PHENYLEPHRINE 10^{-8} M | CARBACHOL + PHENYLEPHRINE | CARBACHOL + ATROPINE 10^{-8} M | PHENYLEPHRINE + ATROPINE | CARBACHOL + PHENYLEPHRINE + ATROPINE |

☐ Histamine   ▨ SRS-A

FIG. 6. The effect of atropine on cholinergic and alpha adrenergic-induced enhancement of the immunologic release of histamine and SRS-A from human lung tissue. Atropine was added to the incubation medium 1 min before the addition of Carbachol, phenylephrine, or both. Antigen was added 3 min after the agonists.

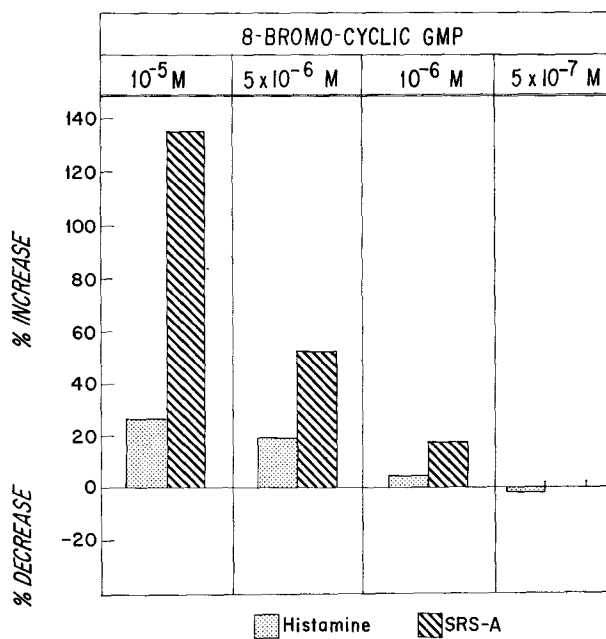| 8-BROMO-CYCLIC GMP			
10^{-5} M	5×10^{-6} M	10^{-6} M	5×10^{-7} M

☐ Histamine   ▨ SRS-A

FIG. 7. The effect of 8-bromo-cyclic GMP on the immunologic release of histamine and SRS-A from human lung tissue. Varying concentrations of 8-bromo-cyclic GMP were added to the incubation medium 5 min before antigen challenge.

cyclic GMP (10^{-5} M) was most evident after 1–5 min and the effect fell off after 15 min. Previous studies with dibutyryl cyclic AMP had revealed a dose-response inhibition of mediator release (3). A comparison of the effects of 8-bromo-cyclic GMP (10^{-6} M) and dibutyryl cyclic AMP (10^{-6} M) introduced 5 min before the antigen-induced release of chemical mediators is shown in Fig. 8. While 8-bromo-cyclic GMP produced a marked enhancement, the dibutyryl derivative of cyclic AMP inhibited the immunologic release of histamine and SRS-A.
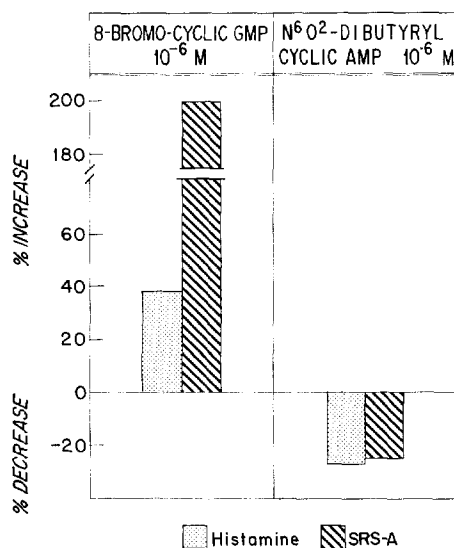

{Fig. 8. Comparison of the effects of 8-bromo-cyclic GMP and dibutyryl cyclic AMP on the immunologic release of histamine and SRS-A from human lung tissue. The same protocol as outlined for Fig. 7 was used.

DISCUSSION

The IgE-mediated release of histamine and SRS-A from human lung tissue is enhanced by both alpha adrenergic and cholinergic stimulation. Previous studies have revealed that agents increasing cellular levels of cyclic AMP, such as beta adrenergic stimulants or methylxanthines, suppress the immunologic release of these mediators. Beta adrenergic blockade of lung tissue subsequently stimulated with catecholamines such as epinephrine or norepinephrine (Fig. 1) which possess both alpha and beta adrenergic activity results in decreases in tissue levels of cyclic AMP and enhancement of the release of mediators (3, 5). Further documentation of the relationship of enhanced mediator release and alpha adrenergic stimulation was achieved using the relatively specific alpha adrenergic agent phenylephrine (Fig. 2). Although this agent alone enhanced the immunologic release of chemical mediators, beta

adrenergic blockade increased its ability and uncovered a dose-response effect. The dose-response enhancement of mediator release by phenylephrine in the presence of propranolol was not associated with a greater depletion of cyclic AMP levels than observed with phenylephrine alone. This discrepancy is attributed to the fact that the level of cyclic AMP relates to the heterogeneous cell population constituting the entire lung fragment, while mediator release involves a discrete subpopulation of target cells. The consistent qualitative relationship between the alpha adrenergic enhancement of mediator release and the decrease in total tissue cyclic AMP was observed whether cyclic AMP was measured by methods based upon the isolation of radiolabeled cyclic AMP after incorporation of adenine-^{14}C into the tissue (7) or by a cyclic AMP binding protein assay (8). In eight experiments studying alpha adrenergic stimulation as achieved by combining equimolar concentrations (10^{-6} M) of norepinephrine and propranolol (Fig. 1), the mean decrease in cyclic AMP was 32%, while the mean enhancement of the immunologic release of histamine was 30% and of SRS-A was 97%. In five experiments phenylephrine (10^{-8} M) produced a mean decrease in the tissue levels of cyclic AMP of 32% and a mean increase in the release of histamine of 48% and of SRS-A of 64%.

Cholinergic enhancement of the immunologic release of chemical mediators was observed with as little as 10^{-10} M acetylcholine (Fig. 3) or 10^{-12} M Carbachol (Fig. 4). This enhancement was not associated with a consistent change in the levels of cyclic AMP (Figs. 3-5). In 16 experiments with Carbachol (10^{-10} M), the mean change in cyclic AMP levels was less than 1% while the mean enhancement of the antigen-induced release of histamine and SRS-A was 44 and 110%, respectively.

The finding that atropine prevents cholinergic enhancement but not comparable alpha adrenergic enhancement is consistent with the view that adrenergic and cholinergic agonists interact with separate receptor sites on the target cells in human lung tissue involved in the immunologic release of chemical mediators (Fig. 6). That the adrenergic effects may be expressed through the levels of cyclic AMP is indicated by the consistent inverse association between the levels of cyclic AMP and mediator release plus the capacity of dibutyryl cyclic AMP to inhibit the release of mediators (Fig. 8) (3). That cholinergic enhancement may be mediated by cyclic GMP (Figs. 7 and 8) is suggested by the recent findings of others that cholinergic stimulation of tissues increases the level of cyclic GMP (10-12) and by the demonstration herein that exogenous 8-bromo-cyclic GMP enhances the immunologic release of histamine and SRS-A in a dose-response fashion. Taken together, these and our previous studies (3, 5) suggest that the target cells in human lung tissue involved in the immunologic release of mediators possess adrenergic and cholinergic receptor sites which when stimulated are capable of modulating a complex immunologic reaction through changes in the level of two cyclic nucleotides, cyclic AMP and cyclic GMP.

SUMMARY

The immunologic release of histamine and slow reacting substance of anaphylaxis (SRS-A) from human lung tissue can be enhanced by stimulation with either alpha adrenergic agents (phenylephrine or norepinephrine in the presence of propranolol) or cholinergic agents (acetylcholine or Carbachol). The finding that atropine prevents cholinergic but not comparable alpha adrenergic enhancement is consistent with the view that cholinergic and alpha adrenergic agonists interact with separate receptor sites on the target cells involved in the immunologic release of chemical mediators. The consistent qualitative relationship between the antigen-induced release of mediators and the level of cyclic adenosine monophosphate (cyclic AMP) as measured by the isolation of ^{14}C-labeled cyclic AMP after incorporation of adenine-^{14}C into the tissues or by the cyclic AMP binding protein assay suggests that changes in the level of this cyclic nucleotide mediate adrenergic modulation of the release of histamine and SRS-A. The addition of 8-bromo-cyclic guanosine monophosphate (cyclic GMP) produces an enhancement of the immunologic release of mediators while dibutyryl cyclic AMP is inhibitory. As cholinergic-induced enhancement was not associated with a measurable change in the levels of cyclic AMP, the possibility is suggested that cyclic GMP may be the intracellular mediator of cholinergic-induced enhancement of the immunologic release of histamine and SRS-A.

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