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MODULATION OF GUINEA-PIG LUNG ADENYLATE CYCLASE BY OVALBUMIN SENSITIZATION

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Abstract—1. The regulation of lung adenylate cyclase was investigated in guinea pigs sensitized with high dose (300–500 $\mu g\ kg^{-1}$) ovalbumin to raise IgG(I) and low dose (2.8–4.0 $\mu g\ kg^{-1}$) to raise to IgG/IgE (II) antibodies.

2. Basal activity of sensitized II (IgG/IgE antibodies) lung adenylate cyclase was approximately double that of the control values.

3. Guanosine 5′-triphosphate (GTP) was a potent activator of adenylate cyclase from the sensitized II (IgG/IgE) lung membranes.

4. The sensitivity of lung membrane adenylate cyclase to stimulation by β-adrenoceptor agonists or VIP was reduced whereas no significant difference was observed with histamine or bradykinin on the sensitized II membranes.

5. Possible mechanisms involved in the increase in basal activity and in the decreased sensitivity to β-adrenoceptor- and VIP-mediated stimulation of adenylate cyclase by ovalbumin sensitization of guinea-pig lung membranes are discussed.

Since extrinsic or allergic bronchial asthma is believed to be caused by an immediate hypersensitivity reaction to allergens and is primarily mediated by IgE antibodies [1], sensitized guinea pigs are often used as animal models of asthma even though the main homocytotropic antibody produced is IgG. Andersson [2] demonstrated that IgE antibodies, in addition to IgG, could be raised in guinea pigs sensitized with low doses of ovalbumin. In the initial phase of our study, we investigated the regulation of lung adenylate cyclase in two guinea-pig models of allergic asthma; one, in which IgG antibodies were raised to higher and another, in which IgE antibodies in addition to IgG were produced by much lower doses of the antigen, ovalbumin.

The basic mechanism triggering bronchial hyperreactivity is not known. A number of theories exist. Chemical mediators released by inflammatory cells or antigen–antibody reactions, reduced functioning of β-adrenoceptors [3] and a functional imbalance between peptidergic-inhibitory and cholinergic-excitatory [4] nerves, have all been implicated in the aetiology of bronchial asthma. Recent experimental and clinical evidence suggests that these mechanisms may be secondary to damage or loss of bronchial epithelium. Modulation by airway epithelial cells could result in alteration of the neurohumoral responsiveness of bronchial smooth muscle [5, 6]. In the second phase, we therefore investigated the effect of β-adrenoceptor agonists as well as histamine and peptides on adenylate cyclase of lung membranes from control and ovalbumin-sensitized guinea pigs within whom the sensitizing antibodies present were identified. Abstracts of this study have been communicated to the Pharmacological and Physiological Societies [7–9].

MATERIALS AND METHODS

Male Redfern guinea pigs were used in all experiments.

Sensitization procedures

(I) *IgG antibodies*. For each experiment 12 guinea pigs were paired into two groups (six control and six experimental). The six experimental guinea pigs (400–500 g) were injected with a single intraperitoneal injection of 2 ml saline (0.9% w/v NaCl solution) containing 200 μg ovalbumin and 4 mg aluminium hydroxide gel. The adjuvant was added to the antigen 30 min before injection. The control animals were injected with adjuvant only. The animals were killed after a 14-day sensitization period.

(II) *IgG and IgE antibodies*. For each experiment 12 guinea-pigs were paired into two groups (six control and six experimental). The six experimental guinea-pigs (250–350 g) were injected with a single intraperitoneal injection of 0.5 ml saline containing 1 μg ovalbumin and 100 mg aluminium hydroxide gel. The adjuvant was added to the antigen 60 min before the injection. The sensitization period was 42 days. Control animals were injected with adjuvant only.

After the respective sensitization periods, the paired control and experimental guinea-pigs were killed by cervical dislocation and blood collected for later analysis of seral reagnic antibody (IgE and IgG) profile by passive cutaneous anaphylaxis [10]. Confirmation of sensitization was obtained using the

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isolated ileum of control and sensitized guinea pigs (Schultz–Dale reaction).

Verification of sensitization

Isolated guinea-pig ileum. During each experiment the isolated ileum of the control and sensitized guinea pig was used to test the presence of cell fixed antibodies in order to assess the success or otherwise of the sensitization procedure. Guinea pigs were killed by cervical dislocation and the terminal ileum close to the ilea–caecal junction was removed. A small strip (20 mm) was mounted in an organ bath containing Tyrode solution (mm: 5.55 glucose, 136 NaCl, 11.9 $NaHCO_3$, 0.32 NaH_2PO_4, 2.6 KCl, 1.8 $CaCl_2$ 0.49 $MgCl_2$; pH 7.4) aerated with 5% CO_2:95% O_2 at 37°. Isotonic tension was recorded on a chart recorder, and the contractile response to ovalbumin was match-assayed against histamine and acetylcholine. A 3-min cycle of drug additions was adopted, and their action was terminated by washing the organ bath with fresh Tyrode solution. Subsequently, in a few experiments, isolated tracheal strips from sham or ovalbumin-injected animals were mounted in the organ bath and tested for sensitization to ovalbumin.

Passive cutaneous anaphylaxis (PCA). Passive cutaneous anaphylaxis was used to assess the presence of antibodies in serum because as yet it is not possible to measure directly circulating IgE antibodies in guinea-pig serum. Reaginic antibodies bind to skin mast cells and interaction with antigen releases mast cell mediators locally. The mediators produce an increase in vascular permeability around the intradermal injection site, which is visualized by using an intravenous dye. Both IgG and IgE antibodies sensitize skin mast cells. IgG antibodies have a short duration of sensitization (~48 hr), whereas it is much longer for IgE antibodies. Consequently, the latent period for mast cell sensitization to occur is 4 hr for IgG antibodies and 14 days for IgE. IgE antibodies are thermolabile, and so confirmation of the presence of this antibody class is obtained by repeating the PCA reaction with serum heated at 56° for 1 hr.

Blood samples taken from control and sensitized guinea pigs were allowed to clot and the serum collected after a brief centrifugation of the tubes at 1600 *g*. The serum from each group was pooled and stored at −70°. Each serum pool was examined by PCA, according to the principles of Watanabe and Ovary [10], with and without heat treatment (56°, 1 hr). Test serum (0.1 ml) serially diluted in saline (0.9% w/v) was injected intradermally into the shaved backs of normal guinea pigs (300–400 g). After a latent period of 4 hr (IgG antibodies) or 14 days (IgE antibodies) the animals were anesthetized with urethane (intraperitoneal 25% w/v; 6 ml kg^{-1}) and the trachea and jugular vein cannulated. The guinea pigs were challenged with an intravenous injection of 1 ml of saline containing 2 mg ovalbumin and 10 mg Evan's blue. The animals were killed after 30 min. The dorsal skin was reflected and the antibody titre estimated by the end point dilution technique (the lowest seral dilution giving a 5 mm diameter blue spot).

Preparation of the enzyme

The lungs were perfused with 0.9% ice-cold saline through the right ventricle. The lung tissue was separated from the larger airways and blood vessels, and tissue showing any evidence of haemorrhage was discarded. For each experiment chopped lung tissue from three animals was homogenized with an Ultraturrax (8N shaft) at full speed for 5 sec in 5 vol. of homogenizing medium (mM: 250 sucrose, 25 KCl, 2 EGTA, 8 theophylline, 50 Tris:HCl; pH 7.4). The homogenate was filtered through a single layer of nylon mesh and the filtrate centrifuged at 1600 *g* for 10 min. The pellet was resuspended in 5 vol. of homogenizing medium and centrifuged for a further 10 min at 1600 *g*. The washed pellet was finally resuspended in 20 vol. of homogenizing medium. Only freshly prepared membrane fractions maintained at 0–4° were used for the measurement of adenylate cyclase.

Measurement of adenylate cyclase

Adenylate cyclase activity was determined using a modification of the method of Albano *et al.* [11]. The standard assay system contained, in a final volume of 75 μl, mM: 2 ATP, 0.01 GTP (except where stated), 3 $MgCl_2$, 10 NaCl, 10 KCl, 1 EDTA, 8 theophylline, 50 Tris–HCl; (pH 7.4). The reaction was started by the addition of 25 μl of enzyme preparation (~20 μg) and incubated at 30°. After 15 min the reaction was terminated by placing the incubation tubes in a Grant heating block (105°) for 3 min. Following freezing and thawing, the samples were resuspended in 1 ml of 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 1600 *g* for 10 min. Fifty microlitre samples were taken for the determination of cyclic AMP by the method of Brown *et al.* [12]. Protein was measured by the method of Lowry *et al.* [13]. All results are expressed as nmol cAMP min^{-1}mg $protein^{-1}$ and are $\bar{\chi}$ + SE of at least three experiments, performed and assayed in triplicate. Adenylate cyclase associated with 1600 *g* lung plasma membranes was found to be linear with respect to time (zero to 30 min) and protein concentration (up to 35 μg).

Statistics

A Student's *t*-test was used to assess significant agonist activation of lung adenylate cyclase from non-treated guinea-pig lung membranes. Data relating differences in the stimulation of cyclase activity of lung membranes from sensitized (I and II) and control (sham-injected) guinea pigs have been analysed by two-way analysis of variance with replication, using the computer programme "Anovar 2R" [14].

Materials

Guanosine 5′-trisphosphate (GTP), guanylyl-5′-imidodiphosphate (GppNHp) and ovalbumin (Grade III) were purchased from Sigma (St Louis, MO). Forskolin was purchased from Calbiochem. Ovalbumin (Grade III), adrenaline bitartrate, isoprenaline bitartrate, histamine dihydrochloride, bombesin and bradykinin were purchased from Sigma and vasoactive intestinal peptide (VIP) from Peninsula. Grateful thanks for the following gifts: Glaxo—salbutamol and ranitidine, ICI—propanolol

and ICI 118551, Reckitt and Colman—RX781094, Astra—terbutaline sulphate. All other compounds were of reagent quality.

RESULTS

Confirmation of sensitization

The isolated ilea of both groups of sensitized guinea pigs contracted when exposed to ovalbumin whereas the antigen had no effect on the ilea of control, sham-injected animals. Isolated tracheal strips from sensitized animals consistently contracted when challenged with ovalbumin. Circulating IgG antibodies (antibody titre:32) were identified by passive cutaneous anaphylaxis in sera collected from guinea pigs injected with 300–500 μg kg^{-1} of ovalbumin. Those sensitized with 2.8–4.0 μg kg^{-1} of antigen, in addition possessed IgE antibodies (antibody titre:4) in their serum. The presence of the IgE antibodies was confirmed by repeating the experiment, using sera that had been heated at 56° for 1 hr, thereby rendering IgE inactive. With the heat-treated serum no reaction to antigen was observed after a 14-day latent period indicating loss of IgE antibodies, even though an IgG-mediated reaction was still present when tested after 4 hr (Table 1: see Andersson [2]).

Regulation of lung membrane adenylate cyclase

There was no difference in the basal activity of lung membrane adenylate cyclase of control (sham-injected) and sensitized I guinea pigs (IgG antibodies). In contrast, there was a highly significant ($P < 0.001$; 175%) increase in the basal activity of lung membranes from sensitized (II, IgG/IgE) animals (Fig. 1). Sodium fluoride (5 mM, NaF) stimulated the adenylate cyclase approximately two-fold above basal values in all groups.

Guanine-nucleotides. Activation of adenylate cyclase by guanosine triphosphate (GTP; 10^{-7} to 5×10^{-3} M) was dose-dependent in sensitized II (Fig. 2) and the paired control lung membranes. However, the magnitude of activation produced by GTP was significantly increased ($P < 0.01$) in the sensitization II (IgG/IgE) membranes (Table 2). The dose–response curve for GTP was shifted to the left

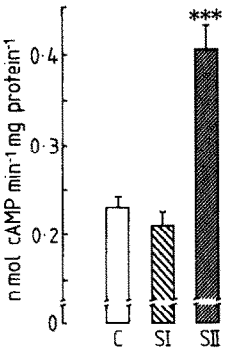

Fig. 1. Basal adenylate cyclase activity of control (c), sensitization I (SI; IgG antibodies only) and sensitization II (SII; IgG and IgE antibodies) guinea-pig lung membranes. Adenylate cyclase activity was measured in the presence of 10 μM GTP. Results are $\bar{x}$ + SE of 8 experiments. Incubations were performed and assayed in triplicate. *** $P < 0.001$.

of the control curve and the maximal stimulation of the enzyme was increased. No difference was observed between the activation of the cyclase between control and sensitized I (IgE) lung membranes by GTP (Table 2). In the absence of GTP there was no difference in basal adenylate cyclase activity of control and sensitized I and II membranes. Guanylyl-5′-imidodiphosphate (GppNHp; a non-hydrolysable analogue of GTP) caused a dose-dependent increase in adenylate cyclase of sensitized I and II and their paired control lung membranes. Neither sensitization (I or II) significantly altered the GppNHp dose–response curves from that observed for the control membranes (Fig. 3; Table 3).

Forskolin and sodium fluoride. Forskolin (0.50 μM) stimulation of adenylate cyclase of control and sensitized I and II lung membranes were examined in the absence and presence of 10 μM GTP (Fig. 4). There was no difference between the forskolin dose–response curves of the sensitized I and II adenylate cyclase and their respective sham-injected controls with or without 10 μM GTP; the forskolin dose–response curves of the control and sensitized

Table 1. Titre of homocytotropic anti-ovalbumin-antibodies from animals sensitized according to procedure (I) 300–500 μg kg^{-1} ovalbumin on (II) 2.8–4 μg kg^{-1} ovalbumin

		PCA latent period			
		4 hr (IgG)		14 days (IgE)	
Sensitizing regimen	Sensitization period (days)	Before heating	After heating 56°, 1 hr	Before heating	After heating 56°, 1 hr
Control I	14	0	0	—	—
Sensitization I	14	32	32	—	—
Control II	42	0	0	0	—
Sensitization II	42	4	4	4	0

The titre given is the average dilution of pooled sera taken from each group of animals after 14 or 42 days which gave a positive PCA-reaction. The reaction was examined after a latent period of 4 hr and 14 days.
—, Experiments not done.

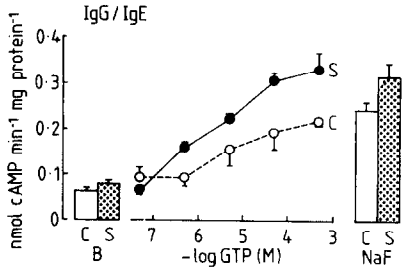

Fig. 2. Effect of GTP on adenylate cyclase of sensitized II (IgG/IgE) guinea-pig lung membranes. Basal (B) activity was measured in the absence of GTP. Symbols indicate the following: control = C, ○——○; sensitized = S, ●——●; NaF = 5 mM sodium fluoride. Results are $\bar{\chi}$ + SE of 3 experiments. Incubations were performed and assayed in triplicate.

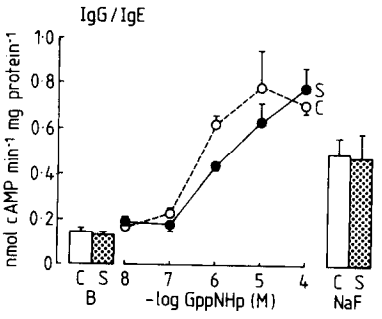

Fig. 3. Comparison of the activation of adenylate cyclase by Gpp(NH)p from paired control and sensitized II (IgG/IgE) lung membranes. Basal (B) activity was measured in the absence of Gpp(NH)p. Symbols indicate the following: Control = C ○---○; sensitized = S, ●——●; NaF = 5 mM sodium fluoride. Results are $\bar{\chi}$ + SE of 3 experiments. Incubations were performed and assayed in triplicate.

II (IgG/IgE) cyclase in the presence of GTP were superimposable if the basal activity was subtracted.

Receptor mediated modulation of lung membrane adenylate cyclase β-adrenoceptor agonists

Adrenaline, isoprenaline, salbutamol and terbutaline (10^{-8}–10^{-4} M) caused a dose-related activation of adenylate cyclase from control lung membranes (Fig. 5). The order of potency (the concentration of drug producing half its maximal response) appeared to be adrenaline (0.10 μM), salbutamol (0.15 μM), terbutaline (0.18 μM) and isoprenaline (0.63 μM). Responses of the lung adenylate cyclase to adrenaline were abolished by L-propanolol, and the selective β_2 antagonists ICI 118551 and butoxamine hydrochloride at a concentration of 10^{-5} M (Fig. 6). Neither phentolamine (10^{-5} M) or a selective α_2-adrenoceptor antagonist RX781094 had a significant effect on the adrenaline stimulated enzyme activity.

Subsequently, adrenaline (10^{-7}–10^{-4} M) activation of adenylate cyclase in control lung membranes was compared with sensitized II (IgG/IgE) and sensitized I (IgG) membranes. When compared to control tissue, adrenaline was relatively ineffective in stimulating the enzyme in the sensitized II (IgG/IgE) membranes. Two-way analysis of variance with replication revealed a highly significant reduction in sensitivity to adrenaline ($P < 0.001$).

GTP is essential for the expression of receptor-mediated activation of adenylate cyclase. Because the basal turnover of the sensitized lung enzyme

Table 2. The effect of GTP on adenylate cyclase from (a) sensitized II (IgG/IgE) and (b) sensitized I (IgG) guinea-pig lung membranes

Source of variation	DF	(a) Sensitized II SOS	V	F	Significance
Between doses	4	149453	37363	15.67	$P < 0.001$
Between treatments	1	21418	21418	8.98	$P < 0.01$
Interaction	4	16172	4043	1.70	
Replication	20	47682	2384		
Total	29	234725			
Source of variation	DF	(b) Sensitized I SOS	V	F	Significance
Between doses	4	193996	48499	15.77	$P < 0.001$
Between treatments	1	300	300	0.10	NS
Interaction	4	2885	721	0.23	NS
Replication	20	61525	3076		
Total	29	258706			

The summary tables of the analysis of variance demonstrate that GTP produces a dose-dependent activation of adenylate cyclase (AC) from both sensitized II and I ($P < 0.001$) membranes. The "between treatments" analysis indicates that GTP significantly enhances the activity of adenylate cyclase from sensitized II ($P < 0.01$) but not sensitized I membranes when compared with membranes AC from their respective paired control animals.

DF, degrees of freedom; SOS, sums of squares; V, variance; F, variance ratio; NS, not significant.

Table 3. Activation of adenylate cyclase by Gpp(NH)p in (a) sensitized II (IgG/IgE) and (b) sensitized I (IgG) guinea-pig lung membranes

Source of variation	DF	(a) Sensitized II SOS	V	F	Significance
Between doses	4	1541951	385488	51.76	$P < 0.001$
Between treatments	1	6308	6308	0.85	NS
Interaction	4	56269	14607	1.89	NS
Replication	20	148961	7448		
Total	29	1753489			

Source of variation	DF	(b) Sensitized I SOS	V	F	Significance
Between doses	4	845942	211486	47.23	$P < 0.001$
Between treatments	1	5576	5576	2.5	NS
Interaction	4	4107	1027	0.23	NS
Replication	20	89558	4478		
Total	29	945184			

The summary tables of the analysis of variance demonstrate that Gpp(NH)p produces a dose-dependent activation of adenylate cyclase (AC) from both sensitized II ($P < 0.001$) and sensitized I ($P < 0.001$) membranes. The "between treatments" analysis indicates no significant enhancement of AC activity in sensitized II or sensitized I membranes by Gpp(NH)p.

DF, degrees of freedom; SOS, sums of squares; V, variance; F, variance ratio; NS, not significant.

showed a GTP-dependent two-fold increase, the reduction in the sensitivity of the enzyme to stimulation by adrenaline was more clearly visualized when the basal values (nmol cAMP min^{-1} mg $protein^{-1}$: control 0.23 ± 0.02; sensitization II IgG/IgE 0.41 ± 0.02) were subtracted (Fig. 7). Similar significant reductions in sensitivity of the cyclase to activation by both isoprenaline and salbutamol were observed in the sensitized II (IgG/IgE) membranes (see Fig. 7). The sensitvity of the lung membrane adenylate cyclase activity of sensitized I (IgG) guinea pigs to stimulation by β-adrenoceptor agonists was also significantly reduced. Although the β-adrenoceptor activation of adenylate cyclase was reduced ($P < 0.01$), the intrinsic activity of the enzyme from IgG sensitized membranes remained unaltered.

Sodium fluoride (NaF) which requires the presence of Ns (the guanine nucleotide regulating

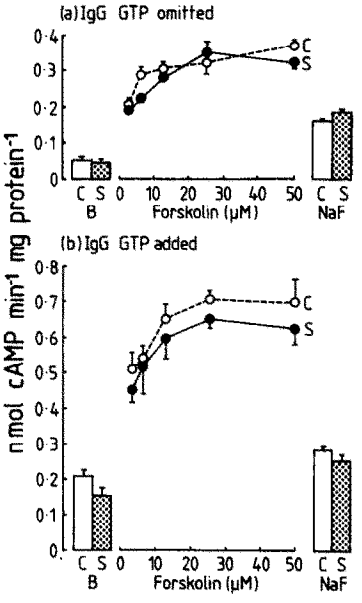

Fig. 4. Dose-dependent effect of forskolin on adenylate cyclase of control (C, ○——○) and sensitized II, IgG/IgE, (S, ●——●) lung membranes (a) in the absence and (b) in the presence of 10 μM GTP. B = basal activity. NaF = 5 mM sodium fluoride. Results are $\bar{x}$ + SE of triplicate incubations assayed in triplicate.

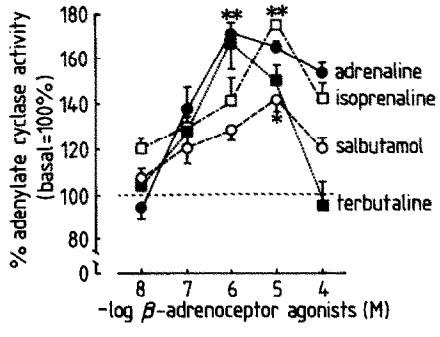

Fig. 5. Activation of guinea-pig lung adenylate cyclase by β-adrenoceptor agonists. Agonist activation of adenylate cyclase is expressed as a percentage of basal activity in absence of drug. Basal enzyme activity for each agonist was as follows in nmol cAMP min^{-1} mg $protein^{-1}$: adrenaline 0.215 ± 0.015 (●——●); isoprenaline 0.250 ± 0.024 (□—·—□); salbutamol 0.215 ± 0.015 (○——○) and terbutaline 0.175 ± 0.012 (■——■). * $P < 0.05$, ** $P < 0.01$.

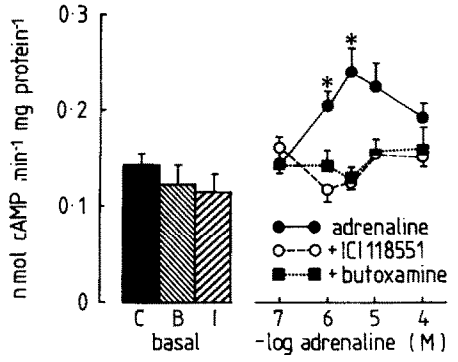

Fig. 6. Effect of selective β_2-adrenoceptor antagonists, ICI 118, 551 (I) and butoxamine hydrochloride (B) on adrenaline-activated lung adenylate cyclase. Adrenaline alone (●——●), ± 10^{-5} M ICI 118551 (○---○) or +10^{-5} M butoxamine hydrochloride (■---■). Basal = basal activity: C = control; B = butoxamine HCl, 10^{-5} M; I = ICI 118551, 10^{-5} M. Results are $\bar{x}$ + SE of triplicate incubations assayed in triplicate. * $P < 0.05$.

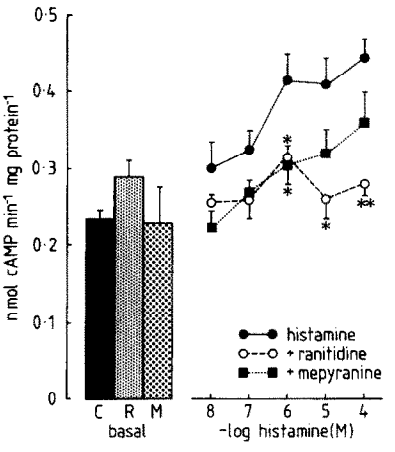

Fig. 8. Effect of mepyramine (H_1) and ranitidine (H_2) on histamine activated lung adenylate cyclase. Histamine alone (●——●); +10^{-5} M ranitidine (○---○) +10^{-5} M mepyramine (■---■). Basal = basal activity; C = control; R = ranitidine, 10^{-5} M. Results are $\bar{x}$ + SE of triplicate incubations assayed in triplicate. * $P < 0.05$; ** $P < 0.01$.

protein) and activates the catalytic site of adenylate cyclase in the absence of cell membrane receptors, produced comparable stimulation of control and the ovalbumin sensitized I and II (IgG; IgG/IgE) lung membranes.

Histamine. Histamine (10^{-8}–10^{-4} M) produced a dose-dependent activation of the enzyme with maximal stimulation of 190% at 10^{-5} M histamine. This activation was abolished in the presence of ranitidine (10^{-5} M), an H_2 receptor antagonist (Fig. 8, see Ref. 15). Mepyramine (10^{-5} M), an H_1 receptor antagonist, was much less effective having a significant inhibitory effect at 10^{-6} M histamine (see Fig. 8). Sensitization II (IgC/IgE) produced no difference in the histamine activation of adenylate cyclase. The histamine dose–response curves of the control and sensitized II enzyme were superimposable if the basal activity was subtracted.

Peptides. Both bradykinin and bombesin (10^{-11}–10^{-7} M) were more potent activators of adenylate cyclase than VIP (10^{-9}–10^{-5} M), in lung membranes from control animals. As illustrated in Fig. 9, VIP produced a greater maximal stimulation of 160% compared with 141% for bradykinin and 137% for bombesin. Stimulation of the enzyme by VIP was reduced ($P < 0.001$) in the sensitized II (IgG/IgE) lung membrane fraction (Table 4; Fig. 10). In contrast, stimulation of the cyclase by bradykinin was unaffected by either type of sensitization (see Fig. 10).

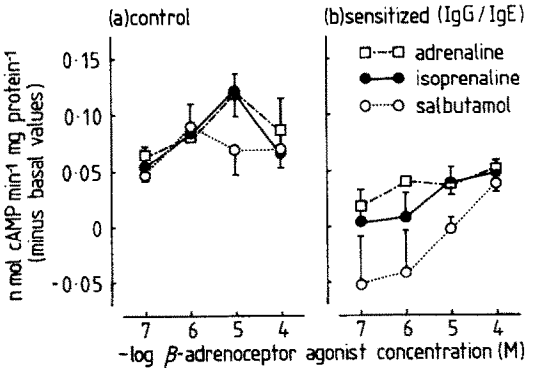

Fig. 7. Activation of (a) control and (b) IgG/IgE sensitized II guinea-pig lung adenylate cyclase by adrenaline (□——□), isoprenaline (●——●) and salbutomal (○---○). Results are $\bar{x}$ SE of at least 3 experiments.

DISCUSSION

An ideal model of allergic bronchial asthma should exhibit most, if not all, the features on human bronchial asthma. The guinea pig is often used as such a model. Sensitized guinea pigs exhibit the typical characteristics of allergic bronchial asthma, including the physiological responses to antigenic challenge,

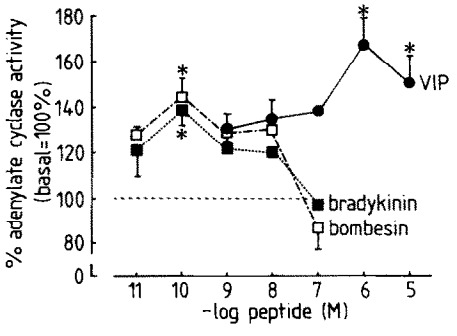

Fig. 9. Activation of adenylate cyclase from control lung membranes by VIP, bradykinin and bombesin. Agonist activation of adenylate cyclase activity is expressed as a percentage of basal activity in absence of any drug. Basal enzyme activity for each agonist as follows in nmol cAMP min^{-1} mg $protein^{-1}$: VIP 0.170 ± 0.01 (●——●); bradykinin 0.215 ± 0.01 (■---■); bombesin 0.145 ± 0.012 (□—·—□). * $P < 0.05$, ** $P < 0.01$.

Table 4. Effect of vasoactive intestinal peptide (VIP) on sensitized II lung membrane adenylate cyclase

Source of variation	Sensitized II (basal activity substracted) DF	SOS	V	F	Significance
VIP					
Between doses	4	24349	6087	9.5	$P < 0.001$
Between treatments	1	7239	7239	11.2	$P < 0.001$
Interaction	4	941	235	0.4	NS
Replication	20	12875	644		
Total	29	45404			

The summary table of the analysis of variance demonstrates that VIP produces a dose-dependent stimulation of adenylate cyclase from sensitized II ($P < 0.001$) membranes. The "between treatments" analysis clearly indicates the reduction in the activation of sensitized II membrane AC by VIP ($P < 0.001$).

DF, degrees of freedom, SOS, sums of squares; V, variance; F, variance ratio; NS, not significant.

the immunological pathways leading to bronchoconstriction and mucociliary dysfunction, and possibly hyper-reactivity to non-specific stimuli [16].

It could be argued that the guinea pigs used in this study may have been in contact with antigens which may have caused immunological sensitization additional to that produced by ovalbumin. However, since paired groups of animals were used for the control and sensitized I or II homogenates, the effect of non-specific antigens was adequately controlled. Because guinea-pig IgE antibodies have yet to be purified to homogeneity and the absence of guinea-pig IgE antiserum, it was not possible to measure directly the total IgE titres. The PCA method provided indirect evidence which confirmed the finding of Andersson [2] that low-dose ovalbumin sensitization raises in the guinea pig, in addition to IgG, circulating IgE antibodies. Many studies using the guinea pig model for asthma have been performed with high-dose ovalbumin only [17, 18]. The effect on basal activity of lung adenylate cyclase is dramatically altered only when a low dose sensitization regime is used. Mammalian adenylate cyclase exhibits basal activity that can be modulated by various agents, and in this respect the guinea-pig lung enzyme is no exception. Mathé *et al.* [17] and Burka and Saad [18] have reported that basal adenylate cyclase activity of guinea-pig lung homogenates was unaffected by ovalbumin sensitization. In the experiments of both groups the guinea-pigs were injected with high doses of ovalbumin (approx. 100 mg/kg^{-1}) which presumably raised IgG antibodies only according to the observations of Andersson [2], although this was not stated in their publications. In our study, similarly, there was no difference in the basal adenylate cyclase of control and high dose sensitized I (IgG) guinea-pig lung membranes, within whom the presence of circulating IgG antibodies was identified. In contrast to this observation, the basal enzyme activity of low-dose sensitized (II) lung membranes showed at least a two-fold increase above control values. The molecular change responsible for this increase in basal activity appears to arise from an increased responsiveness of the enzyme to GTP. Cassel and Selinger [19, 20] demonstrated, in turkey erythrocytes, that the GTP binding site on Ns resided on a GTPase present within the membrane, which hydrolysed the nucleotide thereby terminating the catalytic activity of the enzyme. Interestingly, in our study, activation of the lung adenylate cyclase by GppNHp, a non-hydrolysable analogue of GTP, was similar in both control and IgG/IgE sensitized guinea-pig membranes, thereby suggesting that the sensitizing process reduces the hydrolytic activity of the GTPase located on Ns (guanine nucleotide regulatory protein) resulting in a more effective activation of the enzyme. This view was given credence by the finding that sensitization by ovalbumin did not significantly alter stimulation of adenylate cyclase by forskolin; which is known to directly activate the catalytic unit [21].

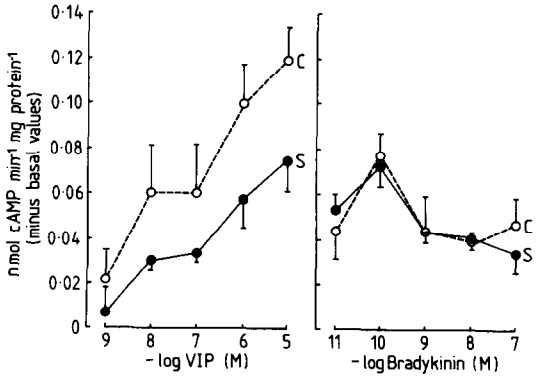

Fig. 10. Comparison of the effect of vasoactive intestinal peptide (VIP) and bradykinin on sensitized II (IgG/IgE) lung membrane adenylate cyclase. Basal activity has been subtracted. Symbols indicate the following: Sensitized II (IgG/IgE) = S ●——●; control = C ○---○. Results are $\bar{x}$ + SE of 3 experiments. Incubations were performed and assayed in triplicate.

Our data clearly indicate that ovalbumin sensitization alters the responsiveness of guinea-pig lung adenylate cyclase to activation by β-adrenoceptor agonists (adrenaline, isoprenaline, terbutaline and salbutamol). On the other hand, a decreased responsiveness to VIP and an increase in basal activity appears to be intimately linked to the presence of IgE antibodies, as this only occurred in the sensitized

II membranes. In contrast, activation of lung adenylate cyclase by bradykinin was unaffected.

Homogenates of lung membranes will obviously contain membranes from several cell types (namely, epithelial, mucosal, mast and smooth muscle cells), all of which may contribute to the adenylate activity measured in this study. Although we cannot provide evidence, in the present experiments, as to the precise cell type and manner in which ovalbumin sensitization affects the guinea-pig lung adenylate cyclase complex, several mechanisms could explain the decreased responsiveness of receptor-coupled lung membrane enzyme to stimulation by β-adrenoceptor agonists and VIP. One explanation could be that the sensitization process selectively alters the coupling of the receptor (R)-guanine nucleotide regulatory protein (Ns)-adenylate cyclase complex. The decreased responsiveness of the guinea-pig lung adenylate cyclase is unlikely to have occurred as a result of a biochemical lesion between the Ns-protein and the catalytic unit, as sodium fluoride stimulation of the cyclase was unaffected by the sensitization process. The reduced responsiveness could result from uncoupling of particular receptors to the adenylate cyclase enzyme complex, similar to that observed in a mutant of mouse S49 lymphoma cells called UNC. The "uncoupled" cell is unresponsive to receptor mediated stimulation of adenylate cyclase, although all of the major components of the complex receptor, Ns-protein and the catalytic unit are present [22]. Ovalbumin sensitization may selectively alter the coupling of β-adrenergic and VIP receptors to lung membrane adenylate cyclase.

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