4.1 RESPONSES OF NORMAL AND SENSITIZED AIRWAY TISSUES TO BRONCHOCONSTRICTORS AND BRONCHODILATORS

4.1.1 Contractile responses to bronchoconstrictors:

The forces generated by tracheal spirals and parenchymal strips after additions of histamine, carbachol, U-44069, LTC_4, and LTD_4 were similar in normal and sensitized guinea pigs (Fig. 5). Since increases in force over baseline tension after additions of histamine were not different in normal and sensitized tissues, all the data were normalized by expressing responses as a percent of the histamine maximum on each tissue obtained in the absence of any modulatory drug (100 μM histamine=100%). The resulting C-R curves were less variable but equivalent to those expressed in absolute force units; i.e., mg force per mg tissue dry weight, data not shown. Histamine was chosen as the reference agonist because of the potential importance of this mediator in allergic asthma.

On the trachea the cholinergic agonist carbachol induced the greatest increase in force at the maximum concentration used (30 μM). Histamine was the next most effective agent followed by the endoperoxide analog U-44069 (Table 7). The C-R curves to the latter agent had a biphasic profile with an initial plateau at about 1 nM. This effect was observed in both normal and sensitized tracheal spirals. LTC_4 and LTD_4 induced the least increases in force when added at concentrations of 0.1 μM, the maximum we could use due to limited availability of these synthetic agents. LTC_4 was equiactive

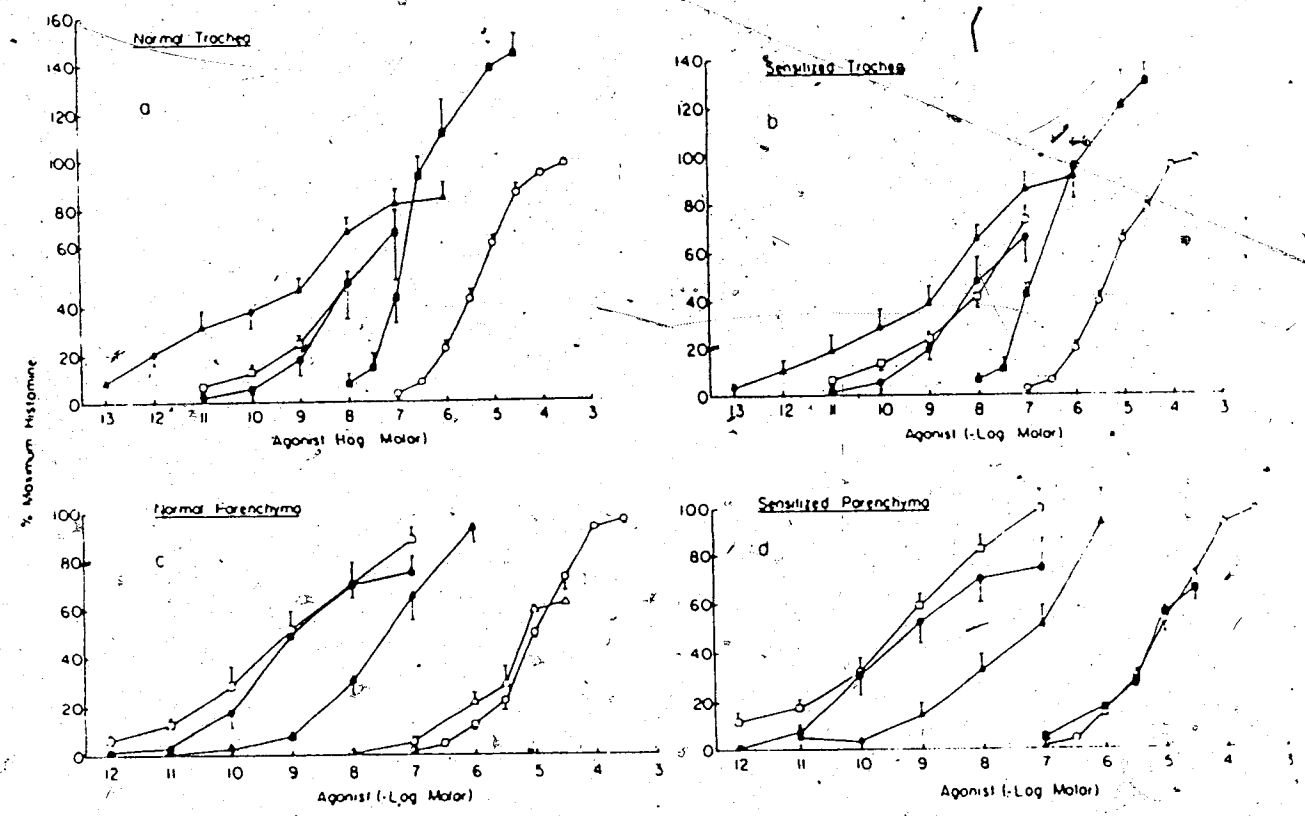


Fig. 5. Concentration-response curves to bronchoconstrictors in tracheal spirals (a and b) and parenchymal strips (c and d) from normal and ovalbumin-sensitized guinea pigs. Values are the mean ± SEM (n=number of animals used for each drug and is shown for normal trachea, sensitized trachea, normal parenchyma, and sensitized parenchyma, respectively after each drug symbol). Bronchoconstrictors were histamine (○) (13,13,10,13), carbachol (■) (6,6,6,10), leukotriene C_4 (●) (3,3,3,3), leukotriene D_4 (□) (5,11,10, 9), and U-44069 (▲) (6,6,5,6).

Table 7. Increases in tension over baseline after the addition of bronchoconstrictor agonists to normal and sensitized airway tissue.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Histamine (300 μM)	154.5±22.9 (13)	166.2±19.6 (13)	13.2±1.9 (10)	11.4±0.94 (13)
Carbachol (30 μM)	288.5±43.0 (6)	256.0±43.0 (6)	8.0±1.7 (5)	6.7±0.6 (10)
U-44069 (1 μM)	152.7±14.6 (6)	159.8±37.2 (6)	9.7±1.2 (5)	11.6±3.1 (6)
LTC_4 (0.1 μM)	129.7±33.9 (3)	102.3±16.3 (3)	9.1±3.6 (3)	7.3±1.3 (3)
LTD_4 (0.1 μM)	126.1±7.42 (5)	140.6±28.0 (6)	9.32±0.88 (10)	9.6±1.2 (9)

NOTE: Mean ± SEM, values in parentheses are number of animals. Values are in mg force per mg of tissue dry weights.

to LTD_4 on normal and sensitized trachea.

Histamine induced a greater contractile force on lung parenchymal strips than carbachol. Since U-44069, LTC_4 and LTD_4 were not used at concentrations sufficiently high to cause maximum responses, their efficacies could not be compared. Nonetheless, at the maximum concentrations used (Figs. 5c, 5d) these agents increased force to levels close to the histamine maximum response (Table 7).

The threshold concentrations of the above agents, as extrapolated from C-R curves, were similar in the appropriate tissues of normal and sensitized animals. However, differences were noted between the trachea (large airways) and the lung parenchyma (small airways). U-44069 was bronchoconstrictor on the trachea at concentrations as low as 10^{-13} M, whereas, on the parenchyma, a contractile effect was observed at about 10^{-11} M. LTD_4 induced contractile effects on the parenchyma at 10^{-12} M, but only caused an equivalent effect (percent histamine maximum) on the trachea at a concentration of 10^{-11} M (Fig. 5).

4.1.2 Sensitivity of tracheal and parenchymal strips to bronchoconstrictors:

The pD_2 and -log EC_{50} histamine maximum values for histamine, carbachol, U-44069, LTC_4, and LTD_4 on normal and sensitized airway tissues are shown in Table 8. There was no observable difference in sensitivity of the airways to the above bronchoconstrictors between

Table 8. Sensitivity of airway tissues from normal and sensitized animals to bronchoconstrictor agents *in vitro*.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Histamine	5.34±0.08[a] (13)	5.4 ±0.08[a] (15)	5.01±0.04[a] (10)	5.17±0.09[a] (14)
Carbachol	6.41±0.13[a] (6)	6.59±0.09[a] (6)	5.58±0.05[a] (10)	5.45±0.11[a] (6)
U-44069	9.7±0.3[a] (5)	9.1 ±0.3[a] (6)	7.14±0.17[b] (6)	7.21±0.24[b] (6)
LTC_4	8.2±0.3[b] (3)	8.17±0.2[b] (3)	8.89±0.06[b] (3)	8.96±0.6[b] (3)
LTD_4	7.82±9.26[b] (5)	7.86±0.15[b] (10)	8.9 ±0.25[b] (10)	8.85±0.28[b] (10)

NOTE: Mean ± SEM. values in parentheses are number of animals.

[a] pD_2 = -log EC_{50} maximum of drug.

[b] -log EC_{50} histamine maximum.

normal and ovalbumin-sensitized animals as determined in vitro (Fig. 5, Table 8). The order of potency of the agonists on normal and sensitized trachea was U-44069 > LTC_4 > LTD_4 > carbachol > histamine. On the parenchyma the order was LTC_4 = LTD_4 > U-44069 > carbachol > histamine.

In all our experiments, challenge of sensitized tissues with OA resulted in a prolonged dose-dependent contraction. Concentrations as low as 0.1 μg/ml were effective with a maximum obtained at about 10 μg/ml. Addition of OA (100 μg/ml) to normal tissues did not induce any observable effects. Hence the basic criterion of hypersensitivity to a specific sensitizing antigen was fulfilled.

4.1.3 Effects of cyclooxygenase and lipoxygenase inhibitors on the responsiveness and sensitivity of airways to bronchoconstrictors:

Indomethacin (8.5 μM), NDGA (30 μM), and phenidone (185 μM) reduced the tone of tracheal spirals but had no effect on the tone of parenchymal strips (Table 9). The tone of tracheal spirals was reduced most markedly by phenidone and least by NDGA. Tone of normal and sensitized tracheal spirals were reduced to a similar extent. The effects of indomethacin, NDGA, and phenidone on responses of the trachea to histamine and carbachol are shown in Fig. 6. For both of these bronchoconstrictors the tension developed to low concentrations was reduced whereas tension developed to higher and maximal

Table 9. Effects of inhibitors on tone and percentage change from preincubation tone.

	Trachea		Parenchyma	
Inhibitors	Normal	Sensitized	Normal	Sensitized
Indomethacin (8.5 μM)	-25.9±3.4% (21)	-29.9±5.9% (26)	-4.1±0.7% (14)	-4.2±0.5% (15)
Phenidone (185 μM)	-44.5±5.4% (21)	-41.9±3.7% (25)	-4.8±1.0% (14)	-5.5±0.7% (16)
NDGA (30 μM)	-15.3±4.3% (22)	-12.2±5.6% (25)	-3.5±0.7% (14)	-3.5±0.9% (15)
Vehicle (control)	±23.8±5.6% (22)	+18.7±6.4 (26)	-2.8±0.5% (14)	-2.6±0.97% (15)

NOTE: Mean ± SEM, values in parentheses are number of animals.

*$p < 0.01$ versus control.

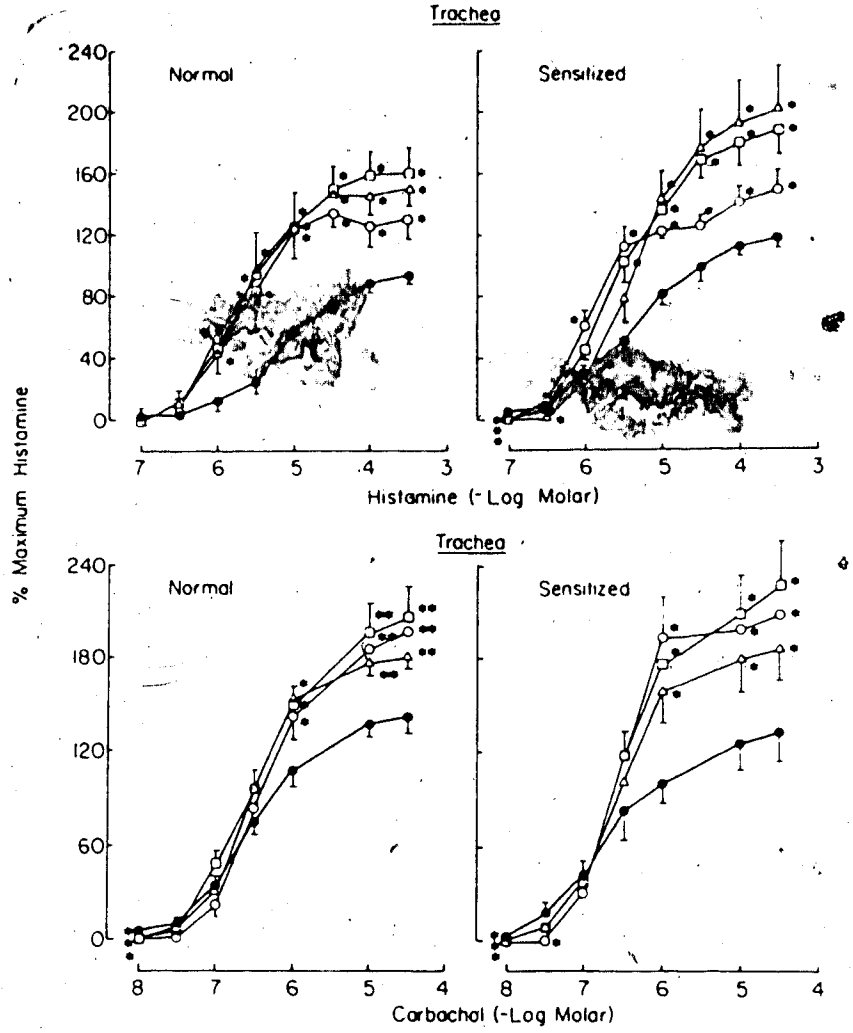


Fig. 6. Concentration-response curves to histamine (upper panel) and carbachol (lower panel) on normal and sensitized guinea-pig tracheal spirals, after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Values are mean ± SEM (n=5 for each curve). Significant differences from paired control responses are as indicated: *$p < 0.05$ and **$p < 0.01$.

concentrations was enhanced significantly ($p<0.05$) when compared with the appropriate paired control tissue. The responses in the presence of the inhibitors were not different between normal and sensitized tissues.

Figure 7 shows the responses of the trachea to LTC_4 and LTD_4 after incubation with the inhibitors. There was a reduction in responses to low concentration of these agents in both normal and sensitized tissue. There was also a tendency towards enhancement to higher concentrations of these agents although this was not statistically significant due to large variability of the observations.

The responses of the trachea from normal and sensitized animals to the endoperoxide analog U-44069 after incubation with indomethacin (0.28, 2.8, and 8.5 μM) are shown in Fig. 8. The responses to lower concentrations of U-44069 were unaffected by prior and continued exposure to indomethacin. On normal trachea the responses to higher concentrations of U-44069 were significantly enhanced ($p<0.05$) after exposure to indomethacin (2.8 and 8.5 μM), whereas no such enhancement was observed on sensitized tracheal spirals. Furthermore, the enhanced responses of normal trachea were significantly ($p<0.05$) different from the comparable responses on sensitized tissues.

The responses of normal and sensitized lung parenchymal strips to histamine, carbachol, and U-44069 were not affected by prior treatment with the inhibitors (Fig. 9).

LTC_4 and LTD_4 C-R curves were not reproducible on the parenchyma

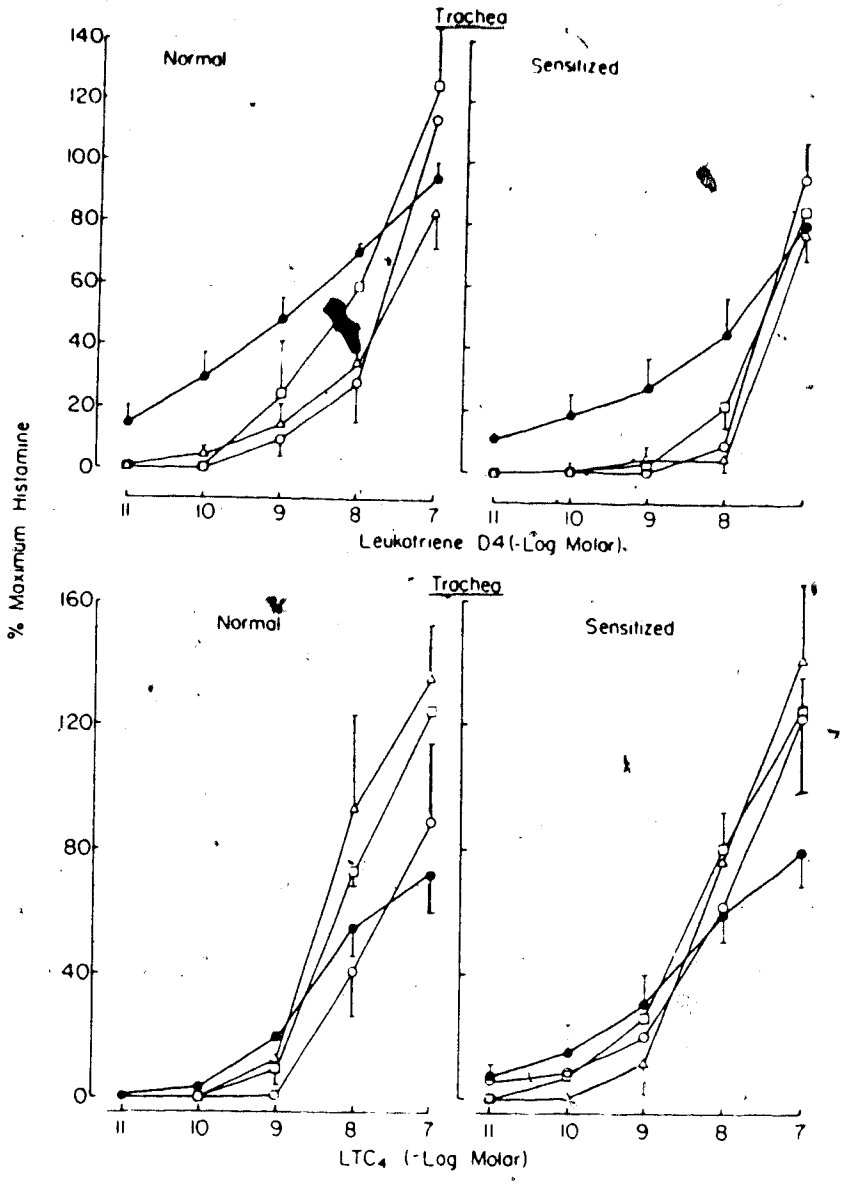


Fig. 7. Concentration-response curves to leukotrienes C_4 and D_4 on normal and sensitized guinea pig tracheal spirals after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Values are mean ± SEM (n=3 animals/curve for leukotriene C_4 and 6 animals/curve for leukotriene D_4).

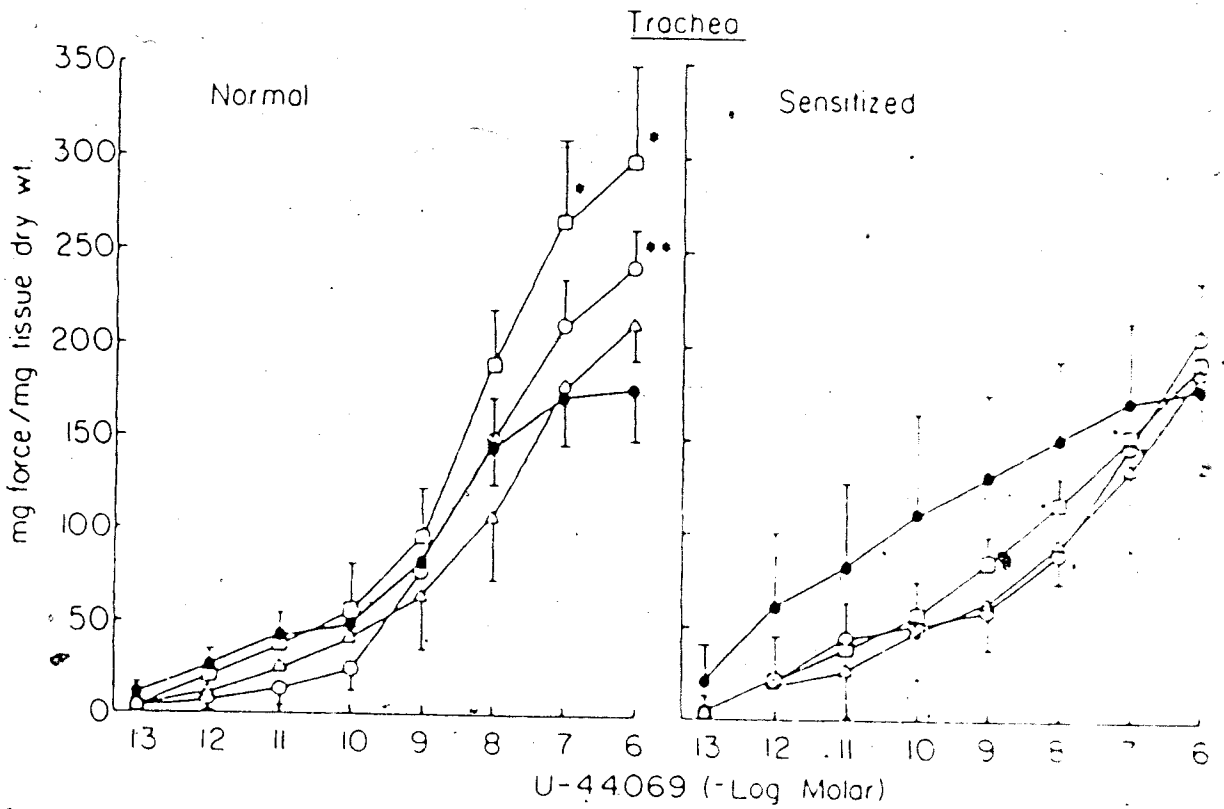


Fig. 8. Concentration-response curves to U-44069 on normal and sensitized tracheal spirals after pretreatment with indomethacin (0.28 μM) (△), (2.8 μM) (○), (8.5 μM) (□), or the paired control tissues (●). Responses are mean±SEM of the absolute force developed over resting tone, divided by the tissue dry weight (n=5 animals for each curve). Significant differences from paired control responses are as indicated: *p<0.05, **p<0.01. Significant differences between similarly treated tissues from normal and sensitized guinea pigs are also indicated in the sensitized tissues panel (*p<0.05).

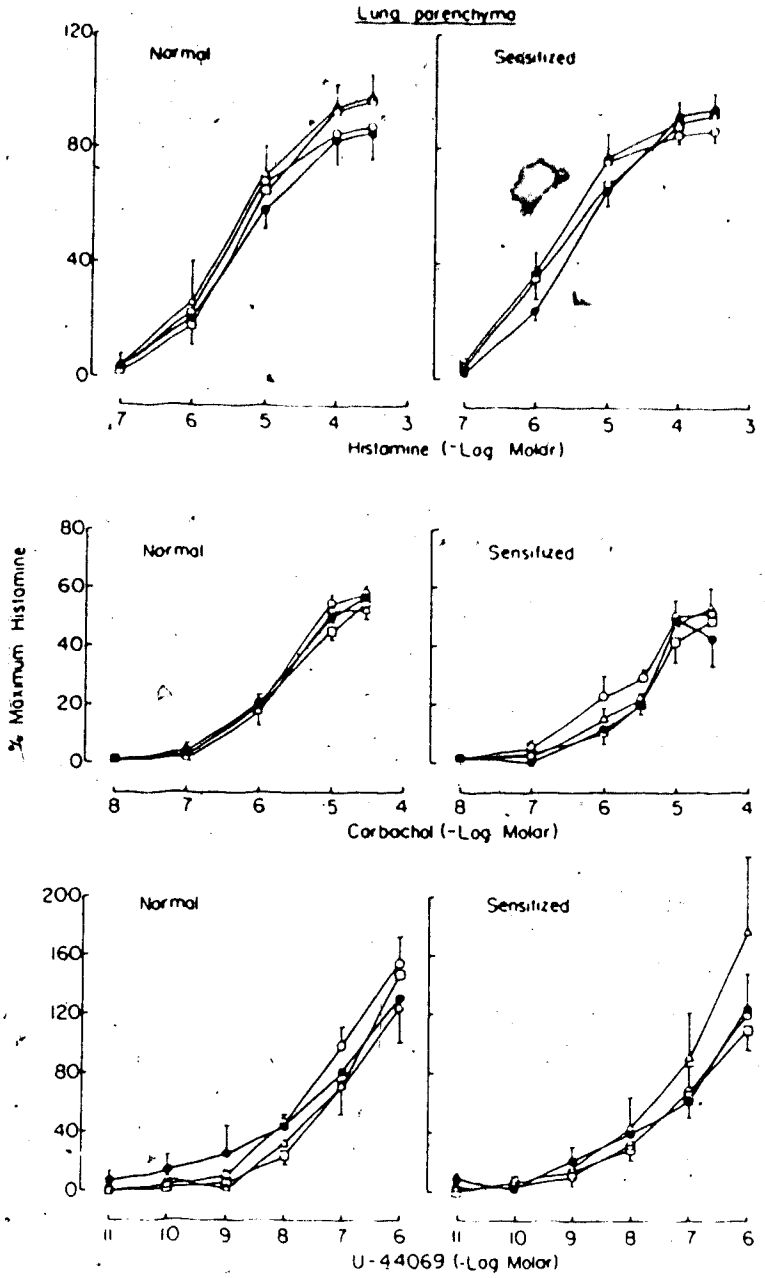


Fig. 9. Concentration-response curves to histamine (top panel) and carbachol (middle panel) on normal and sensitized guinea pig lung parenchymal strips after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Responses to U-44069 (bottom panel) after indomethacin pretreatment (0.28 μM) (△), (2.8 μM) (○), (8.5 μM) (□), or the paired control responses (●) also are shown. Results are presented as mean±SEM (n>5 for each curve).

as mentioned earlier. The tissues were primed, then incubated wi
the inhibitors (vehicle for the paired control tissue), and then a
C-R curve was generated on each tissue. Figure 10 shows the respon
of normal and sensitized parenchyma to LTC_4 and LTD_4 after the abo
treatment. All the inhibitors reduced the contractile response to hi
concentrations of LTC_4 and LTD_4 (10^{-10} to 10^{-7} M). Responses to l
concentrations of these agonists (10^{-12}, 10^{-11} M) were also reduced

Concentration-response curves for the bronchoconstrictors i
presence of modulatory drugs were the same if established from
reduced basal tone which is a consequence of these drugs or
established from a tone readjusted mechanically to 1 g tension. T
ensured that changes in the responsiveness and sensitivity, as
consequence of cyclooxygenase and lipoxygenase inhibitors, were
due to changes in tone.

The sensitivities of normal and sensitized trachea to histami
carbachol, and U-44069 were not signfiicantly different from those
subsequently generated paired time-control curves. The pD_2 val
from the initial C-R curves for histamine and carbachol on norm
trachea were 5.47±0.13 (n=8) and 6.57±0.08 (n=10) (mean±SEM), wher
the comparable pD_2 values derived after generation of a second
curve were 5.21±0.06 (n=8) and 6.52±0.09 (n=10) ($p>0.05$).

The pD_2 values for histamine were unchanged after treatment
indomethacin, phenidone, and NDGA on normal and sensitized tra
(Table 10). Sensitivity of normal and sensitized trachea to carba

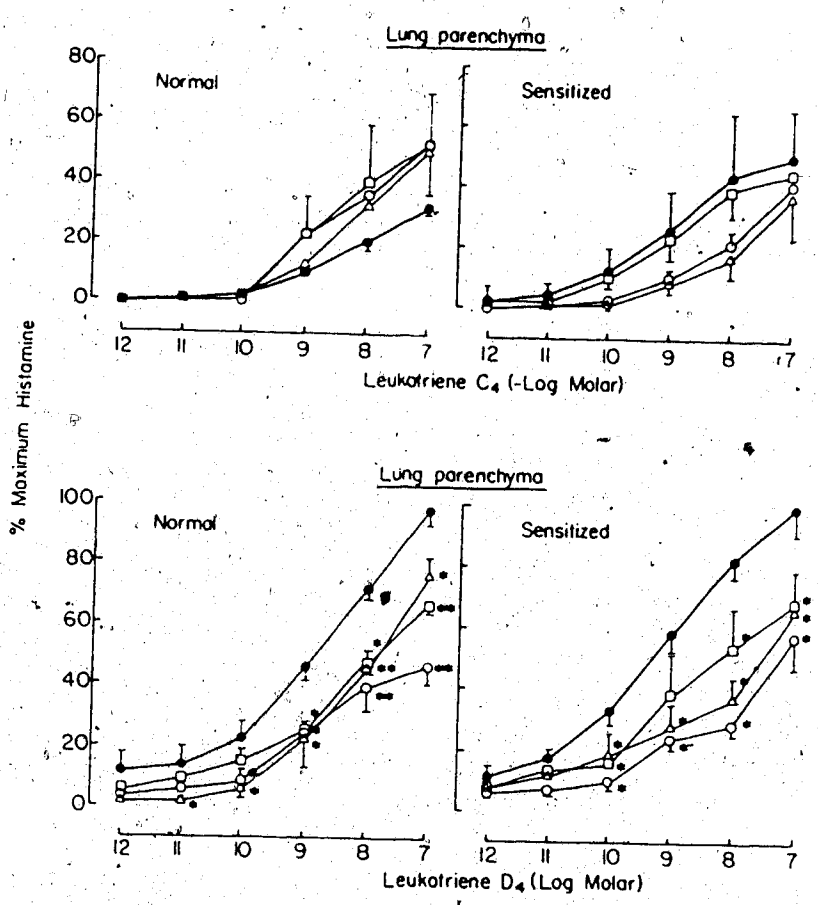


Fig. 10. Concentration-response curves to leukotrienes C_4 (n=3) and D_4 (n=6) on normal and sensitized lung parenchymal strips, after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). The responses to leukotriene C_4 (upper panel) were those obtained after generation of an initial concentration-response curve. Results are mean±SEM. Significant differences from paired control responses are as indicated: *$p<0.05$ and **$p<0.01$.

Table 10. Effect of inhibitors on sensitivity (pD_2) of tracheal spirals to histamine and carbachol.

Inhibitors	Histamine		Carbachol	
	Normal	Sensitized	Normal	Sensitized
Indomethacin (8.5 μM)	5.33±0.13 (8)	5.37±0.08 (12)	6.41±0.08 (9)	6.53±0.09 (9)
Phenidone (185 μM)	5.47±0.16 (8)	5.45±0.12 (9)	6.26±0.08* (8)	6.32±0.05** (8)
NDGA (30 μM)	5.32±0.16 (8)	5.22±0.06 (11)	6.53±0.07 (9)	6.56±0.06 (9)
Time and vehicle control	5.21±0.06 (8)	5.19±0.20 (12)	6.52±0.09 (10)	6.57±0.07 (9)

NOTE: Mean ± SEM, values in parentheses are number of animals.

*$p < 0.05$, **$p<0.01$., relative to control tissue.

was significantly reduced ($p<0.05$) following incubation with phenidone (185 μM). The sensitivity of tracheal spirals and parenchymal strips to U-44069 was unaffected by indomethacin treatment.

4.1.4 Relaxant responses to bronchodilators:

Isoproterenol, VIP, PGE_2 and forskolin caused a dose-dependent relaxation of the guinea-pig trachea and the lung parenchymal strips (Fig. 11). There was no difference between normal and sensitized tissues in the relaxant responses to all four drugs used. The rank order of potency of these agents on the airways was VIP > isoproterenol > PGE_2 > forskolin (Table 11). The parenchyma was more sensitive than the trachea to these drugs. Normal and sensitized tissues exhibited similar sensitivity to these agents. Isoproterenol was the most efficacious drug used on the trachea causing the most relaxation (Fig.11, Table 12). Forskolin was the next most efficacious drug with VIP and PGE_2 being equivalent. PGE_2 relaxed airways at low concentrations. However, at higher concentrations (>1 μM) this agent sometimes contracted the lung parenchymal strip (Fig. 11).

4.1.5 Effects of indomethacin-pretreatment on drug-induced relaxations:

Indomethacin (8.5 μM)-pretreatment did not significantly change

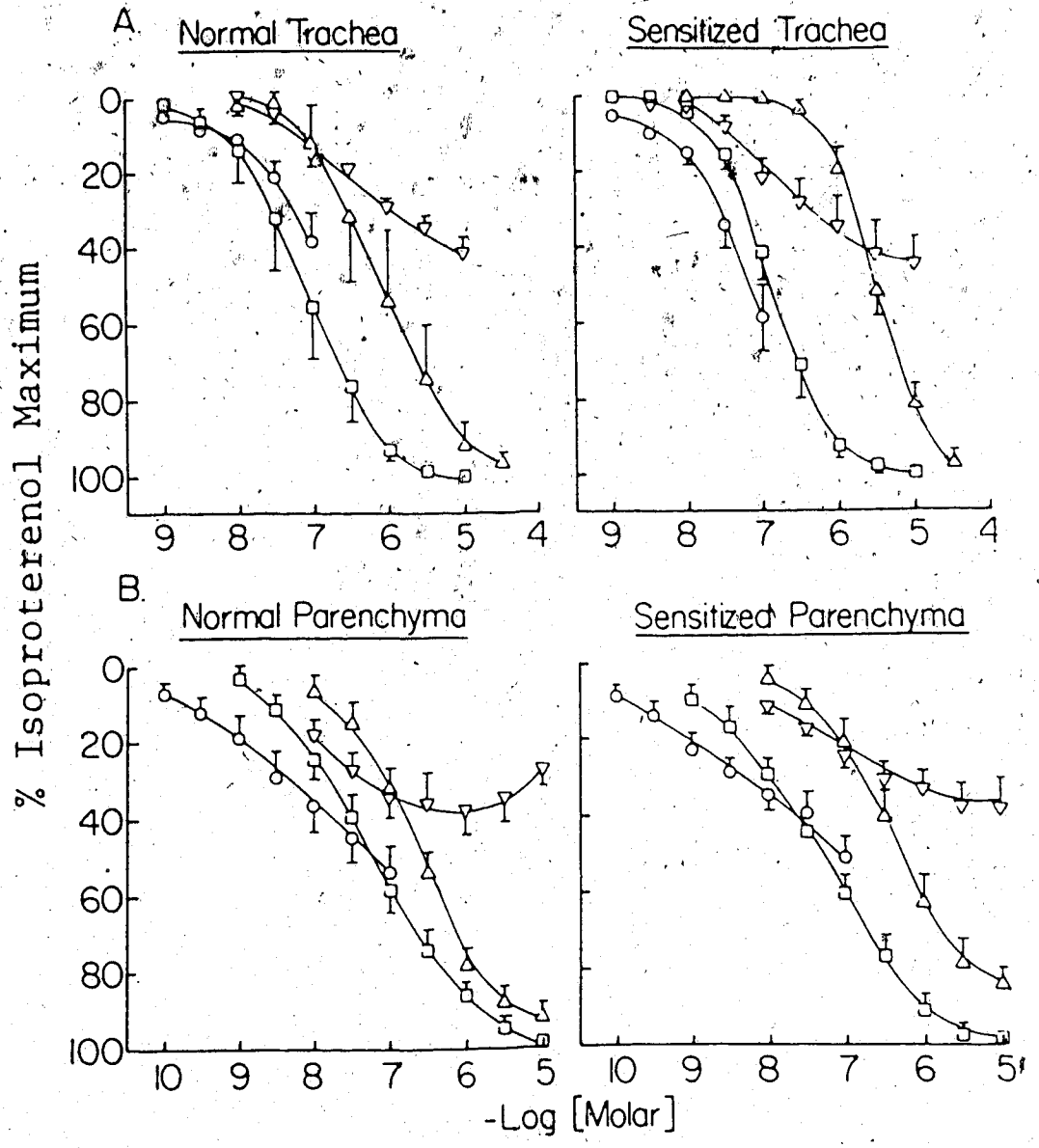


Fig. 11. The relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals (a) and lung parenchymal strips (b). Symbols indicate the following: (□) isoproterenol, (△) forskolin, (○) vasoactive intestinal peptide and (▽) prostaglandin E_2. Tracheal spirals were precontracted with carbachol (1 μM) and lung parenchymal strips were mechanically adjusted 1 g to baseline tension. Results are mean±SEM (n=5).

Table 11. Sensitivity[a] of airway tissues from normal and sensitized animals to relaxant agents in vitro.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol	7.11±0.27[b]	6.80±0.13	7.19±0.17	7.30±0.11
Forskolin	6.10±0.30	5.44±0.13	6.66±0.09	6.40±0.13
VIP	7.66±0.06	7.59±0.05	8.51±0.18	8.73±0.14
PGE_2	6.54±0.15	6.83±0.28	7.62±0.24	7.37±0.21

[a] = Negative logarithm of the concentration of each drug required to achieve 50% of the maximum response to that drug.

[b]Mean±SEM (n=5).

Table 12. Maximum control relaxations (mg force mg^{-1} tissue dry weight) of airway tissues from normal and ovalbumin-sensitized guinea pigs after addition of relaxant drugs in vitro.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol (10 μM)	120.8±21.4[a]	111.9±18.5	7.9±0.7	9.3±1.1
Forskolin (30 μM)	106.7±17.8	86.8±10.2	10.8±1.4	11.7±0.8
VIP (0.1 μM)	35.7±10.3	69.7±18.8	3.3±0.5	4.6±1.0
PGE_2 (trachea: 30 μM) or (parenchyma: 10 μM)	39.2± 8.3	55.9±16.0	2.6±0.8	3.1±0.7

[a] = Mean±SEM of 5 experiments.

the efficacy of isoproterenol, VIP, PGE_2 or forskolin in causing relaxations of the normal or sensitized trachea (Fig. 12). Furthermore, the sensitivity of the tissues to the drugs (expressed as the negative logarithm of the EC_{50}) was unchanged. All comparisons were relative to the paired time and vehicle control tissue (Table 13).

Indomethacin (8.5 μM)-pretreatment significantly ($p<0.05$) reduced the magnitude of the relaxation of parenchyma induced by high concentrations of isoproterenol (1-10 μM), forkskolin (1-10 μM) and PGE_2 (0.1-10 μM) on normal and sensitized lung parenchyma (Fig. 13). However, the EC_{50} of each drug was unchanged when compared to control. As with the trachea, all comparisons are relative to the paired time and vehicle control tissue (Table 13).

4.1.6 Effects of bronchodilators on adenylate cyclase activity in control and indomethacin-pretreated lung membranes:

The adenylate cyclase of normal and sensitized guinea-pig lung had basal activities (measured in the absence of any drugs) of 54.8± 9.6 and 60.3±8.2 pmol cyclic AMP mg^{-1} protein min^{-1}, respectively. Responses of lung adenylate cyclase to isoproterenol were hormone-specific (Fig. 14) since they were abolished by the β-adrenoceptor antagonist propranolol (1 μM), whereas the responses to PGE_2, VIP and forskolin were unaffected by propranolol. Isoproterenol, VIP, PGE_2 and forskolin stimulated normal and sensitized guinea-pig lung adenylate cyclase activity. There was no difference in the magnitude

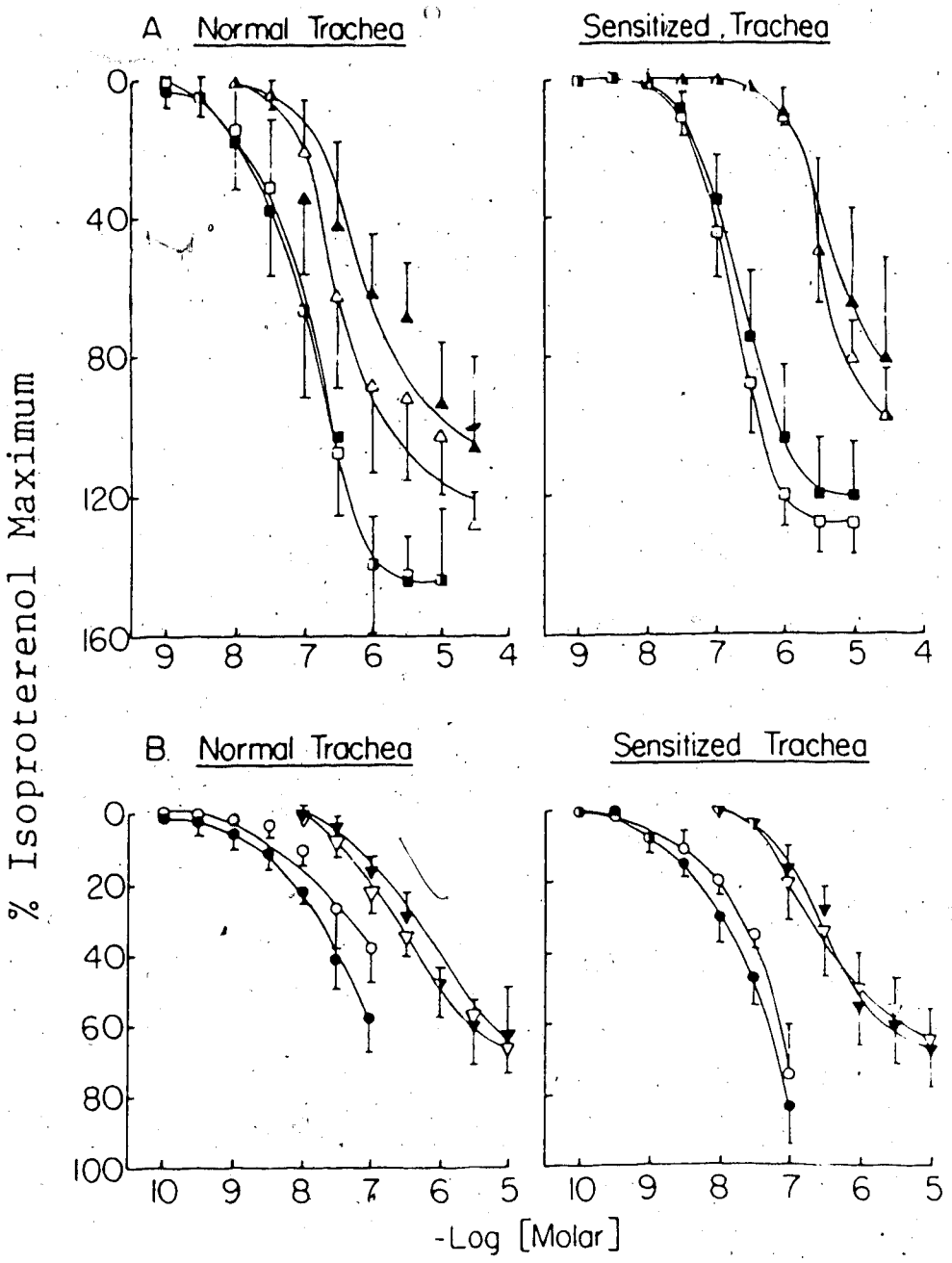


Fig. 12. Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals to isoproterenol (□,■) forkskolin (△,▲) (a) and vascoactive intestinal peptide (○,●), and prostaglandin E_2 (▽,▼) (b). Open symbols represents the responses of the time and vehicle control tissues and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissues. Results are mean±SEM (n=5).

Table 13. Sensitivity[a] of airway tissue from normal and sensitized animals to relaxant agents in vitro. Effect of indomethacin pretreatment.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol	6.90±2.22[b]	6.80±0.13	7.10±0.20	7.30±0.19
	(7.17±0.37)[c]	(6.60±0.19)	(7.14±0.17)	(7.21±0.17)
Forskolin	6.24±0.5	5.50±0.14	6.26±0.33	6.25±0.20
	(6.10±0.39)	(5.51±0.26)	(6.37±0.29)	(5.81±0.25)
VIP	7.71±0.16	7.51±0.05	8.45±0.26	8.67±0.24
	(7.85±0.14)	(7.65±0.13)	(8.44±0.28)	(8.96±0.49)
PGE_2	6.62±0.24	6.53±0.23	6.78±0.09	7.26±0.19
	(6.40±0.07)	(6.66±0.22)	(not determined)	(not determined)

[a] = -Logarithm of EC_{50}

[b] = Time control tissue

[c] = Indomethacin (8.5 μM) treated.

values are mean±SEM (n=5).

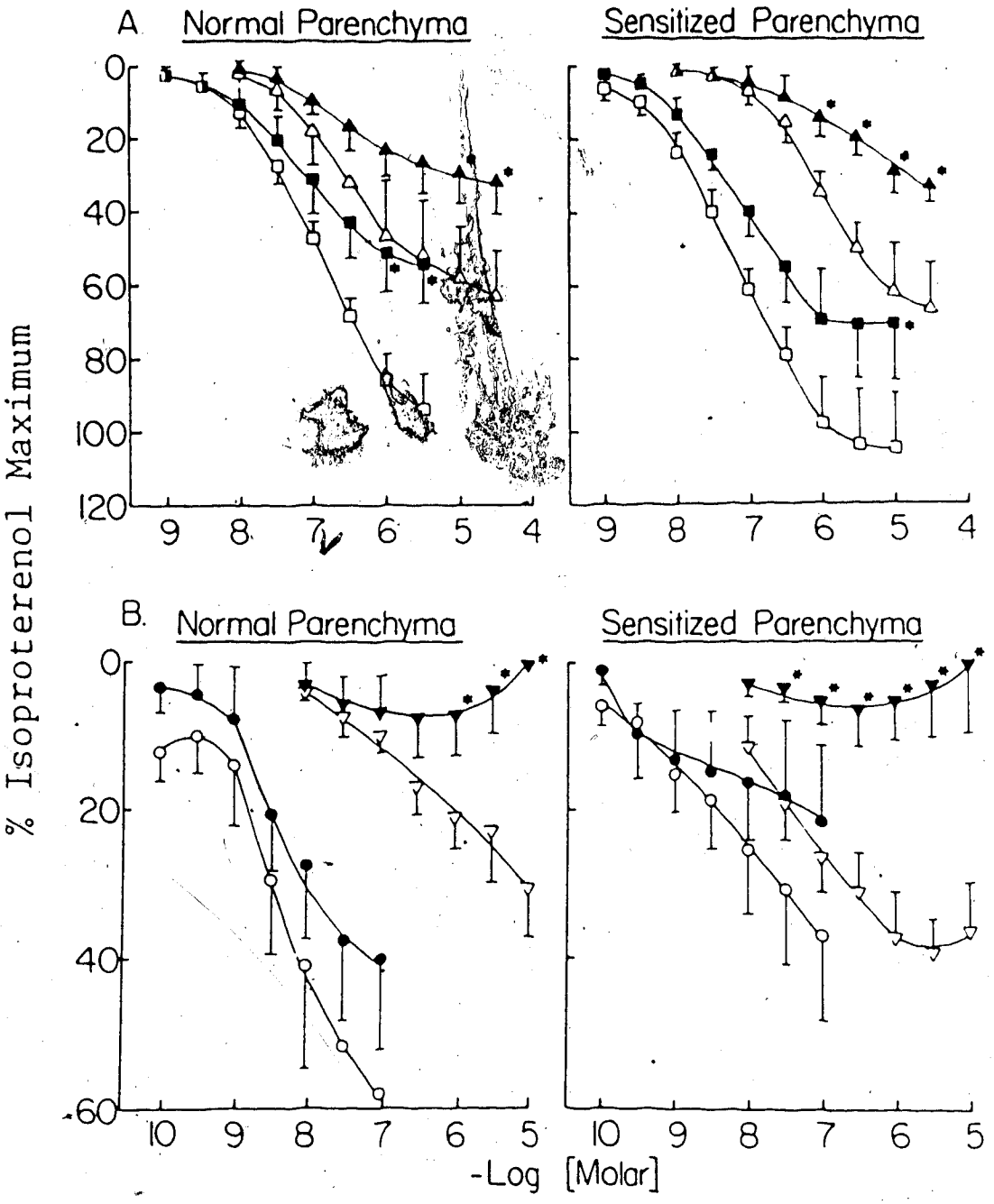


Fig. 13. Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig lung parenchymal strips to isoproterenol (□, ■), forskolin (△, ▲) (a) and VIP (○, ●), and PGE_2 (▽, ▼) (b). Open symbols represent the responses of the time and vehicle control tissue and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissues. *Significantly different ($p<0.05$) from the time and vehicle control tissue responses. Results are mean±SEM (n=5).

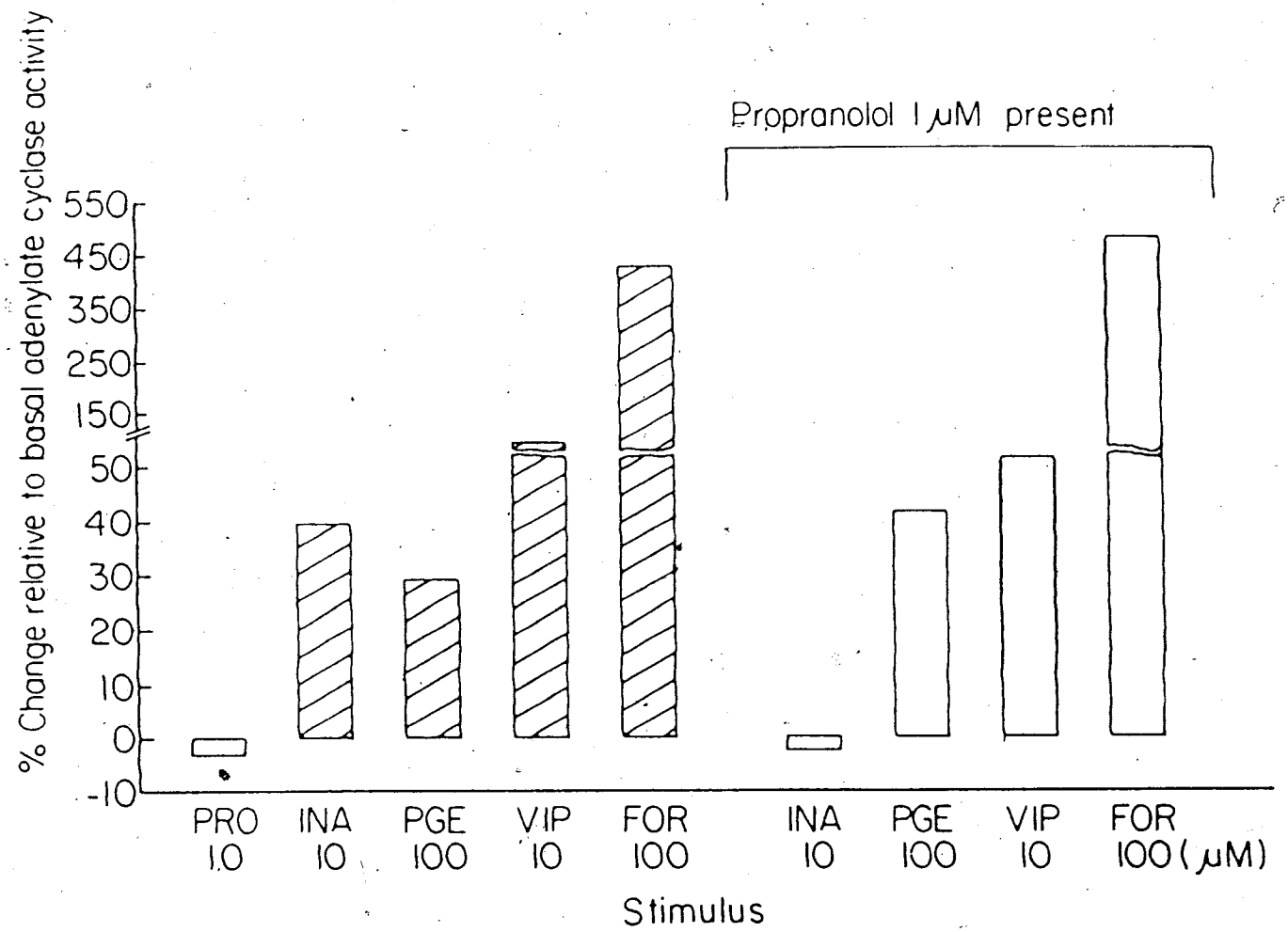


Fig. 14. Hormonal specificity of the responses of guinea-pig lung parenchymal adenylate cyclase. Activation of adenylate cyclase by isoproterenol (INA), prostaglandin E_2 (PGE_2), vasoactive intestinal peptide (VIP) and forskolin (For) in the absence and presence of propranolol (1 μM) is shown. The assays, which were carried out in duplicate, are the mean of results from 2 preparations. Hatched bars are responses in the absence of propranolol and open bars are responses with propranolol (Pro, 1 μM) present.

of the stimulation between normal and sensitized lung (Fig. 15 and 16).

The stimulation of adenylate cyclase by the above drugs was concentration-dependent, and occurred at low concentrations (Fig. 15 and 16). The rank order of efficacy of these drugs on lung adenylate cyclase activation was forskolin>isoproterenol=VIP>PGE_2. Forskolin was the most efficacious drug on lung adenylate cyclase. Basal lung adenylate cyclase activity was increased to 500.0±50.5% of basal activity with a forskolin concentration 100 μM (Fig. 16).

4.1.6.1 Effect of indomethacin-pretreatment on adenylate cyclase activation:

Indomethacin (8.5 μM)-pretreatment of guinea-pig lung parenchyma did not significantly change basal activity or drug-stimulated activity of the enzyme (Fig. 16, Table 14). The effects of indomethacin-pretreatment on the forskolin-induced activation of adenylate cyclase of normal and sensitized lung are shown in Fig. 16.

4.2 ANTIGEN-, A23187-, AND AA-INDUCED CONTRACTIONS OF NORMAL AND SENSITIZED AIRWAY TISSUES.

4.2.1 Effects of indomethacin, NDGA, or mepacrine:

A23187 contracted normal and sensitized tissues in a concentration-dependent fashion (Figs. 17 and 18). There was no difference in the magnitude of the induced contraction between normal

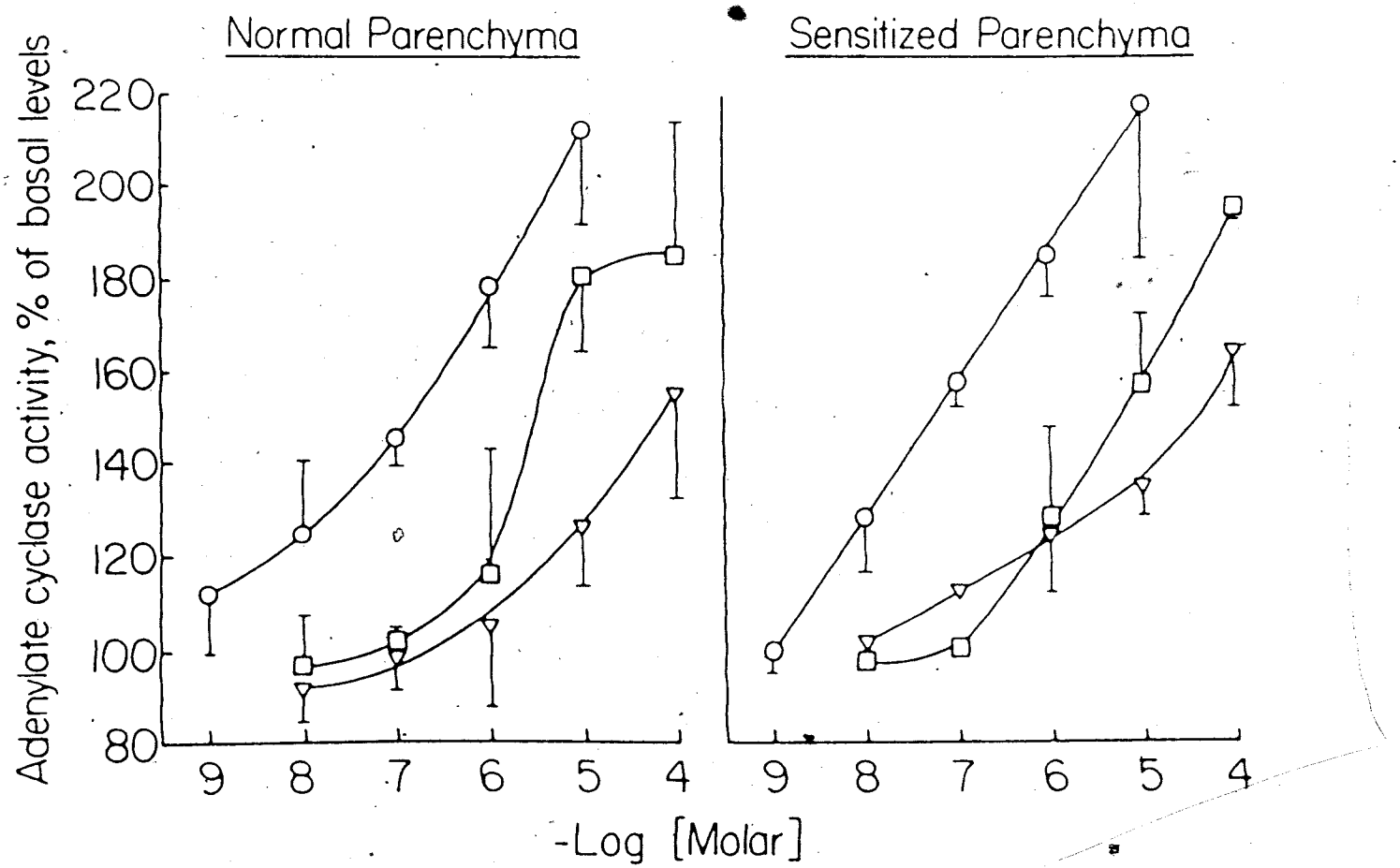


Fig. 15. Activation of normal (a) and ovalbumin-sensitized (b) guinea-pig lung adenylate cyclase by isoproterenol (□), VIP (○) and PGE_2 (▽). Assays were carried out in duplicate and the results are the mean±SEM of experiments using 4 different preparations each of normal and sensitized lung. Adenylate cyclase activity is expressed as a percentage of the basal activity measured in the absence of any drug.

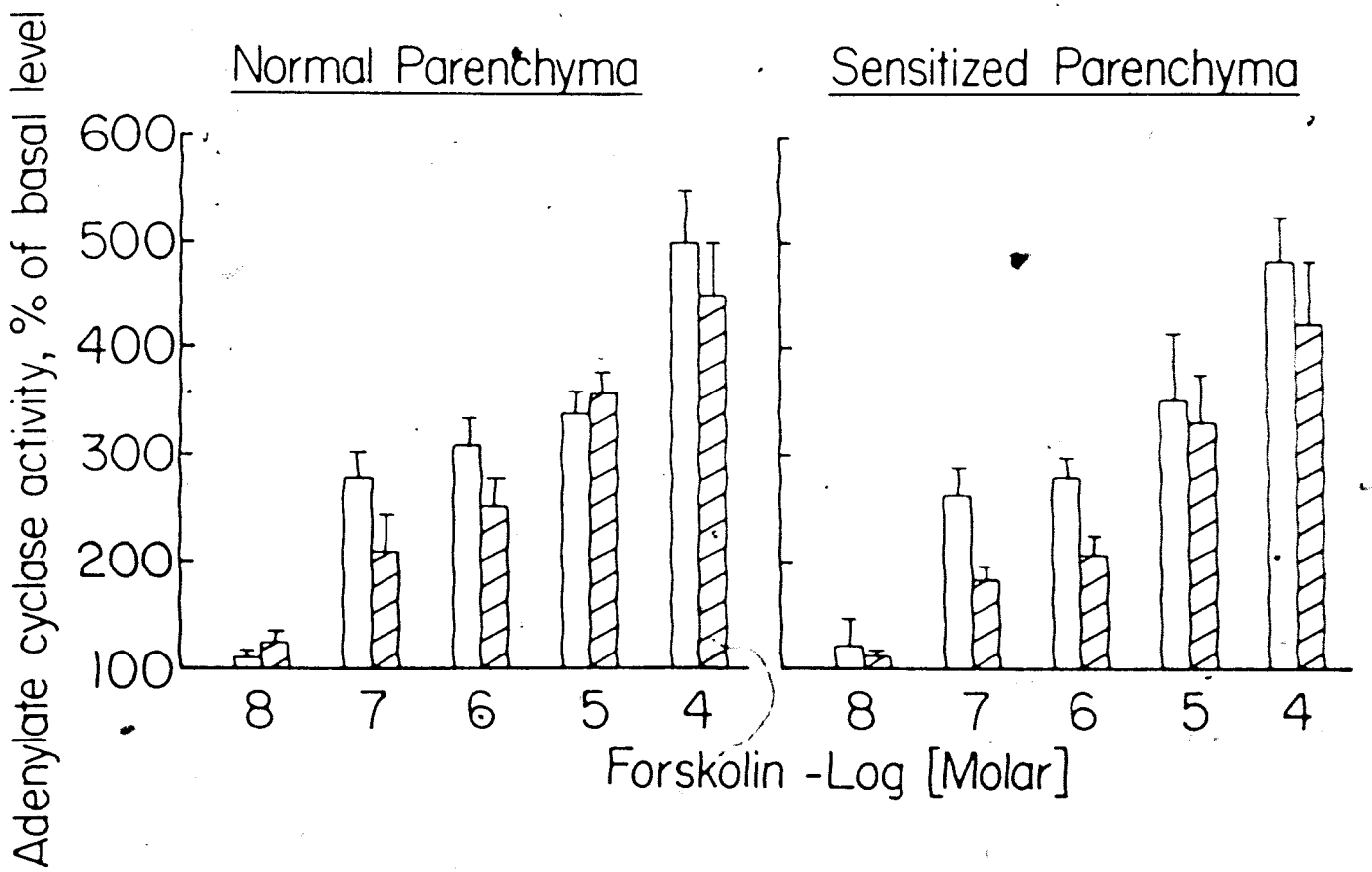


Fig. 16. Effects of indomethacin (8.5 μM)-pretreatment on normal (a) and sensitizied (b) guinea-pig lung adenylate cyclase activation induced by forskolin. Open bars are responses without, and hatched bars are responses after indomethacin (8.5 μM)-pretreatment. Assays were carried out in duplicate and the results are the mean±SEM of experiments using 3 different preparations each of normal and sensitized lung. Adenylate cyclase activity is expressed as a percentage of the basal activity measured in the absence of any drug

Table 14. Adenylate cyclase activity of control and indomethacin (8.5 μM)-pretreated lung membranes. Results are mean±SEM of 4 preparations.

	Normal Lung		Sensitized lung	
	Control	Indomethacin	Control	Indomethacin
Basal activity (Pmoles mg^{-1} min^{-1})	54.8± 9.6	41.8± 3.8	62.3± 9.3	50.5± 7.2
% of basal activity				
Isoproterenol (10 μM)	181.1±16.6	180.6±16.1	158.4±15.3	157.7±15.8
PGE_2 (100 μM)	156.6±21.9	146.1±16.4	165.1±11.6	156.9± 6.1
VIP (1 μM)	179.0±13.5	180.0±27.7	186.3± 9.6	171.7±18.5

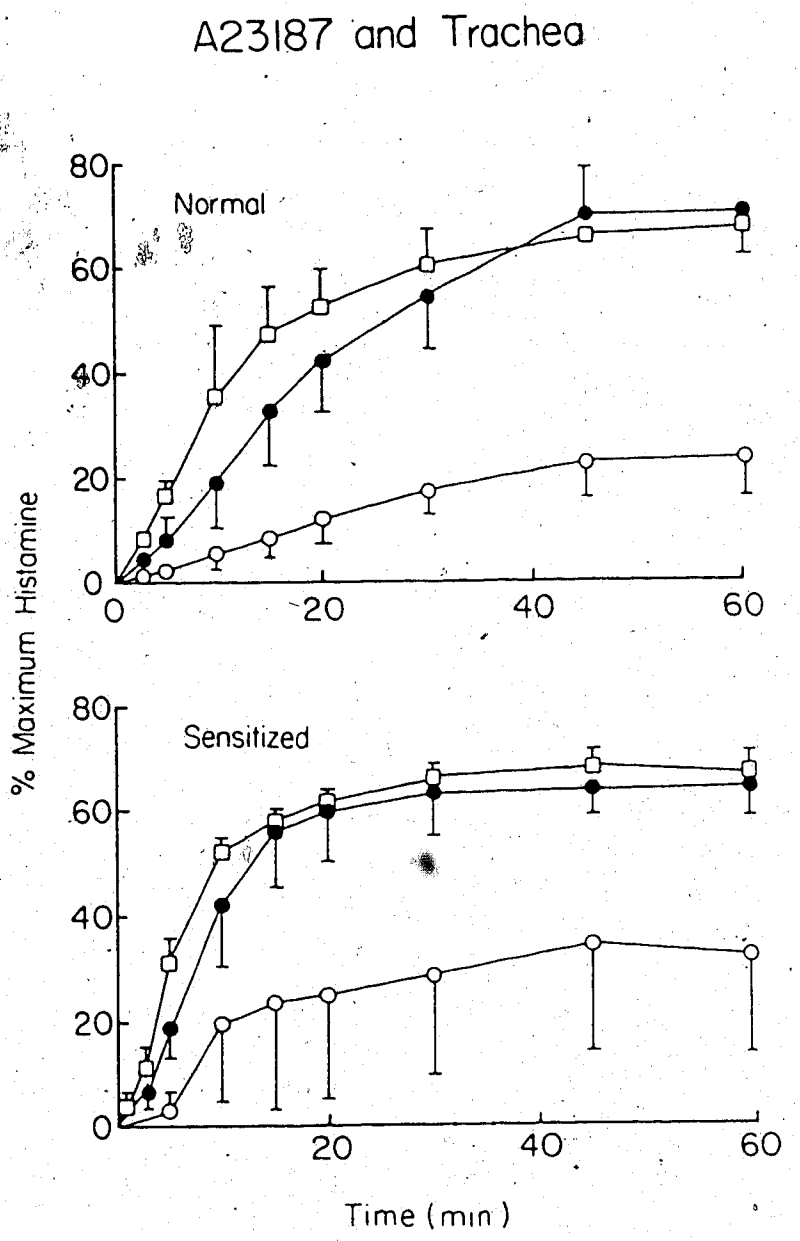


Fig. 17. Contraction of normal and sensitized trachea by A23187 at 0.19 μM (○), 1.9 μM (●), and 5.7 μM (□). Responses are expressed as a percentage of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

A23187 and Parenchyma

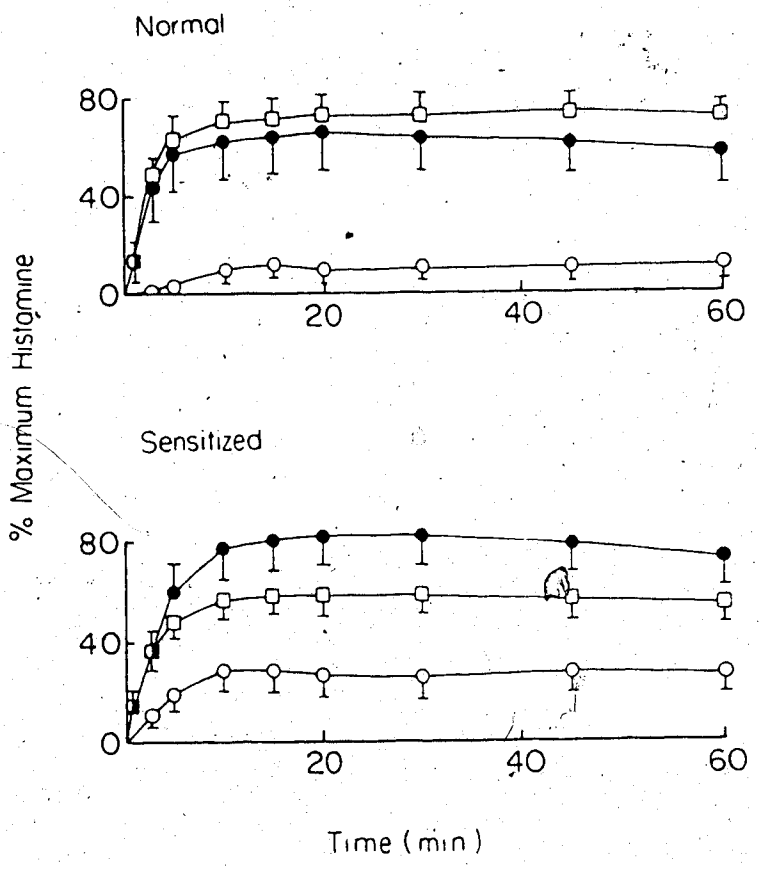


Fig. 18. Contraction of normal and sensitized lung parenchymal strips by A23187 at 0.19 μM (○), 1.9 μM (●), and 5.7 μM (□). Responses are expressed as percentage of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

and sensitized tissues (trachea or parenchyma). The contractions induced by A23187 were prolonged and the profile of contractions were different from those induced by OA (Fig. 19). The maximal contractile responses induced by A23187 or OA were at 5.7 μM and 3 μg/ml, respectively. These concentrations were used in further experiments.

Indomethacin (8.5 μM)-pretreatment enhanced both types of contractions significantly ($p<0.05$) (Fig. 20). The presence of AA (32.8 μM) did not further alter the responses of indomethacin pretreated tissues to either OA or A23187. This concentration of AA contracts the tissues on its own to about 30% of maximal histamine (data not shown).

NDGA (50 μM) pretreatment either completely abolished or reduced OA- and A23187-induced contractions of the trachea (Fig. 20).

AA slightly relaxed tracheal spirals and contracted lung parenchymal strips before indomethacin-pretreated. AA (66 μM)-induced contractions after indomethacin-pretreatment were unaffected by mepacrine (210 μM) (a phospholipase A_2 inhibitor)-pretreatment of normal and sensitized trachea (Fig. 21) and parenchyma (Fig. 22). The extent of contraction to AA (66 μM) after indomethacin-pretreatment is shown for normal and sensitized trachea (Fig. 23) and for normal and sensitized lung parenchyma (Fig. 24). The trachea (93.3±20.1% histamine maximum) was more responsive to AA than the lung strip (32.5±5.1% histamine maximum), contracting to a greater extent with respect to the maximal contraction obtained with histamine. This concentration of AA induces approximately 80% of the respective maximum

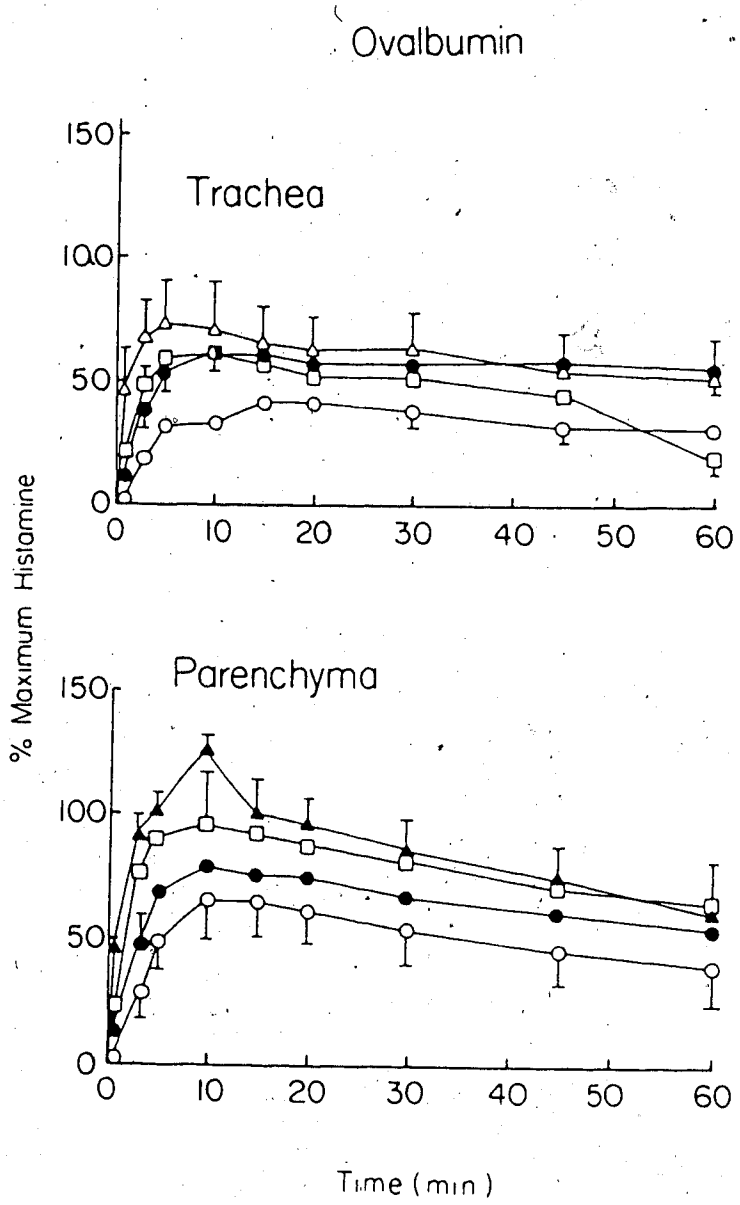


Fig. 19. Responses of sensitized airway tissue to ovalbumin 0.1 μg/ml (○), 1.0 μg/ml (●), 3.0 μg/ml (□), and 10 μg/ml (▲). Responses are expressed as a % of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

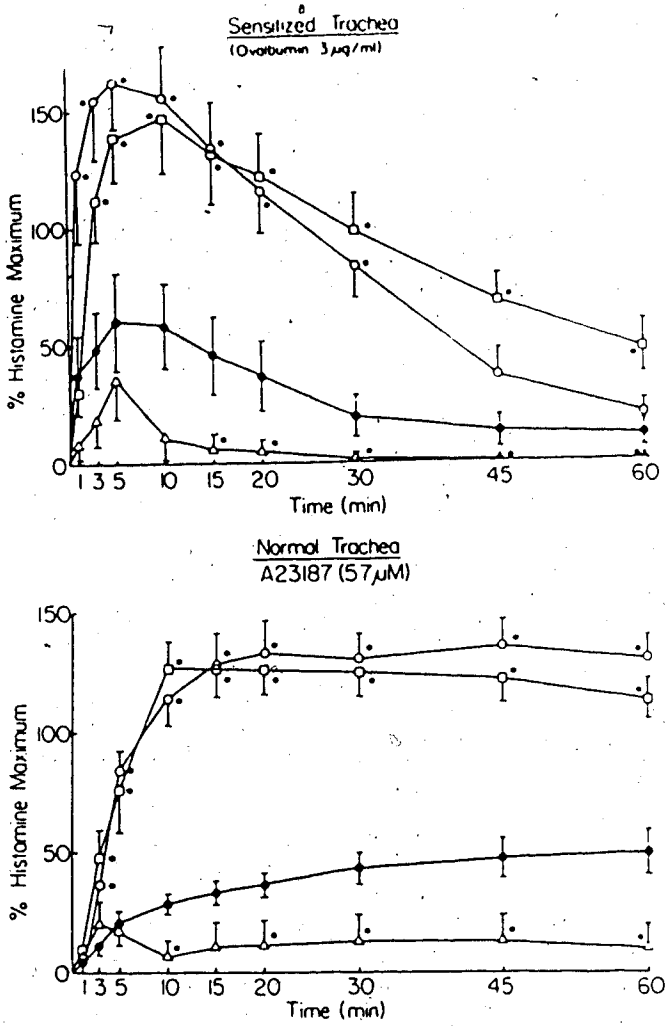


Fig. 20. Contractile response of normal and sensitized trachea to A23187 and ovalbumin. Control responses (●), indomethacin (8.5 μM) pretreated tissues (○), indomethacin and arachidonic acid (32.8 μM) (□) and nordihydroguaiaretic acid (50 μM) pretreated tissues (△). Values are mean ± SEM (n=6 for each curve). Statistical significance was assessed by comparison with the appropriate control and are as indicated: *$p<0.05$.

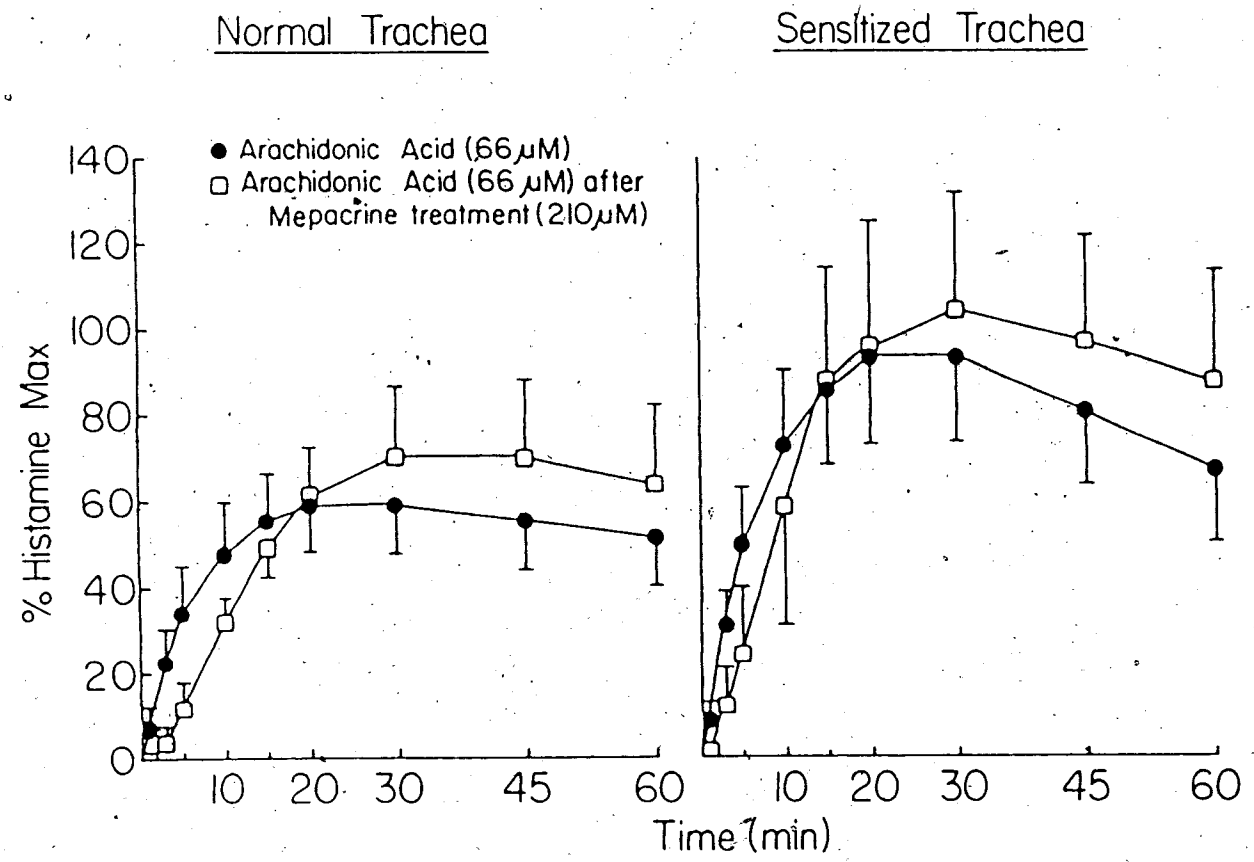


Fig. 21. The responses of normal and sensitized trachea to AA (66 μM). Tissues were pretreated with indomethacin (8.5 μM). The effects of mepacrine (210 μM)-pretreatment are shown. Results are mean±SEM (n=5).

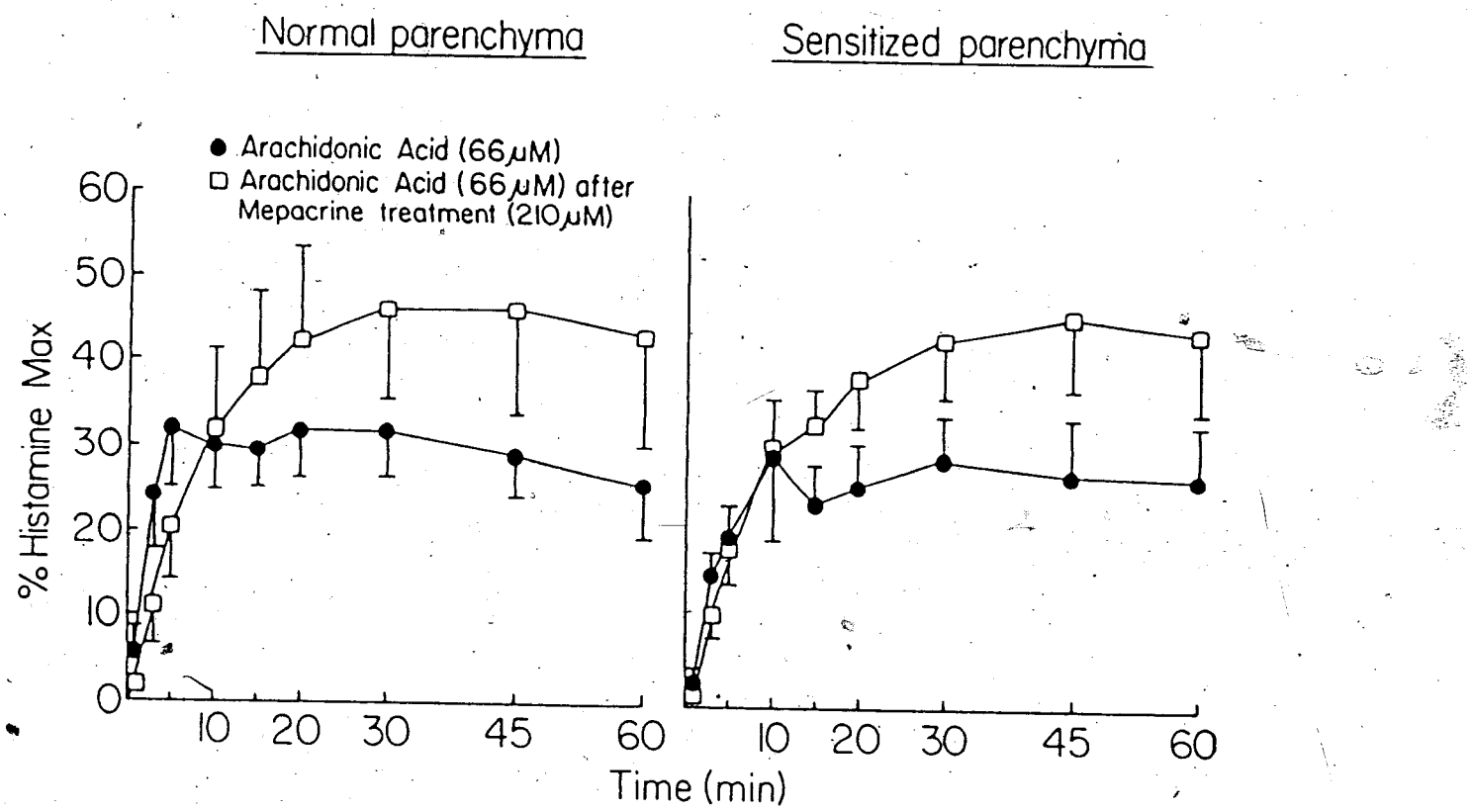


Fig. 22. The responses of normal and sensitized parenchymal strips to AA (66 μM). Tissues were pretreated with indomethacin (8.5 μM). The effects of mepacrine (210 μM)-pretreatment are shown. Results are mean±SEM (n=5).

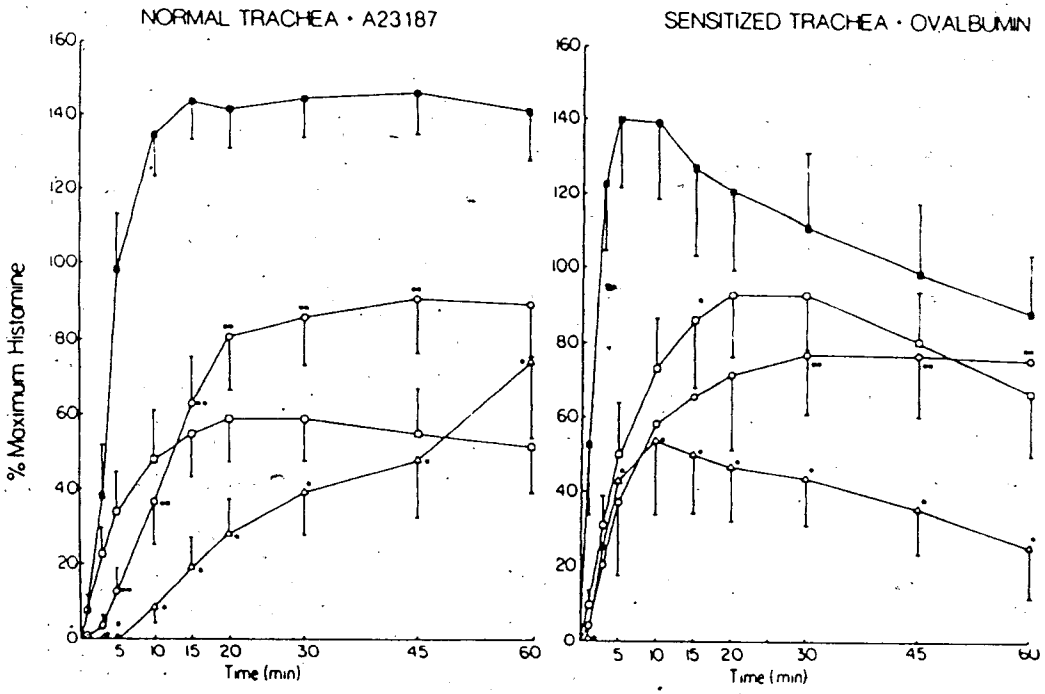


Fig. 23. The contractile responses of indomethacin-treated tracheal spirals to AA, A23187 or OA: (a) normal trachea plus A23187: (b) sensitized trachea plus OA. Results are the mean±SEM of at least 5 experiments. Symbols indicate the following: (□) AA alone; (■) A23187 or OA alone; (△) mepacrine and A23187 or mepacrine and OA; and (○) mepacrine AA and A23187 or mepacrine, AA and OA. *Significantly different ($p<0.05$) from contractions to A23187 (a) or OA alone (b) indicating that mepacrine partly inhibits contractions induced by A23187 or OA; **significantly different ($p<0.05$) from contractions to mepacrine-treated tissues stimulated with A23187 (a) or OA alone (b), indicating that addition of exogenous substrate (i.e. AA) enhanced contractions previously inhibited by mepacrine.

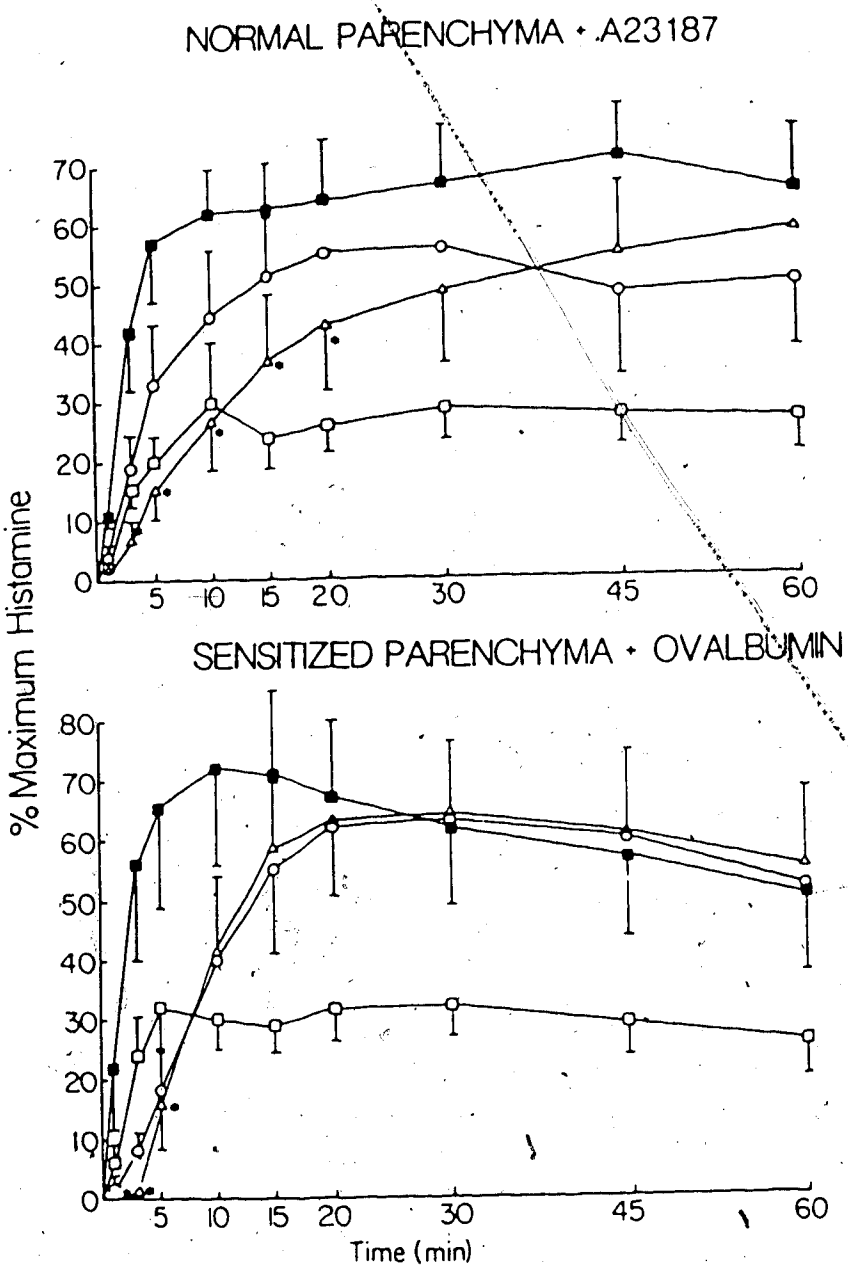


Fig. 24. The contractile responses of indomethacin-treated lung parenchymal strips to AA, A23187 or OA; (a) normal parenchyma plus A23187, (b) sensitized parenchymal plus OA. Results are the mean±SEM of at least 5 experiments. Symbols indicate the following; (□) AA alone; (■) A23187 or OA alone; (△) mepacrine and A23187 or mepacrine and ovalbumin; and (○) mepacrine, AA and A23187 or mepacrine, AA and OA. *Significantly different ($p<0.05$) from contractions to A23187 or OA alone, indicating that mepacrine partly inhibits contractions induced by A23187 or OA.

effect possible on trachea or parenchyma. There was no statistically significant difference in the extent and magnitude of AA-induced contractions between normal and sensitized tissues (Figs. 23 and 24).

NDGA (100 μM), a lipoxygenase inhibitor, significantly ($p<0.05$) reduced the magnitude of the contraction at most time points studied (Figs. 25 and 26). Phenidone (185 μM), another lipoxygenase inhibitor, exhibited similar effects (2 experiments). A23187-induced contractions (normal trachea) and OA-induced contractions (sensitized trachea) were significantly reduced) ($p<0.05$) after mepacrine pretreatment (Fig. 23). Addition of exogenous AA to mepacrine-pretreated trachea increased the magnitude of the contraction to A23187 or OA after (mepacrine-pretreatment), but not to the level observed in the absence of mepacrine (Fig. 23). Mepacrine pretreatment significantly reduced the extent of contraction of lung parenchyma to A23187 or OA in the initial period (0-10 min) after stimulus addition. Exogenous AA did not further increase the magnitude of the contractions (Fig. 24).

NDGA (100 μM) pretreatment of airway tissues markedly reduced the extent of contraction to AA and A23187 or AA and OA (normal or sensitized tissues) (Figs. 25 and 26).

4.2.2 High performance liquid chromatography:

Analysis of the material initially purified with SEP-PAKs by HPLC indicated the presence of peaks cochromatographing with synthetic LTC_4 and LTD_4 (Fig. 27). The retention times for these substances were 5.27

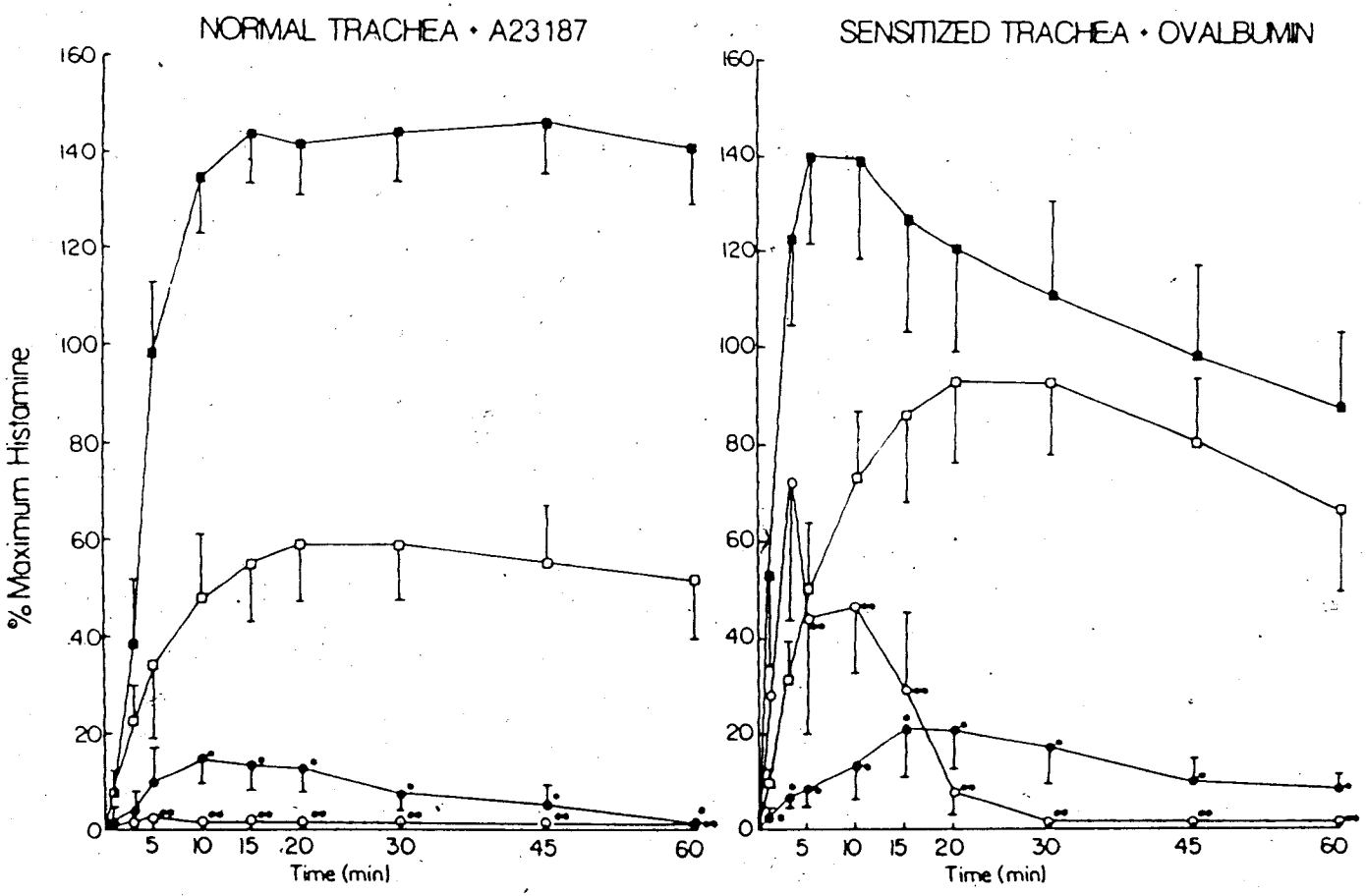


Fig. 25. The contractile responses of indomethacin-treated tracheal spirals to or AA, A23187 or OA (a) normal trachea plus A23187; (b) sensitized trachea plus OA. Results are mean±SEM. Symbols indicate the following: (□) AA alone (n=8); (■) A23187 or ovalbumin alone (n=5); (●) nordihydroguaiaretic acid (NDGA) and AA (n=5); and (○) NDGA and A23187 and AA or OA and AA (n=3). *Significantly different ($p<0.05$) from contractions to AA alone and **significantly different ($p<0.05$) from contractions to A23187 or AA alone.

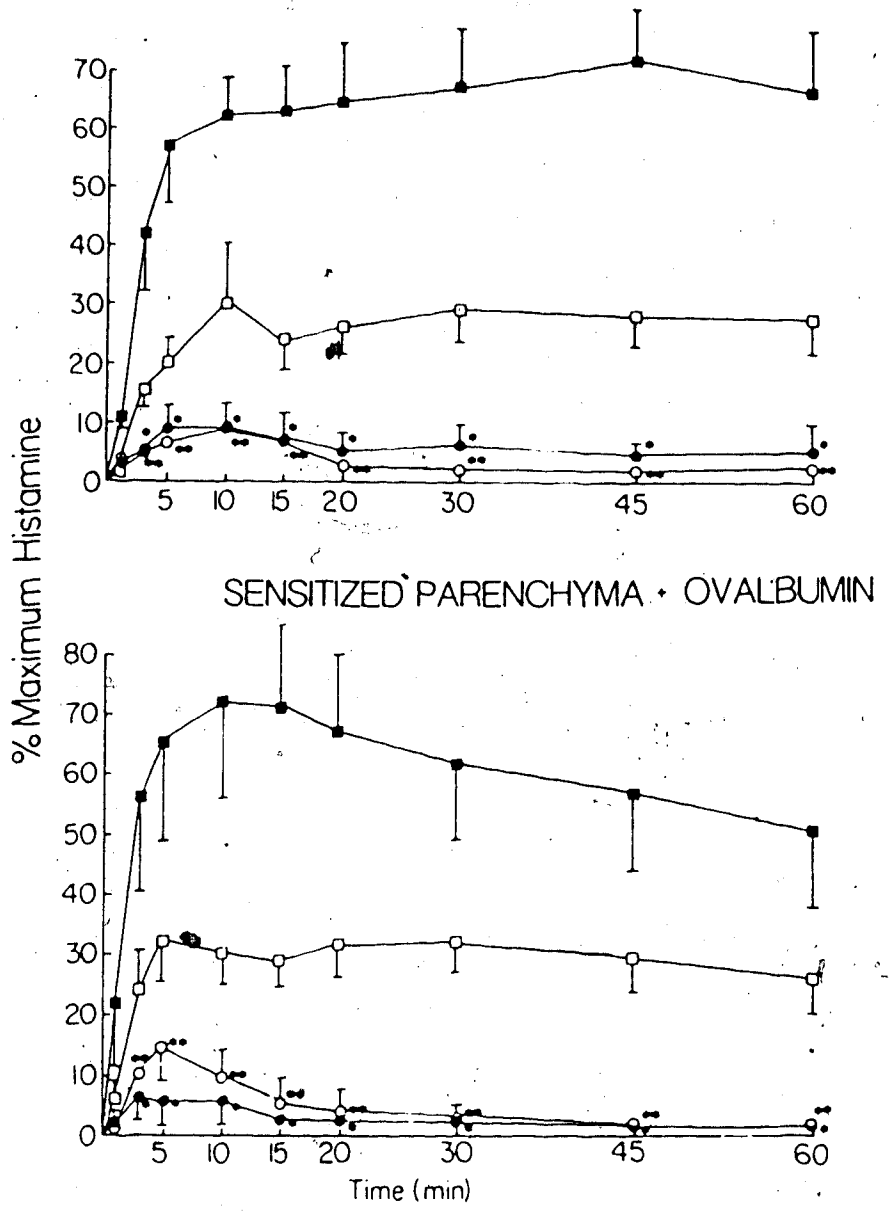


Fig. 26. The contractile responses of indomethacin-treated lung parenchymal strips to AA, A23187 or OA: (a) normal parenchyma plus A23187; (b) sensitized parenchyma plus OA. Results are mean±SEM. Symbols indicate the following; (□) AA alone (n=6); (■) A23187 or ovalbumin alone (n=5); (●) nordihydroguaiaretic acid (NDGA) and AA (n=5); and (○) NDGA and A23187 and AA or OA and AA (n=3). *Significantly different ($p<0.05$) from contractions to AA alone and **significantly different ($p<0.05$) from contractions to A23187 or ovalbumin alone.

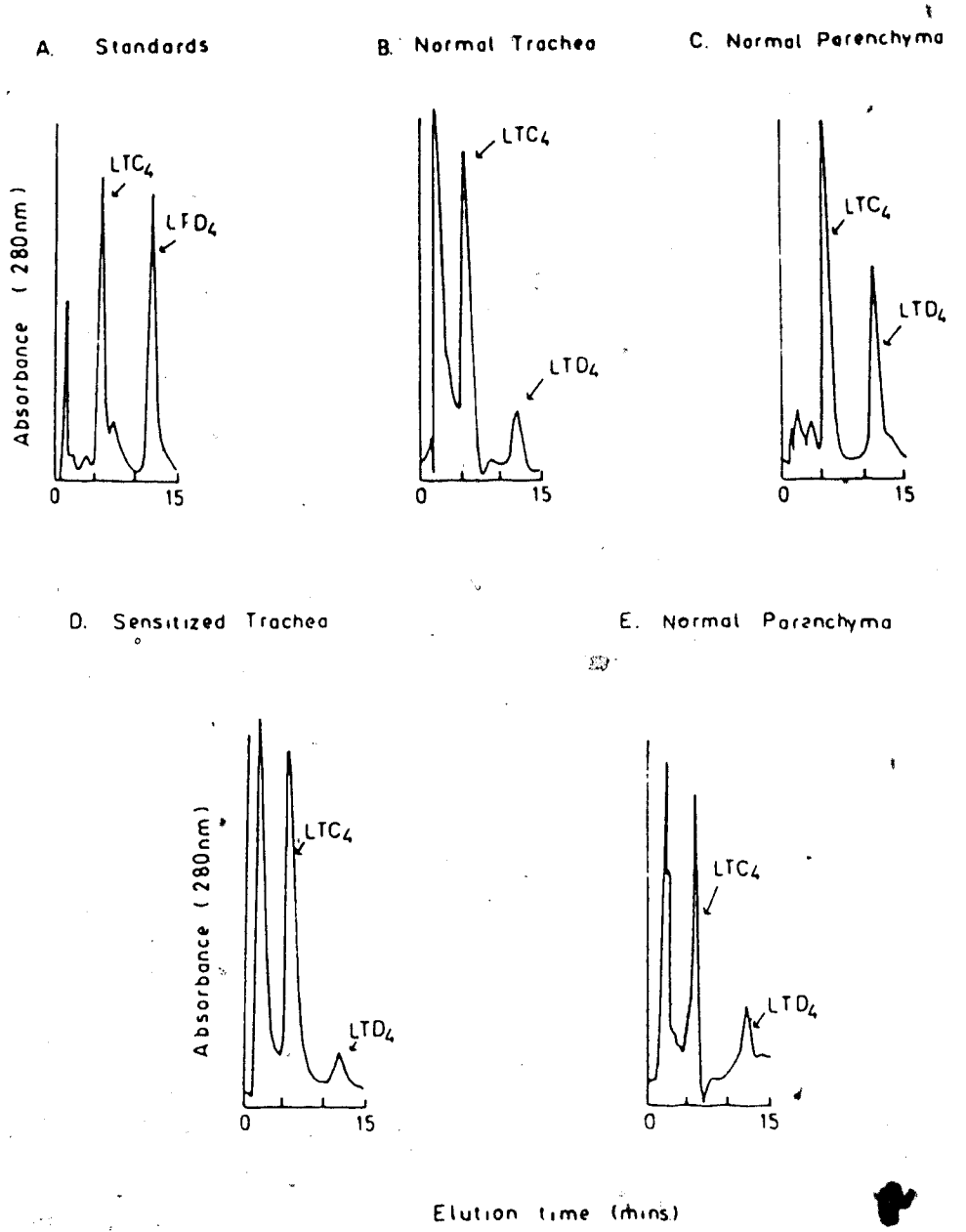


Fig. 27. Typical HPLC chromatograms obtained after analysis of medium surrounding airway tissues after appropri stimulation. Synthetic standards LTC_4 and LTD_4 are shown (A); Normal trachea after stimulation with AA in (B); Nor parenchyma after AA stimulation in (C); sensitized trac after mepacrine treatment and AA stimulation with OA in (and normal parenchyma after A23187 in (E). All absorba were measured at 280 nm.

and 11.50 min, respectively. The peaks had an ultraviolet absorption spectrum with a maximum at 278-280 nm with typical shoulders (Fig. 28) characteristic of the the conjugated triene structure of LTs. Numerous samples were subjected to HPLC and the results shown in Figs.27 and 28 are representative.

4.2.3 Bioassay of released LTs:

The crude SEP-PAK extracts of the bath fluid, and the chromatographed samples that coincided with either LTC_4 or LTD_4 in retention time, exhibited spasmogenic activity on guinea-pig ileum and lung parenchymal strips. Both contractile activities were prevented or reversed by the LT antagonist FPL55712 (Fig. 29).

From the HPLC studies, it was noted that LTC_4 was the predominant mediator released from the airways (LTC_4:LTD_4=20:1 ratio) using AA and as the stimulus. This is based on % composition of samples determined by integration of the area under the HPLC peaks. We used synthetic LTC_4 as the standard for bioassay on longitudinal strips of guinea pig ileum. Similar amounts of LTs were released from normal and sensitized tissues under the same conditions. NDGA pretreatment completely abolished or markedly reduced release of LT-like activity (Table 15). Samples obtained from mepacrine-treated tissues could not be bioassayed because the mepacrine residue after initial sample purification relaxed the ileum. Hence the data were considered unreliable. It can be seen from Table 15 that indomethacin-pretreatment reduced the

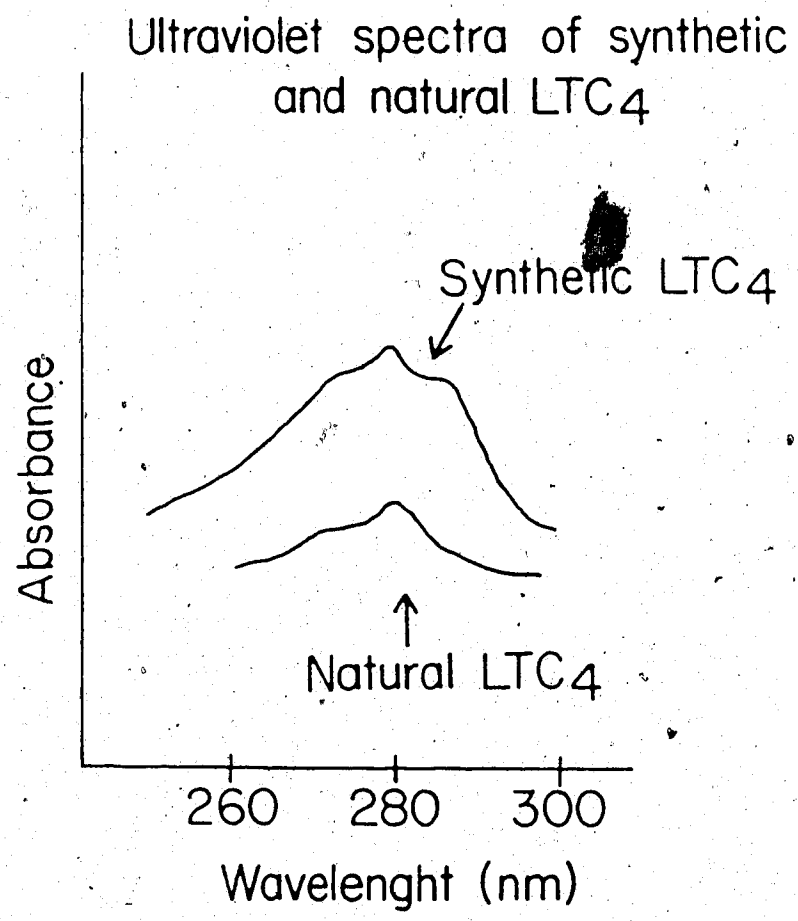


Fig. 28. Ultraviolet spectra of synthetic and natural LTC_4 (released from trachea) recorded in methanol.

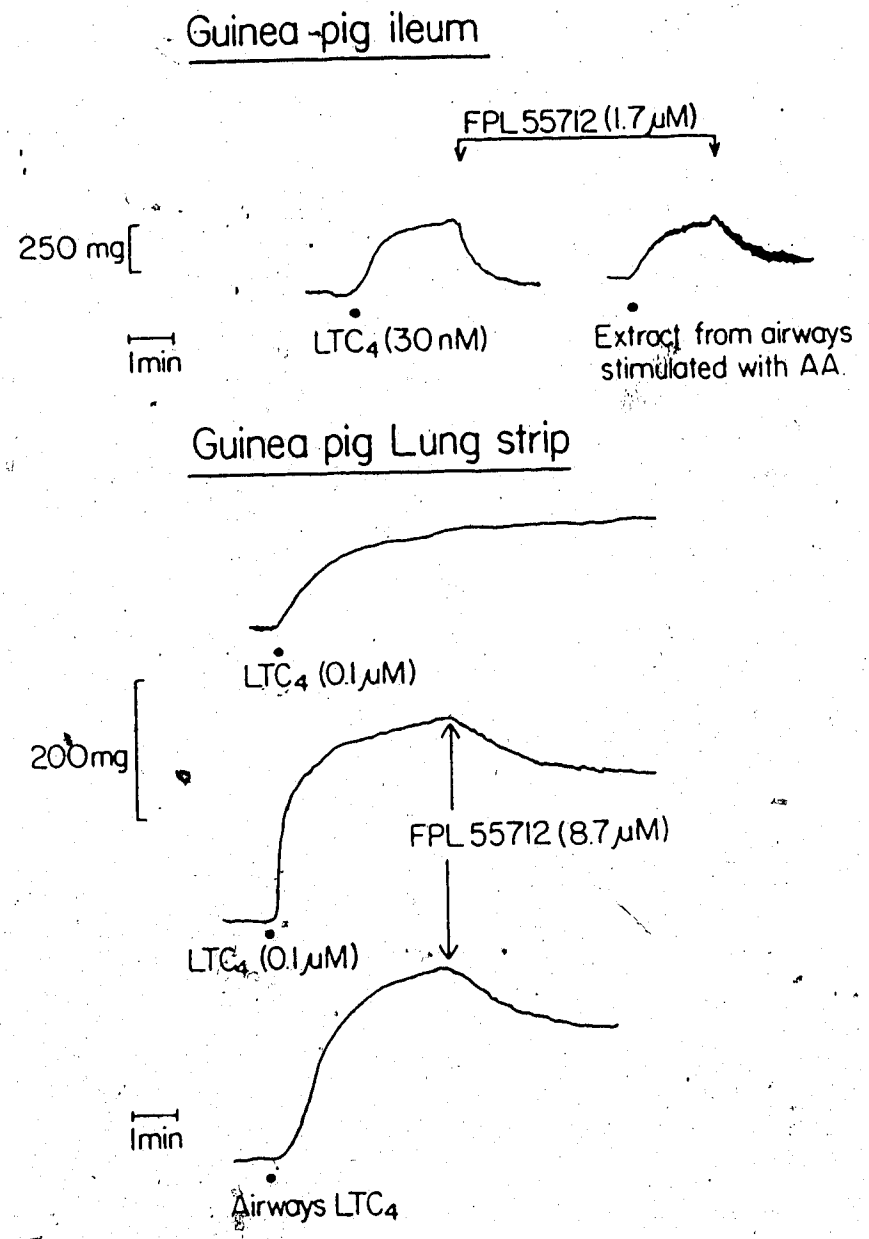


Fig. 29. Bioassay of released LTC_4 on guinea-pig ileum (a) and lung parenchymal strips (b). Synthetic LTC_4 was used as a standard. FPL55712 (1.7 μM) was used to reverse contractions on the ileum. A higher concentration of FPL55712 was necessary to partly reverse contractions on the lung strip. The bioassay was carried out in the presence of atropine (1 μM) and mepyramine (1 μM).

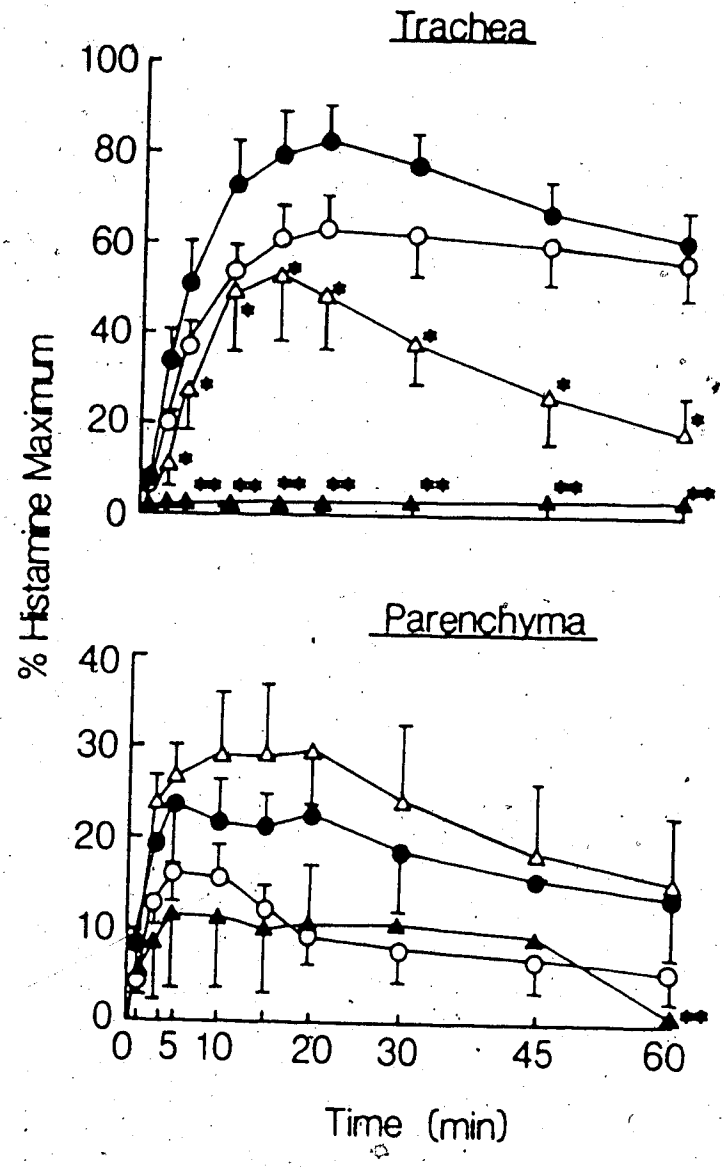


Fig. 30. The effects of calcium and TMB-8 on AA-induced contractions of trachea and parenchyma. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (△) Ca^{2+}-free (○); Ca^{2+} (8.8 mM); and (▲) Ca^{2+}-free plus TMB-8 (100 μM). *Indicates a significant difference from control ($p<0.05$). **Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p<0.05$). Results are the means±SEM ($n=5$).

The AA-induced contraction of the trachea in the presence of KHS containing Ca^{2+} (8.8 mM) was not significantly different from the contraction observed in normal KHS. However, after 30 min in Ca^{2+}-free KHS, the tracheal response to AA was significantly reduced ($p<0.05$) at 3-60 min from control (Fig. 30). However, the response was not completely abolished. The response (as % of histamine maximum) at 20 min in Ca^{2+}-free KHS was 48.2±10.0% whereas at the same time time point in normal KHS the response was 82.1±9.0%. The intracellular calcium antagonist, TMB-8 (100 μM), completely abolished the response of trachea to AA in Ca^{2+}-free KHS.

On the parenchyma, AA-induced contractions in Ca^{2+}-free KHS were not significantly different from contractions in normal KHS. In fact, the contractions in Ca^{2+}-free KHS were slightly enhanced (Fig. 30). In KHS containing Ca^{2+} (8.8 mM) there was a slight, but not significant reduction of the response to AA (Fig. 30). TMB-8 reduced the magnitude of the contraction to AA in Ca^{2+}-free KHS. However, this was not significantly different from contractions in Ca^{2+}-free KHS until 60 min following addition of AA. Addition of calcium (2.2 mM) to trachea and parenchyma stimulated with AA in calcium-free KHS in the presence of TMB-8 did not further increase the response of these tissues (data not shown).

4.3.2. Effects of nitrendipine and verapamil:

The contractions of trachea and parenchyma to AA (66 μM) were

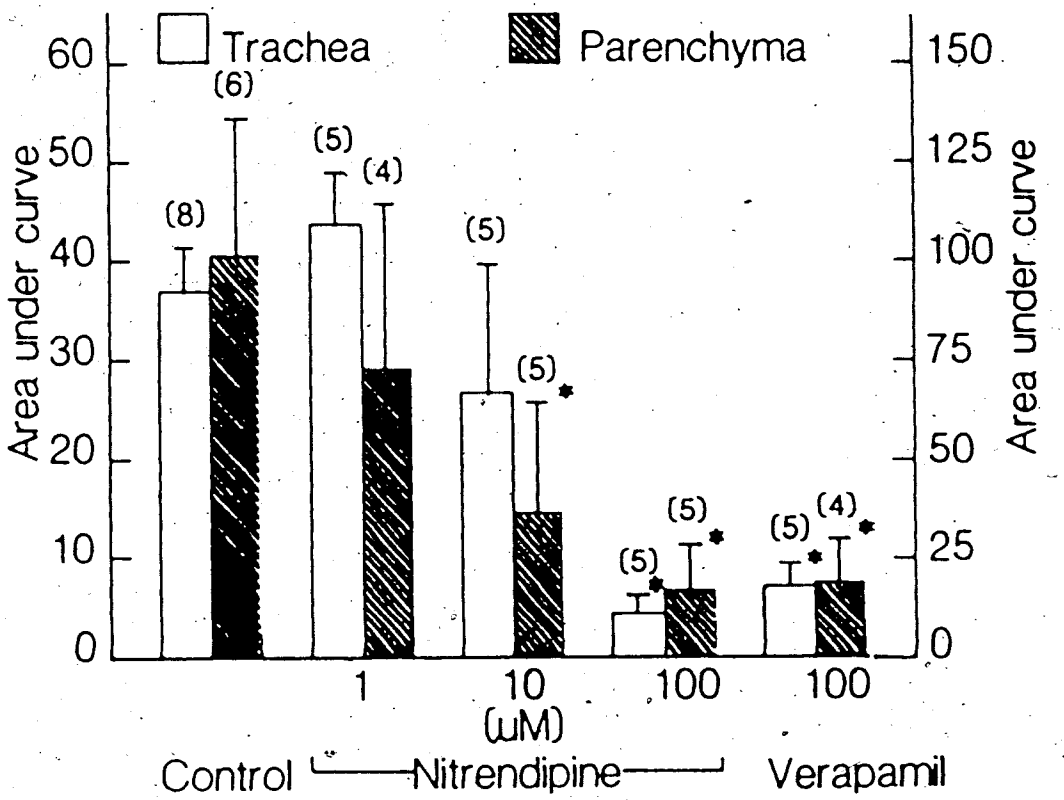


Fig. 31. Effects of nitrendipine and verapamil on AA-induced contractions of trachea (□) and parenchyma (▨). Contractions are expressed as areas under the curve obtained by plotting percentage of maximum histamine against time. *Indicates a significant difference from control ($p<0.05$). Values are mean±SEM of number of experimensts shown above each bar.

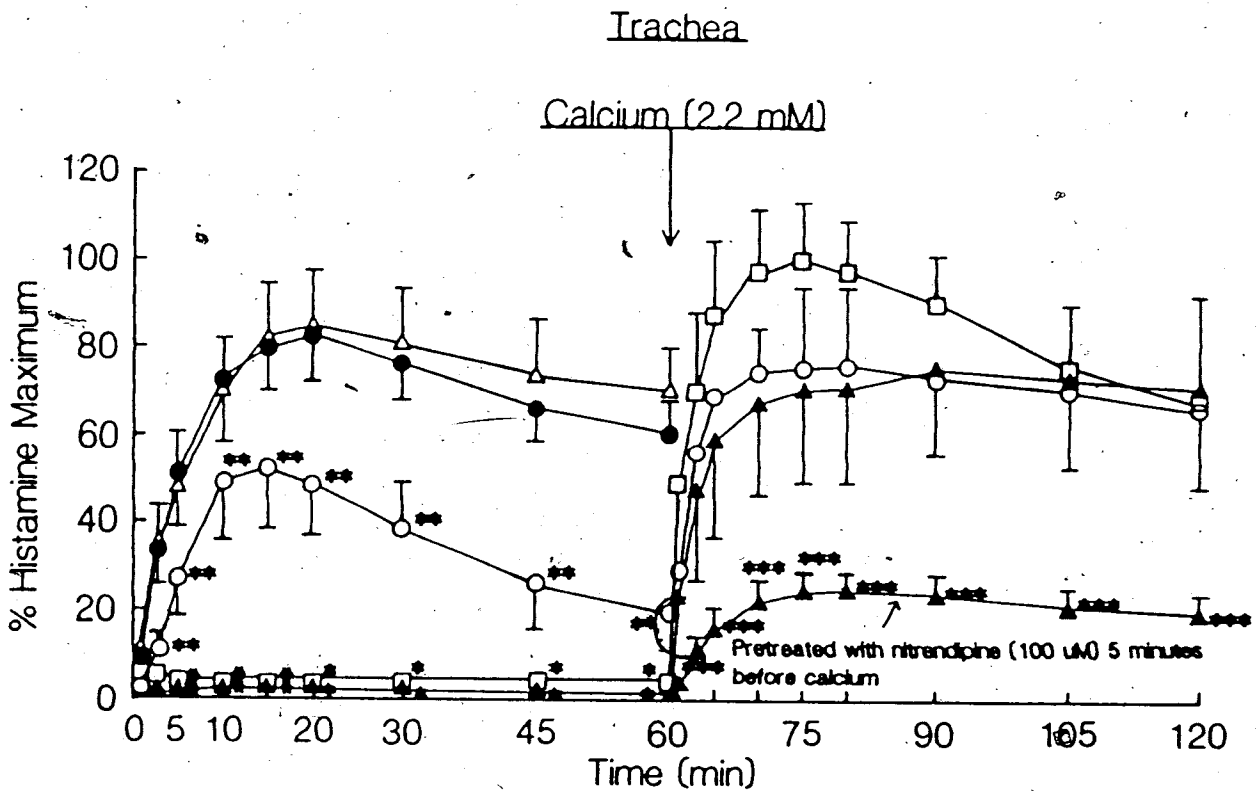


Fig. 32. Effects of lanthanum chloride and EDTA on AA-induced contractions of trachea. Ca^{2+} (2.2 mM) was added at 60 min to the appropriate tissues. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); (□) Ca^{2+}-free plus EDTA (300 μM); and (△) Ca^{2+} (2.2 mM) plus lanthanum chloride (1 mM). *Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p < 0.05$). **Indicates a significant difference from control contractions and ($p<0.05$) and ***indicates a significant difference from contractions obtained after calcium was added to lanthanum treated tissues in Ca^{2+}-free KHS ($p<0.05$). The results are the mean±SEM (n=5).

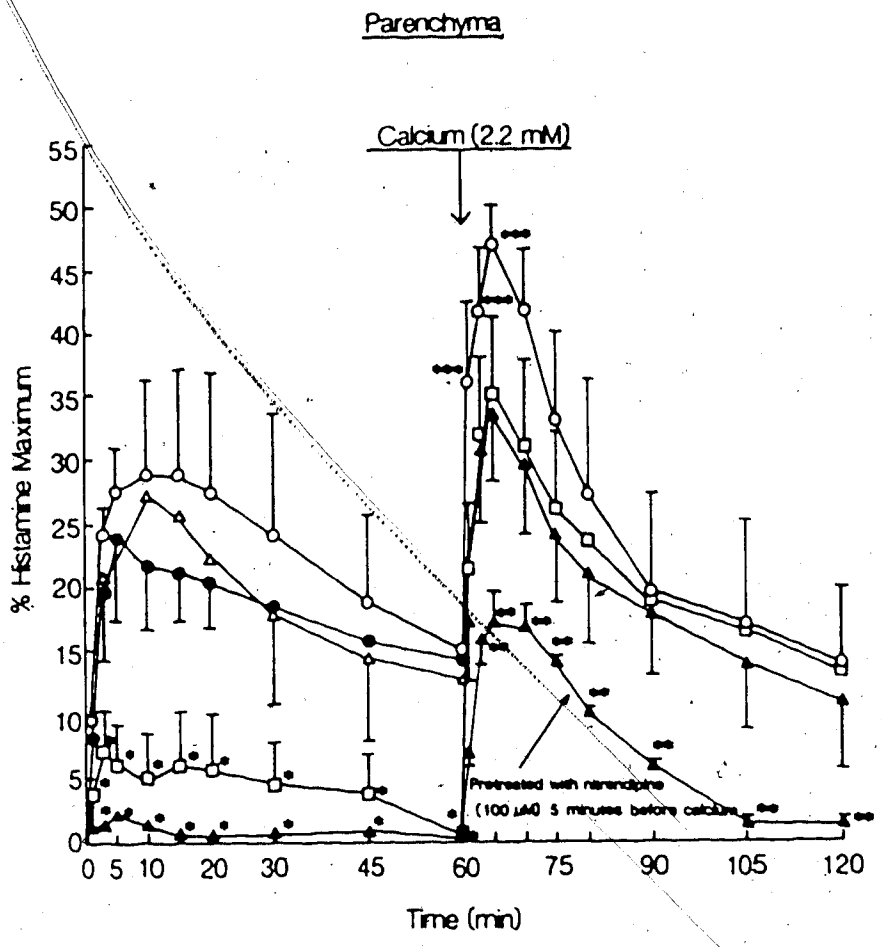


Fig. 33. Effects of lanthanum chloride and EDTA on AA-induced contractions of parenchyma. Ca^{2+} (2.2 mM) was added at 60 min to the appropriate tissues. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); (□) Ca^{2+}-free plus EDTA (300 μM); and (△) Ca^{2+} (2.2 mM) plus lanthanum chloride (1 mM). *Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p<0.05$), **indicates a significant difference from contractions obtained after calcium was added to lanthanum-treated tissues in Ca^{2+}-free KHS ($p<0.05$), and ***indicates a significant difference from control contractions. The results are the mean±SEM ($n=5$).

parenchyma, addition of Ca^{2+} (2.2 mM) to tissues in Ca^{2+}-free KHS caused a statistically significant greater response at early time points after Ca^{2+} addition than that observed with control tissues (Fig. 33). Pretreatment of trachea and parenchyma with nitrendipine (100 μM) 5 min before Ca^{2+} re-addition significantly attentuated the contractile response to this cation (Figs. 32 and 33). In these experiments only tissues stimulated with AA in the presence of lanthanum chloride (1 mM) and Ca^{2+}-free KHS were used. Addition of Ca^{2+} (2.2 mM) to tissues stimulated with AA in normal KHS caused little or no further response of these tissues. Furthermore, addition of more Ca^{2+} to tissues contracting in Ca^{2+}-free KHS after the initial 2.2 mM stimulus at 60 min did not cause any further response (data not shown)

4.3.4 Concentration-response curves to LTC_4:

The concentration-response curves to LTC_4 on the trachea and parenchyma are shown in Fig. 34. Nitrendipine (100 μM) did not significantly affect the response of the trachea to LTC_4 when compared to control. Similar results were obtained with the parenchymal strip. The responses of the trachea to LTC_4 in Ca^{2+}-free KHS and in Ca^{2+}-free

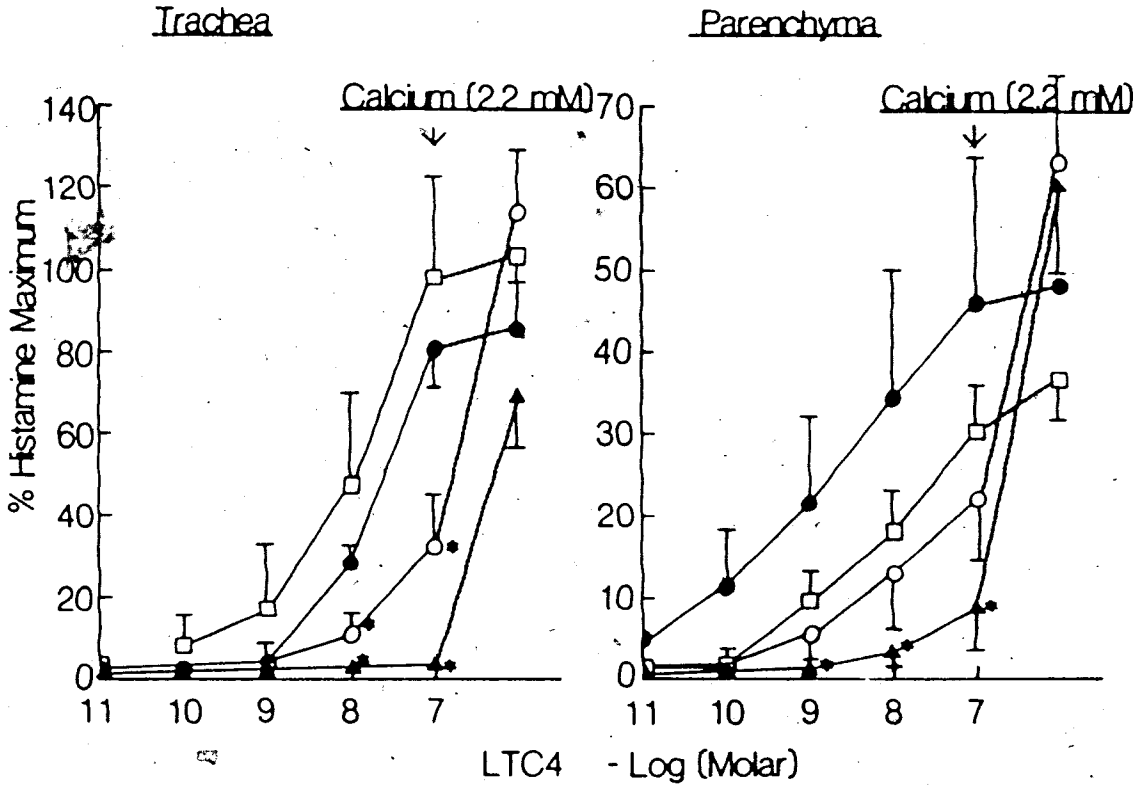


Fig. 34. Contraction of trachea and parenchyma to LTC_4. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); and (□) Ca^{2+} (2.2 mM) plus nitrendipine (100 μM). Ca^{2+} (2.2 mM) was added when the response to LTC_4 (0.1 μM) has reached a maximum. *Indicates a significant difference from control responses ($p<0.05$). Results are mean±SEM (n=5).

tion of LTC_4 used (0.1 μM) had plateaued, resulted in an additional contraction of both trachea and parenchyma in Ca^{2+}-free KHS to the control level. Further Ca^{2+} addition did not give further increases in response (data not shown).

4.4 EFFECTS OF LTD_4 ON LUNG AND CEREBELLAR ADENYLATE CYCLASE ACTIVITY

The results presented in this section were calculated as m±SEM. The SEM between duplicate or triplicate determinations did exceed 5-10% of the mean value. Hence, in some of the figures the s have not been displayed in order to enhance clarity. Most experiments have been done at least three times utilizing different membrane preparations.

4.4.1 Initial studies:

The conversion of [^{32}P]-ATP into [^{32}P]-cyclic AMP by guinea-pig lung adenylate cyclase was linear with time (Fig. 35) and protein concentration (up to 1 mg/ml) (Fig. 36). Lung enzyme activity was increased by GTP, isoproterenol, and forskolin (Fig. 37). Forskolin increased enzyme activity by a far greater degree than either GTP or isoproterenol. The effects of GTP were additive to those of forskolin and isoproterenol (Fig. 37).

Figs. 38 and 39 show the concentration-dependent stimulation of lung adenylate cyclase induced by forskolin and isoproterenol. The effects of forskolin were Mg^{2+}-dependent (Fig. 38) since at Mg^{2+} con-

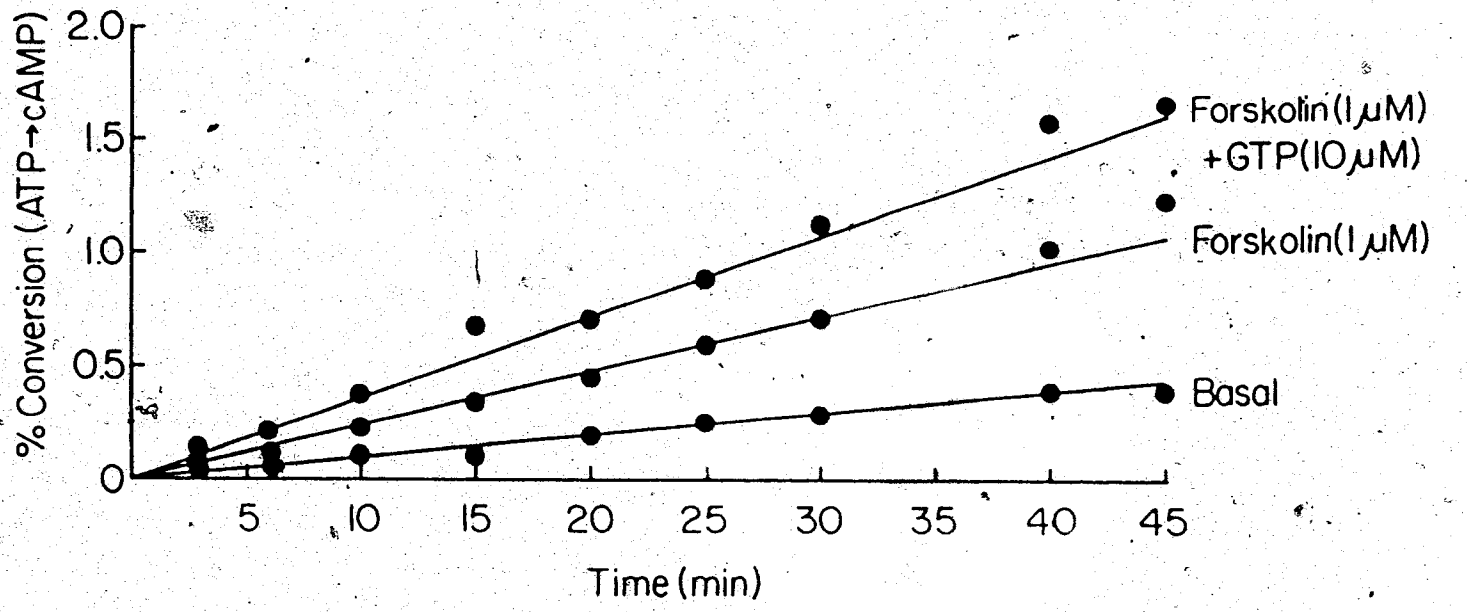


Fig. 35. Time course of activation of lung adenylate cyclase at 20°C. Activation of the enzyme by forskolin and GTP are shown. Assay points are means of duplicates.

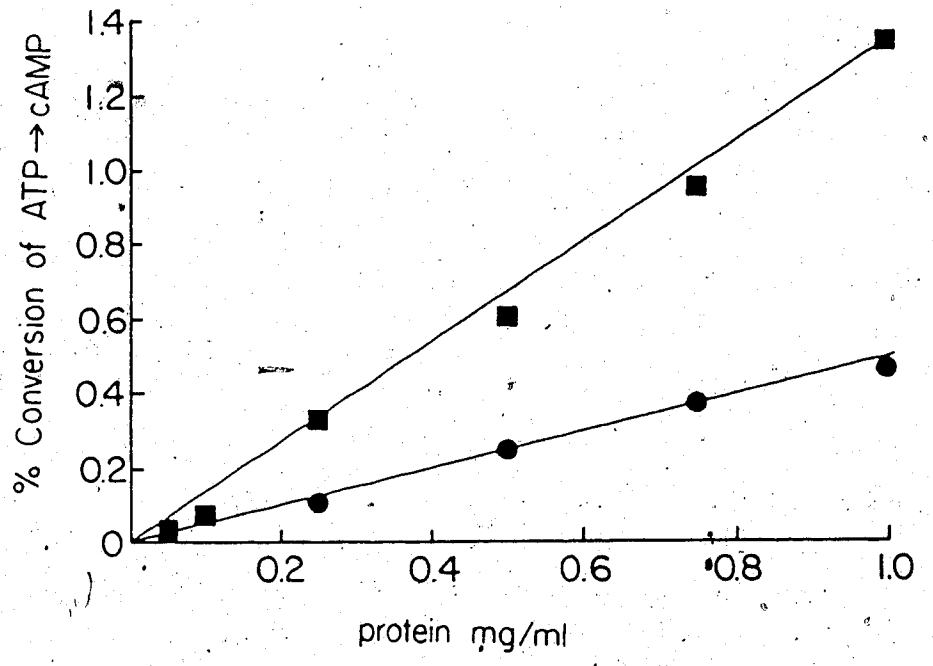


Fig. 36. Protein linearity of adenylate cyclase assays. The percent conversion of [^{32}P]-ATP into [^{32}P]-cyclic AMP at 20°C is shown as a function of added lung protein in the assay. The symbols represent basal activity (●); and forskolin (1 μM) (■) stimulated activity. Values are the means of duplicates.

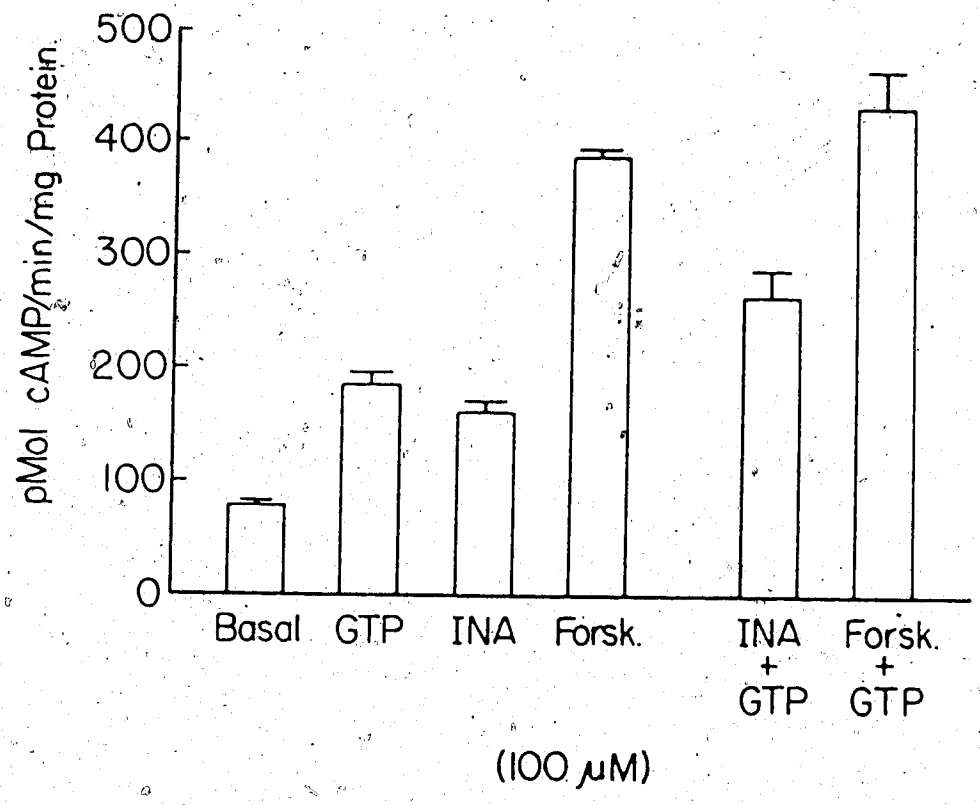


Fig. 37. Activation of lung adenylate cyclase at 37°C by GTP, isoproterenol (INA), and forskolin (Forsk) alone or in combination as shown. Results are mean±SEM of duplicates.

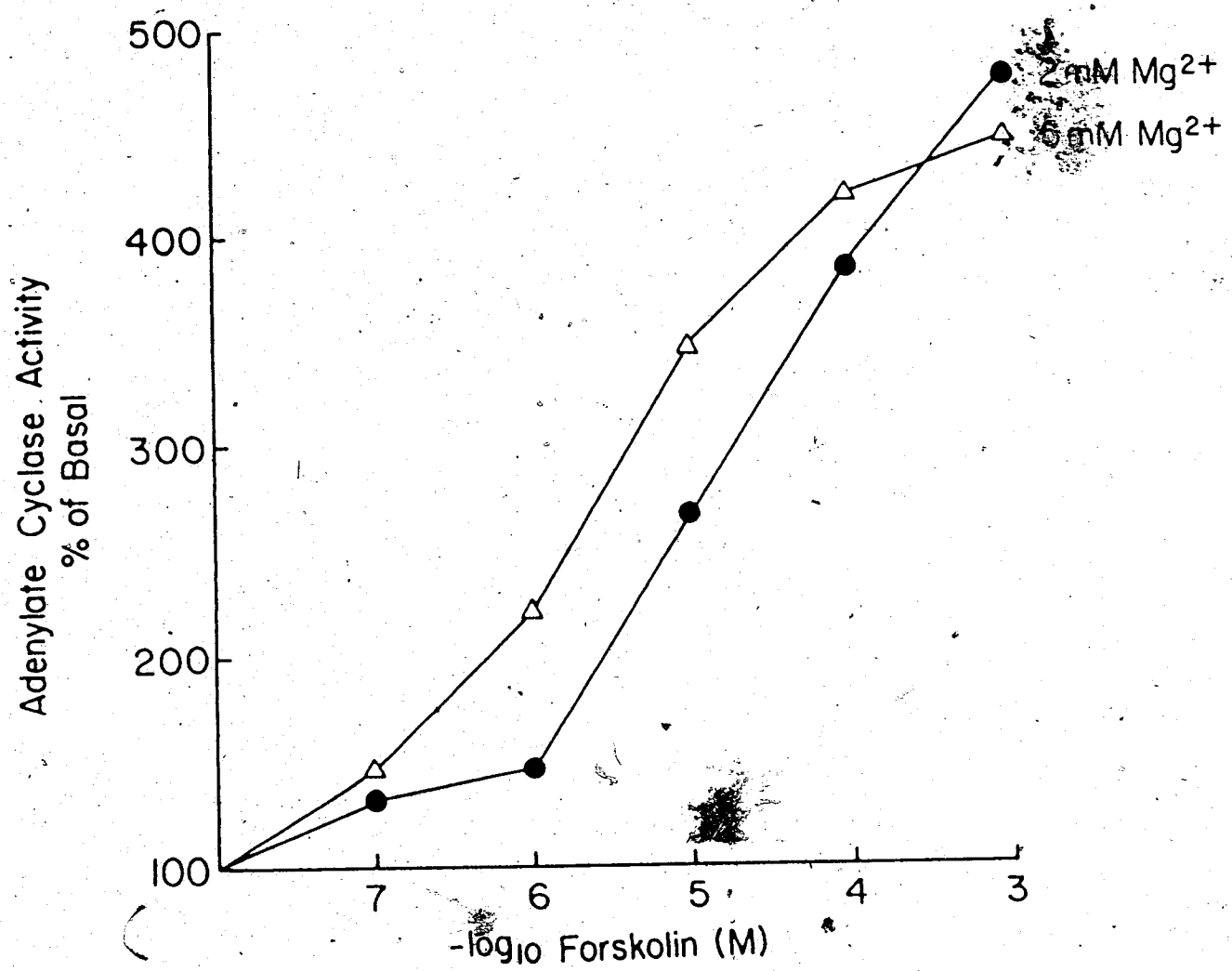


Fig. 38. Stimulation of lung adenylate cyclase by forskolin at 37°C in the presence of Mg^{2+} (2 mM) or (5 mM). The results are the means of duplicates.

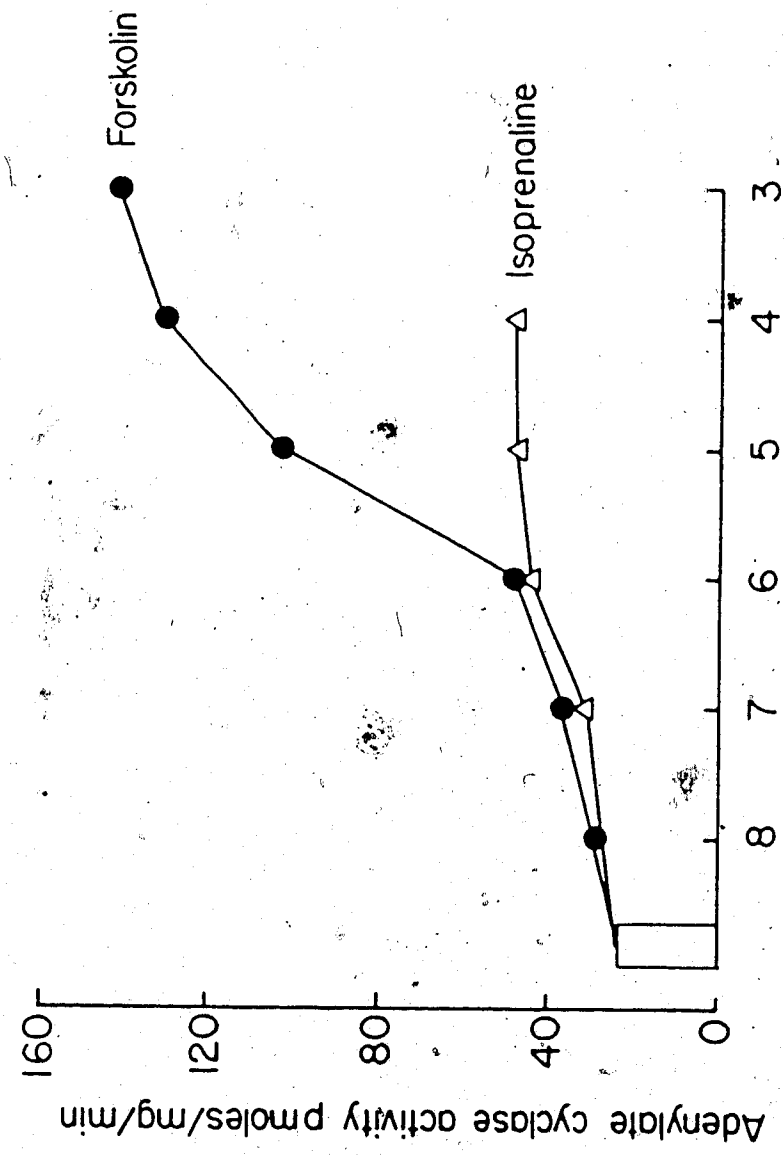


Fig. 39. Stimulation of lung adenylate cyclase at 20°C by forskolin and isoproterenol. The Mg^{2+} concentration was 2 mM. The bar represents the basal unstimulated enzyme activity. Results are the means of duplicates.

centrations of 2 mM the effects of forskolin (expressed as a percentage of basal activity) were less than at Mg^{2+} concentrations of 5 mM. Fig. 39 shows the relatively weak stimulation of the enzyme induced by isoproterenol at 20°C and the dramatic stimulation induced by forskolin. The EC_{50} of forskolin in stimulating the lung enzyme was approximately 10 μM (Figs. 38 and 39).

The activation of lung adenylate cyclase by GTP in the absence and presence of LTD_4 (0.1 μM) is shown in Fig. 40. Table 16 shows the effects of adenosine deaminase (10 U/ml) on the GTP-stimulated enzyme. Adenosine deaminase was included in order to determine whether adenosine was being formed endogenously in the assay. This is a common problem in studies of adenylate cyclase because endogenous adenosine may mask stimulation or inhibition of the enzyme by other agents. Adenosine deaminase will deaminate adenosine to produce inosine which is virtually inactive at adenosine receptors. As can be seen from Table 16 inclusion of adenosine deaminase did not significantly alter the profile of GTP stimulation of the enzyme.

Inclusion of adenosine deaminase in rat cerebellar cyclase assays completely changed the profile of the effects of GTP (Figs. 41 and 42). It can be seen in Fig. 41 that the higher the amount of enzyme (protein concentration) added, the greater was the inhibitory effect of GTP at any given concentration. However, if adenosine deaminase (2.5 or 5 U/ml) was also included, the inhibitory effects of GTP were reduced (Fig. 41). Hence it seemed that endogenously formed adenosine

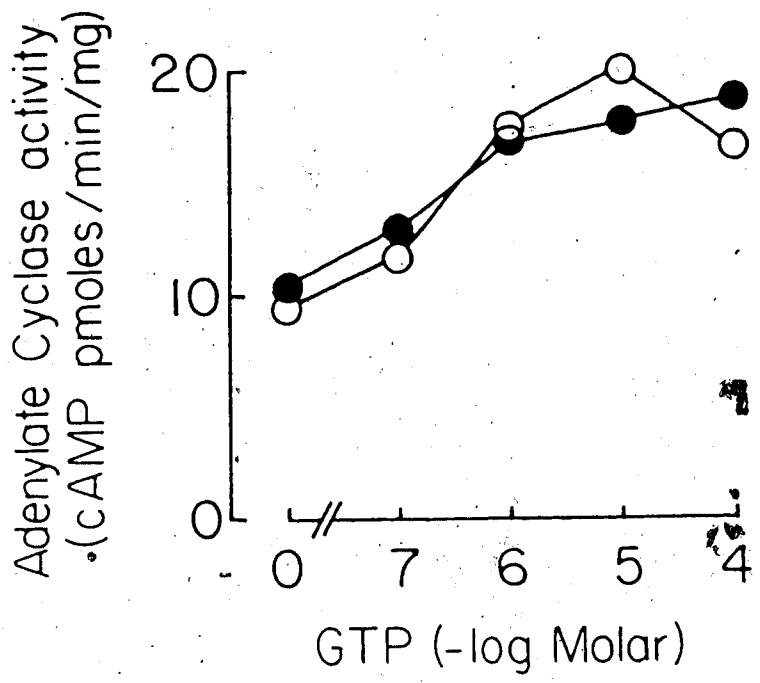


Fig. 40. Activation of lung adenylate cyclase by GTP in the absence (●) and presence of LTD_4 (0.1 μM) (○) at 20°C. The points are means of duplicates.

Table 16. Effects of GTP on lung adenylate cyclase in the presence or absence of Adenosine deaminase (10 U/ml).

	Control	+ Adenosine deaminase (10 U/ml)
	Pmoles cyclic AMP/min/mg protein	
Basal activity	11.5±1.2[a]	10.4±1.2
GTP 0.1 (μM)	14.2±0.6	12.6±0.1
GTP 1.0 (μM)	17.3±0.1	16.7±1.5
GTP 10.0 (μM)	21.1±0.7	17.2±0.7
GTP 100.0 (μM)	20.5±0.4	18.5±0.9

[a] = values are mean±SEM of duplicates.

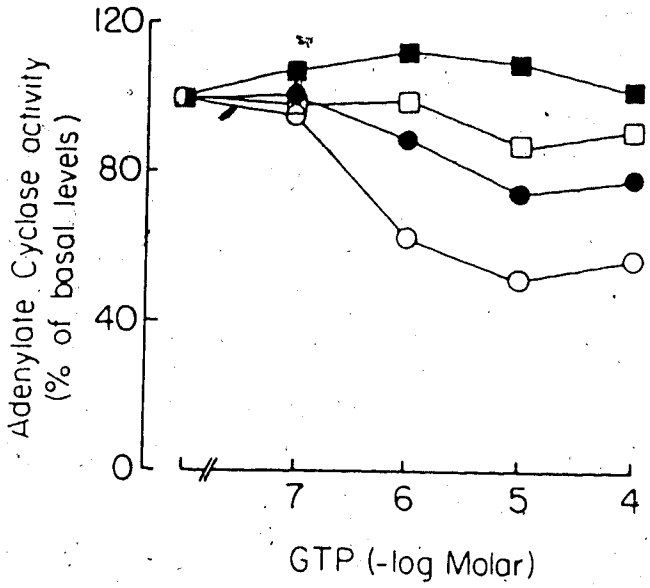


Fig. 41. The effects of adenosine deaminase on the responses of rat cerebellar adenylate cyclase to GTP. The symbols represent the following: (○), 0.4 mg/ml protein; (●), 0.2 mg/ml protein; (□), 0.4 mg/ml protein plus adenosine deaminase 2.5 U/ml; (■), 0.4 mg/ml protein plus adenosine deaminase 5 U/ml. The experiment was performed at 20°C. Each point is the mean of duplicates.

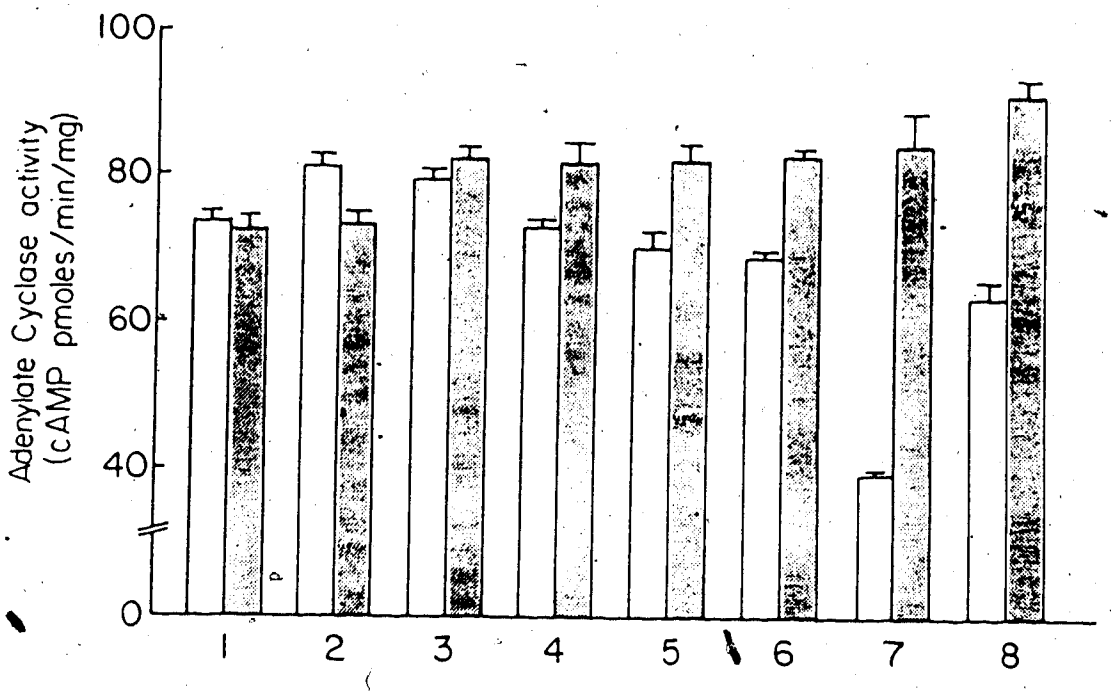


Fig. 42. Effects of adenosine deaminase on the responses of rat cerebellar adenylate cyclase to GTP and adenosine (10 μM). The clear bars are controls. The hatched bars are in the presence of adenosine deaminase (5 U/ml). The numbers indicate the following: 1: Basal activity; 2: adenosine; 3: GTP (0.1 μM); 4: GTP (0.1 μM) + adenosine; 5: GTP (1 μM); 6: GTP (10 μM); 7: GTP (10 μM) + adenosine; 8: GTP (100 μM). The results are mean±SEM of duplicates.

was inhibiting the enzyme in a GTP-dependent fashion. This was confirmed by the experiment shown in Fig. 42. Exogenously added adenosine inhibited the enzyme (Bars 6 and 7) in the presence of GTP and absence of adenosine deaminase. However, if adenosine deaminase (5 U/ml) was present, the inhibitory effects of adenosine were abolished and the slight stimulatory effects of GTP on rat cerebellar adenylate cyclase became apparent (Figs. 41 and 42). Therefore, in all further experiments with cerebellar adenylate cyclase only low amounts (<0.2 mg/ml) of protein were used. Furthermore, adenosine deaminase (5 U/ml) was present in the assay mixture. It is apparent that endogenously produced adenosine would inhibit the enzyme and mask the effects of other inhibitors.

Table 17 shows the basal (unstimulated) adenylate cyclase activities of lung and cerebellum. The influence of Mg^{2+}, Mn^{2+}, temperature and substrate concentration are shown. As shown cerebellar cyclase specific activity was about 8 fold higher than for lung cyclase under similar conditions (Table 17). Furthermore, the specific activity of both enzymes was stimulated by an increase in temperature and divalent cation concentration. Mn^{2+} was more effective than Mg^{2+} in stimulating specific cyclase activity. Finally, a reduction of substrate concentration (from 0.1 mM to 0.05 mM) only slightly reduced the specific activity of cerebellar cyclase. This was done in order to further reduce the formation of endogenous adenosine in cerebellar cyclase assays.

Table 17. Basal Adenylate cyclase activities (cyclic AMP pmoles/min/mg)

Tissue	Cation (mM)	Temp (°C)	ATP (mM)	Adenylate cyclase activity
Guinea pig	Mg^{2+} 2.0	20.0	0.1	14.2±1.1 (16)[a]
lung	Mg^{2+} 2.0	37.0	0.1	33.1±5.6 (6)
	Mg^{2+} 5.0	37.0	0.1	64.8±3.6 (12)
	Mn^{2+} 1.0	20.0	0.1	31.9±1.7 (5)
Rat cerebellum	Mg^{2+} 2.0	20.0	0.1	107.1± 7.3 (12)
(+AD 5 U/ml)	Mg^{2+} 2.0	20.0	0.05	95.6± 7.9 (5)
	Mg^{2+} 5.0	20.0	0.05	138.2±11.0 (5)
	Mg^{2+} 1.0	20.0	0.05	230.0±13.3 (3)

[a] = mean±SEM (number of preparations).

4.4.2 Effects of LTD_4 on lung adenylate cyclase:

A major problem in these studies was a difficulty in obtaining reproducible data. This difficulty was compounded by the fact that the LTD_4 effects on the enzyme were small. In experiments performed during this work, LTD_4 effects varied from 0 to 60 % inhibition with the latter occurring very rarely. However, inhibitory effects of about 10-15 % occurred regularly. Representative results are included of all these situations.

The lack of effect of LTD_4 (0.1 μM) on the forskolin and isoproterenol-stimulated lung enzyme is shown in Fig. 43. As can be seen LTD_4 did not affect the degree of stimulation by either forskolin or isoproterenol. In the presence of GTP (10 μM) and forskolin (10 μM) (Fig. 44) LTD_4 slightly inhibited the enzyme with a maximal effect occurring at about 0.1 μM. As can be seen from the figure there was a variable effect of LTD_4 between three different preparations of the enzyme. Furthermore, higher concentrations of LTD_4 (1 μM) tended to inhibit less than lower concentrations.

The effects of LTD_4 on the forskolin (10 μM) stimulated enzyme in the presence of GTP (10 μM) or GTP (10 μM) and NaCl (100 mM) are shown in Fig. 45. As shown, LTD_4 inhibited the enzyme with somewhat greater effects in the presence of NaCl. NaCl also reduced enzyme activity on its own. This effect of LTD_4 shown in Fig. 45 was difficult to reproduce in the same preparation or in other preparations. Similarly the experiment shown in Fig. 46 was difficult to

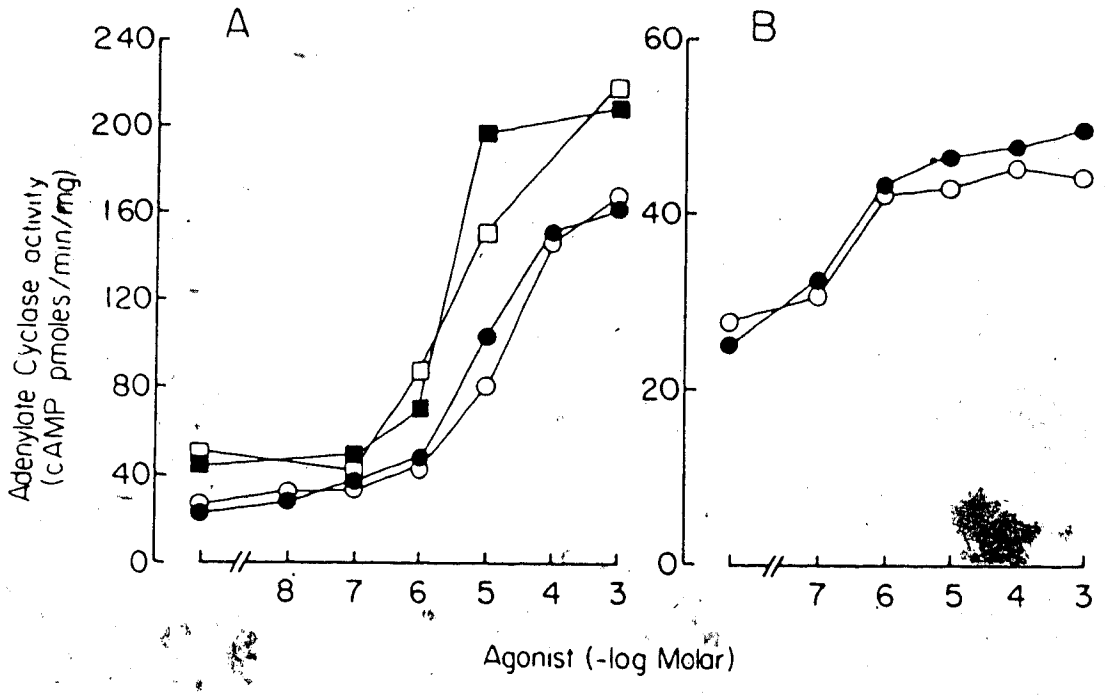


Fig. 43. Lack of effect of LTD_4 (0.1 μM) on forskolin (panel A) or isoproterenol stimulated lung adenylate cyclase (panel B) at 20°C. Forskolin stimulation was measured at Mg^{2+} (2 mM) (●) or Mg^{2+} (5 mM) (■). Isoproterenol stimulation was measured at Mg^{2+} (2 mM). The shaded symbols were the controls and the open symbols were in the presence of LTD_4 (0.1 μM). Each point is the mean of duplicates.

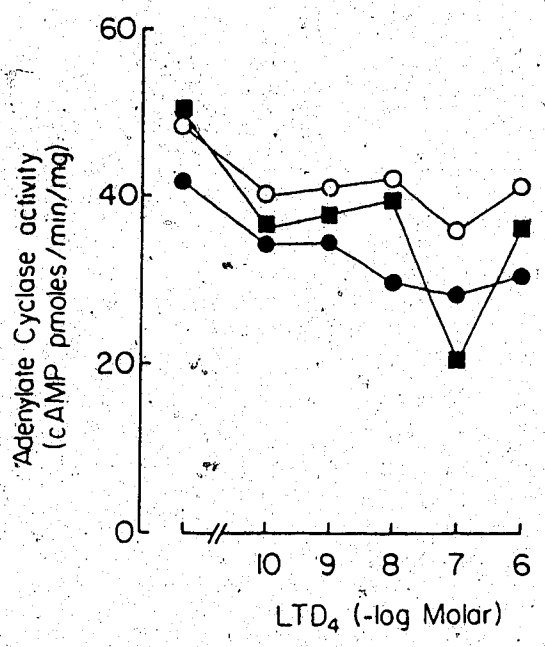


Fig. 44. Effect of LTD_4 on forskolin-stimulated lung adenylate cyclase. GTP (10 μM) was also present. The three different symbols represent three different preparations. Each point is the mean of duplicates.

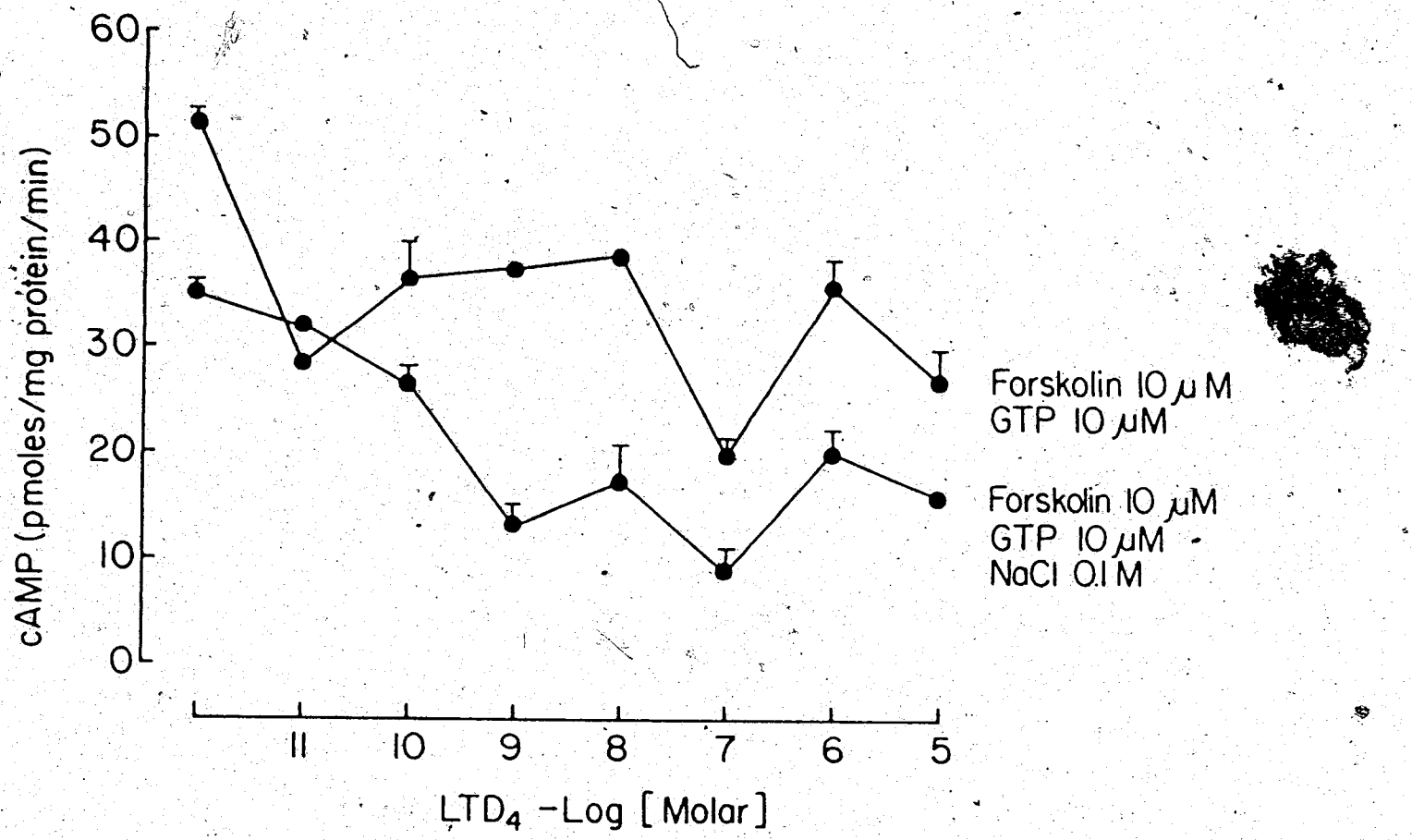


Fig. 45. Effect of LTD_4 on lung adenylate cyclase at 20°C in the presence of Mg^{2+} (2 mM). Each point is the mean±SEM of triplicates. See text for details.

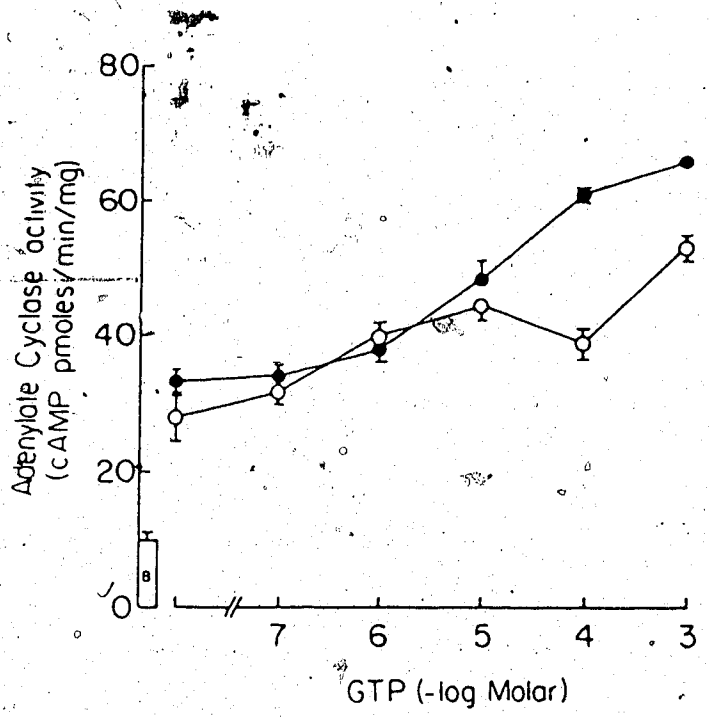


Fig. 46. Effect of GTP on LTD_4-induced inhibition of lung adenylate cyclase. Forskolin (1 μM) was present in each tube. The symbols indicate control (●) and LTD_4 (0.1 μM) (○). The points are mean±SEM of duplicates. The assay was at 20°C in the presence of Mg^{2+} (2 mM). The enzyme's basal activity is indicated by the bar.

reproduce. This experiment was done to demonstrate the GTP dependence of LTD_4 (0.1 μM) inhibition of lung adenylate cyclase.

The effects of LTD_4 on the forskolin (10 μM)-stimulated enzyme in the presence of GTP 0.1, 1.0 and 10 (μM) are shown in Fig. 47. This experiment was performed at 37°C in the presence of Mg^{2+} (5 mM). As shown, LTD_4 inhibited the enzyme with no apparent concentration dependeney. However, the magnitude of inhibition was greater at the higher GTP concentrations (1 and 10 μM). Yet again, these results were difficult to reproduce.

The effect of LTD_4 on the lung enzyme at 20°C in the presence of Mn^{2+} (1 mM) and GTP (0.1 μM and 10 μM) are shown in Fig. 48. Also shown is the effect of LTD_4 on the basal activity in the presence of Mg^{2+} (2 mM). Small inhibitory and stimulatory effects of LTD_4 could be demonstrated. Mn^{2+} (1 mM) increased the basal activity of the enzyme to 3 fold the level observed with Mg^{2+} (Fig. 48). In the presence of GTP (10 μM); LTD_4 (10 nM) slightly inhibited the enzyme. It is noteworthy that the same concentration of LTD_4 slightly stimulated the enzyme in the absence or presence of low concentrations of GTP (0.1 μM) or inhibited the enzyme slightly in the presence of Mg^{2+} (Fig. 48).

In summary, LTD_4-induced inhibition of lung adenylate cyclase was small and difficult to reproduce. However, an effect occurred in most of the experiments performed even though this effect was sometimes small. This is illustrated in Fig. 49 which should be

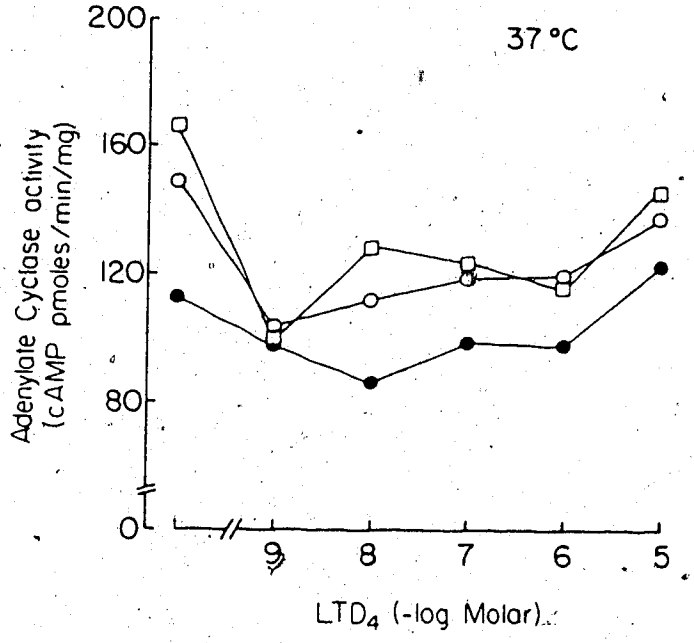


Fig. 47. Effect of LTD_4 on lung adenylate cyclase activity. Forskolin (10 μM) was present in each tube. The symbols indicate the presence of GTP (0.1 μM) (●), (1.0 μM) (○), and (10 μM) (□). The assay was conducted at 37°C in the presence of Mg^{2+} (5 mM). Each point is the mean of duplicates.

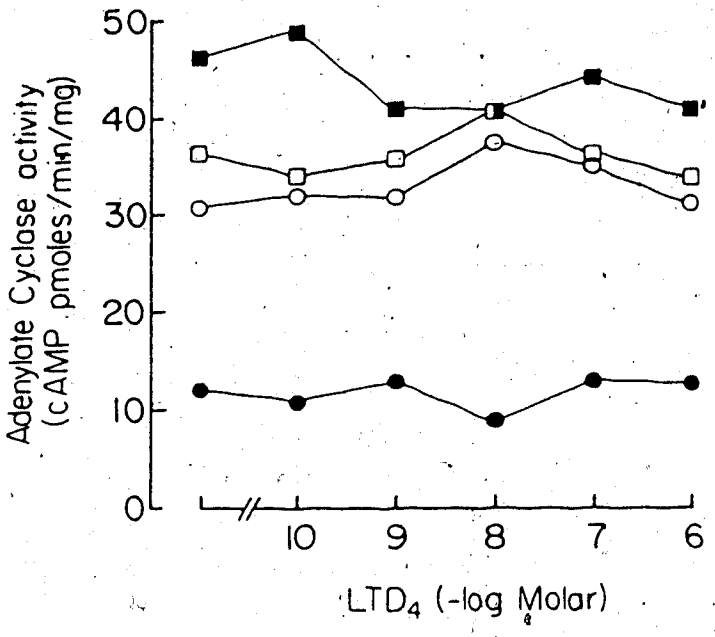


Fig. 48. Effect of LTD_4 on lung adenylate cyclase activity in the presence of Mg^{2+} (2 mM) or Mn^{2+} (1 mM). The symbols indicate Mg^{2+} (●), Mn^{2+} (○), Mn^{2+} and GTP (0.1 μM) (□), and Mn^{2+} and GTP (10 μM) (■). The assay was at 20°C. Each point is the mean of duplicates.

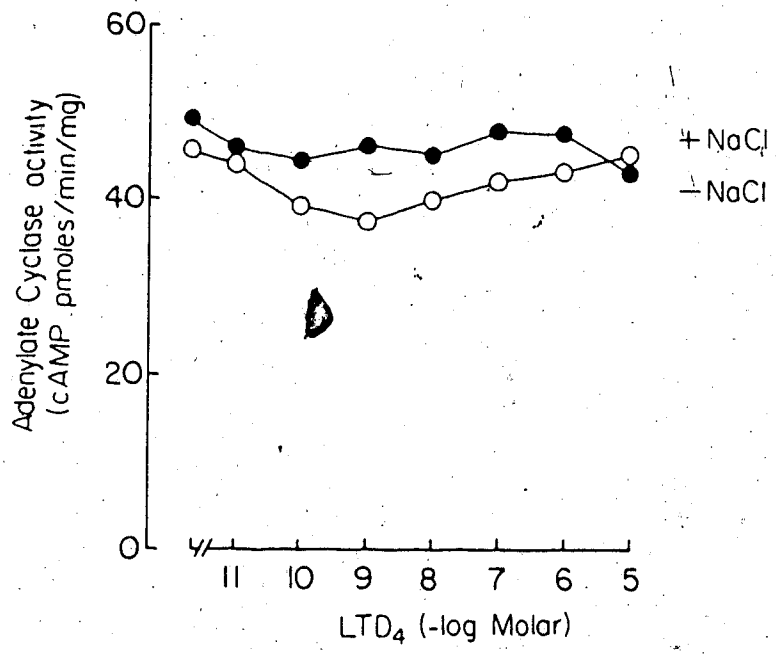


Fig. 49. Effect of LTD_4 on lung adenylate cyclase at 20°C in the presence of Mg^{2+} (2 mM). Each tube contained 10 μM each of forskolin and GTP and NaCl 100 mM as indicated in the figure. Each point is the mean of duplicates.

compared with the experiment in Fig. 45. Both experiments were done under identical conditions as indicated in the figure legends.

In order to determine the causes of variation and lack of reproducibility of the data presented above, a variety of experimental approaches to membrane preparation were undertaken. These included very gentle homogenization procedures which utilized hand homogenization of the tissue or homogenization at low motor speeds. Furthermore, the pH of the homogenization medium was varied from pH 5.5 to pH 8.5. None of these approaches yielded greater inhibition by LTD_4.

Another approach taken was to test the hypothesis that the LTD_4 may not be binding to its receptors in the membranes used for assay of adenylate cyclase activity. Hence, this would lead to little inhibitory effects on enzyme activity. Therefore, the specific binding of $[^3H]$-LTC_4 to guinea-pig lung membranes was studied. The method involved incubation of $[^3H]$-LTC_4 with lung membranes under conditions exactly similar to adenylate cyclase assay conditions. $[^3H]$-LTC_4 bound specifically and with high affinity to lung membranes (Fig. 50). The B_{max} and k_D value demonstrated for the binding interaction are similar to published values (Pong & DeHaven, 1984).

Finally, since divalent cations (mM concentrations) have been shown to stimulate binding of LTs to their receptors, the effects of Ca^{2+} on adenylate cyclase activity of guinea-pig lung (Fig. 51) and rat cerebellum (Fig. 52) were investigated. The effects of Ca^{2+} on

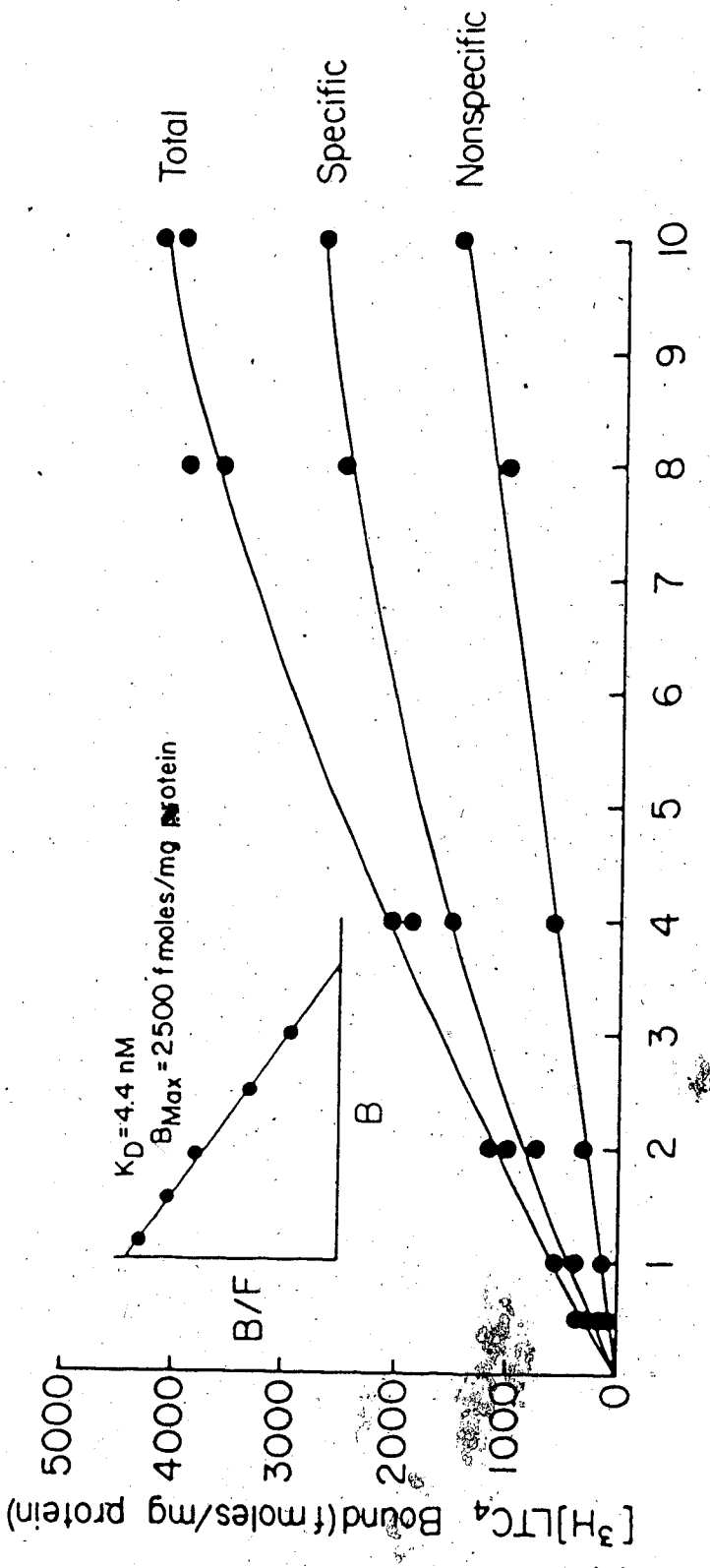


Fig. 50. Binding of [^{3}H]-LTC$_4$ to guinea pig lung membranes at 20°C. The points are means of duplicate values. The inset shows the Scatchard representation of the data.

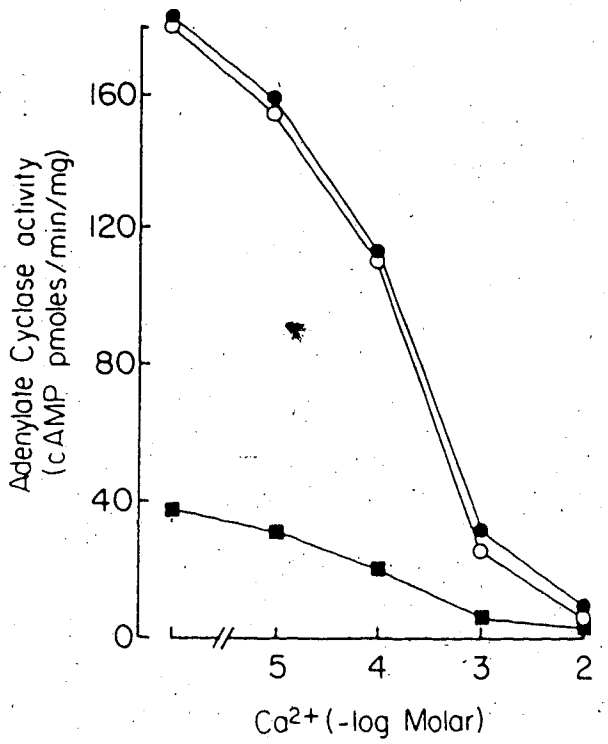


Fig. 51. Inhibition lung adenylate cyclase activity by Ca^{2+}. The symbols represent basal activity (■); forskolin and GTP (10 μM) (●), and forskolin and GTP (10 μM each) with LTD_4 (0.1 μM) (○). The points are the means of duplicates. The assay was carried out at 37°C.

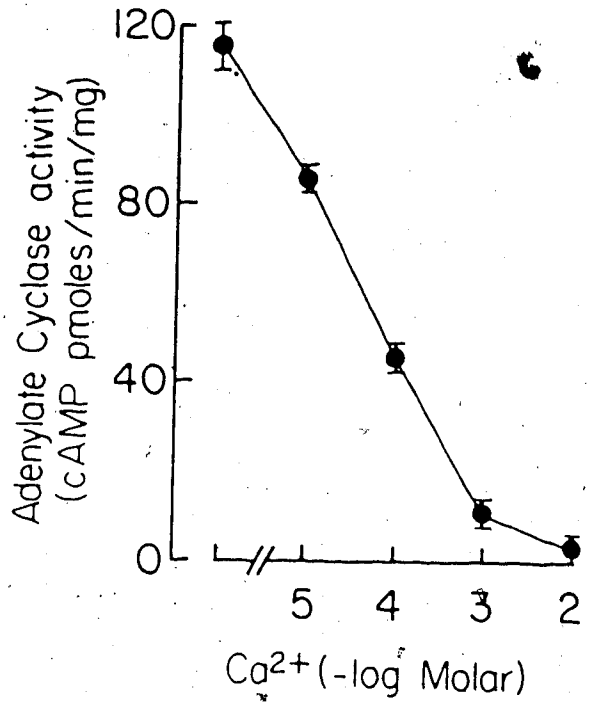


Fig. 52. Inhibition of cerebellar basal adenylate cyclase activity by Ca^{2+}. The points are mean±SEM of duplicates. The assay was carried out at 20°C in the presence of Mg^{2+} (2 mM).

LTD_4 action on lung cyclase was also studied (Fig. 51). Ca^{2+} inhibited basal and forskolin stimulated activity of lung adenylate cyclase and basal activity of cerebellar adenylate cyclase with an IC_{50} of about 100 μM (Figs. 51 and 52). Hence Ca^{2+} ions could not be used to stimulate the binding of LTD_4 to its receptors in order to facilitate the effect on adenylate cyclase.

4.4.3 Effect of GTPγS on guinea-pig lung and rat cerebellar adenylate cyclase:

As mentioned in section 1.2.3.1.1. GTPγS-induced inhibition of adenylate cyclase activity is an index of the presence and function of Ni (Seamon & Daly, 1982; Jakobs et al. 1984). This effect of GTPγS was utilized in these studies to demonstrate Ni functions in both cyclase systems studied. Furthermore, this effect was used to demonstrate optimal conditions for inhibiton of both enzymes.

The effects of GTPγS were determined against the basal and forskolin stimulated activity of lung and cerebellar cyclases at 20°C and 37°C. As shown in Fig. 53, GTPγS slightly inhibited forskolin stimulated but not basal adenylate cyclase activity of lung at 20°C. The magnitude of adenylate cyclase inhibition was 16.3±3.4% (n=3) (Table 18). This maximal inhibitory effect of GTPγS occurred at 1 nM. Higher concentrations stimulated enzyme activity (Fig. 53). The presence of LTD_4 (0.1 μM) did not modify GTPγS effects (Fig. 53).

GTPγS inhibition of forskolin-stimulated lung adenylate cyclase

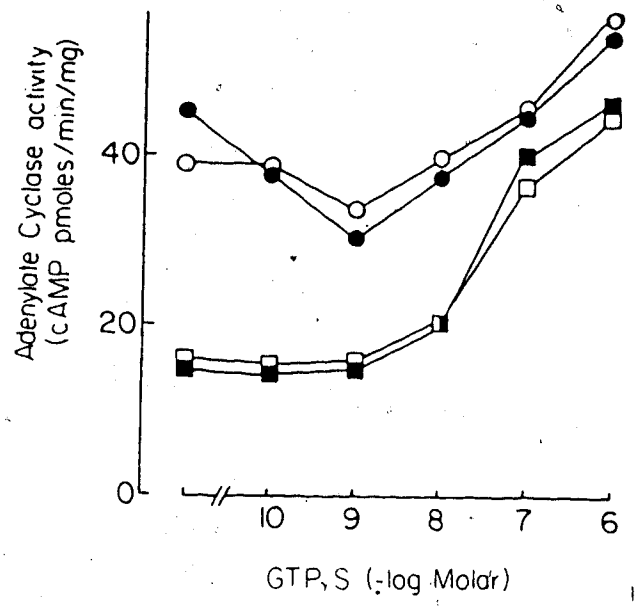


Fig. 53. Inhibition and stimulation of lung adenylate cyclase activity by GTPγS at 20°C. The Mg^{2+} concentration was 2 mM. The squares represent basal unstimulated activity. The circles represent stimulation with forksolin (10 μM). Open symbols represent activity determined in the presence of LTD_4 (0.1 μM) and the shaded symbols, control activity. Each point is the mean of duplicates.

Table 18. GTPγS inhibition[a] of adenylate cyclase activity.

Tissue	Temp (°C)	ATP (mM)	% inhibition of adenylate cyclase activity
Rat cerebellum	20.0	0.1	60.2±3.6 (6)[b]
+			
GTPγS (0.1 μM)	20.0	0.1 + AD[c] 5 U/ml	62.6±4.4 (4)
	20.0	0.05 + AD 5 U/ml	61.3±1.8 (4)
Guinea pig lung	20.0	0.1	16.0±3.4 (3)[d]
+			
GTPγS (1 nM)	37.0	0.1	3.9±1.3 (2)[c]

a = Measured in the presence of Mg^{2+} (2 mM).

b = Mean±SEM (number of preparations).

c = AD = adenosine deaminase.

d = Measured in the presence of forskolin (100 μM).

activity at 37°C was very small 3.9±1.3% (n=2) (Table 18, Fig. 54). Concentrations over 1 nM stimulated the enzyme and LTD_4 (0.1 μM) did not modify this effect (Fig. 54).

Fig. 55 shows the effects of GTPγS on lung and cerebellar adenylate cyclase activities at 37°C. As shown GTPγS inhibited and stimulated enzyme activity in a concentration dependent fashion. Low concentrations (0.1 μM) inhibited cerebellar adenylate cyclase stimulated with forskolin (100 μM) to about 50% of the control level. The maximal inhibition of cerebellar adenylate cyclase in the absence of forskolin stimulation was less (Fig. 55) and occurred at a lower concentration (10 μM) of GTPγS. GTPγS stimulated adenylate cyclase activity of cerebellar membranes at higher concentrations (Fig. 55).

The time course of GTPγS inhibition of basal cerebellar adenylate cyclase activity at 20°C in the presence of low Mg^{2+} (2 mM) concentrations is shown in Fig. 56. The inhibition proceeded after an initial lag phase of 5 minutes. The magnitude of inhibition at the maximally effective concentration (0.1 μM) was 62.6±4.4% (n=4) (Table 17).

The small inhibitory effect of GTPγS on lung adenylate cyclase relative to cerebellar adenylate cyclase (Table 18) implies that Ni function or concentrations are relatively smaller in lung than in cerebellar membranes. It was important to determine whether this situation was intrinsic to lung membranes and is not an artifact of lung membrane preparation. Ni effects are reduced by proteases (Jakobs et al. 1984) and homogenization of lung and other tissues may

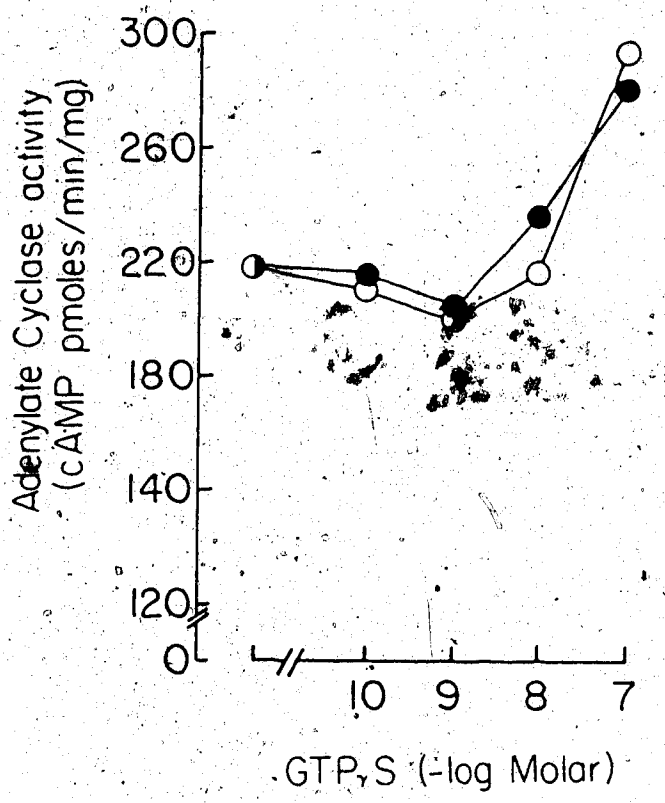


Fig. 54. Effect of GTPγS on forskolin (100 μM) stimulated lung adenylate cyclase activity at 37°C. The Mg^{2+} concentration was 2 mM. Open symbols represent activity in the presence of LTD_4 (0.1 μM). Shaded symbols represent control activity. Each point is the mean of duplicates.

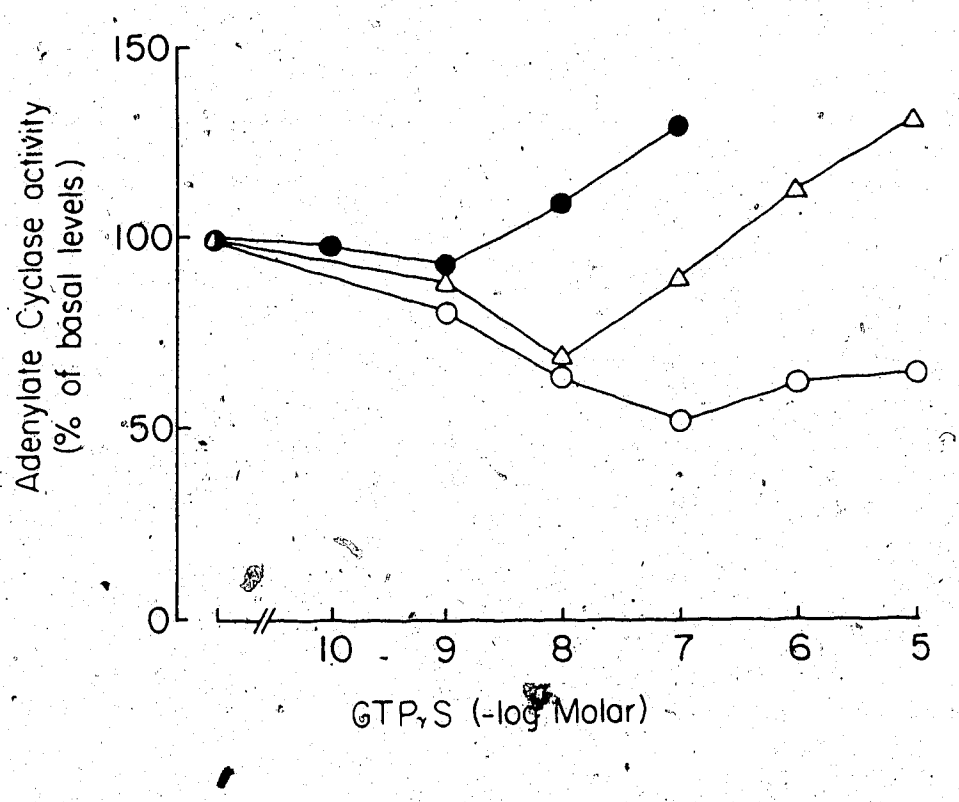


Fig. 55. Effect of GTPγS on lung and cerebellar adenylate cyclase activities at 37°C. The Mg^{2+} concentration was 2 mM. The symbols represent lung adenylate cyclase stimulated with forskolin (●); cerebellar adenylate cyclase at basal activity (△); and cerebellar adenylate cyclase stimulated with forskolin (100 μM) (○). The control adenylate cyclase activities were 218.5 ±3.0, 147.0 ±10.1, and 964.5 ±24.1 pmoles cyclic AMP/min/mg, respectively. Each point is the mean of duplicates.

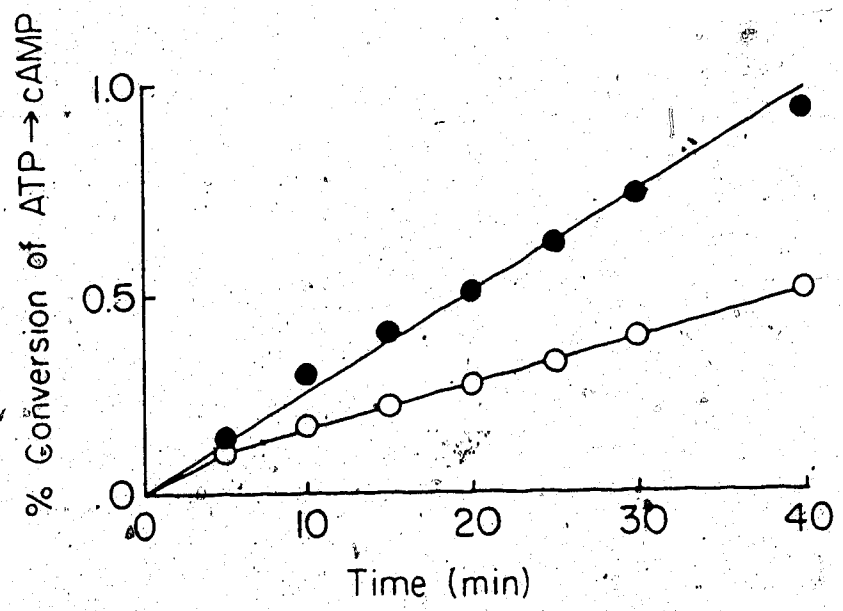


Fig. 56. Time course of GTPγS (0.1 μM) (○) inhibition of basal (●) cerebellar adenylate cyclase activity at 20°C. The Mg^{2+} concentration was 2 mM. Each point is the mean of duplicates.

release proteases that will affect Ni function. Therefore, to show that such a situation was not occurring in these experiments, the supernatant from a lung homogenate was taken and incubated with half a rat cerebellum. The tissue was homogenized in this medium and membranes prepared as described in the Methods. The other half of the cerebellum was treated exactly the same way with buffer that had not been exposed to lung tissue. Subsequently, both membrane preparations were assayed for GTPγS-induced inhibition of adenylate cyclase activity at 20°C. GTPγS inhibited basal adenylate cyclase activities of both preparations with exactly similar percentage inhibition at all concentrations (Fig. 57). The basal activities for both preparations were also very similar (Fig. 57).

Hence using this bioassay approach it is likely that the small Ni-mediated inhibition of lung adenylate cyclase is an intrinsic quality of the membranes and is not due to artefacts arising from lung homogenization. Furthermore, the use of protease inhibitors including polymethylsulphonyl fluoride (0.3 mM), soybean trypsin inhibitor (20 μg/ml), leupeptin (1 μg/ml) and bacitracin (100 μM) in the homogenization buffer did not increase the magnitude of GTPγS inhibition of lung adenylate cyclase activity (Fig. 58). However, basal activities and forskolin stimulated activities were slightly increased after homogenization in the presence of protease inhibitors (Fig. 58).

Therefore, from the above experiments it seems likely that the small degree of LTD_4-induced inhibition of lung adenylate cyclase may

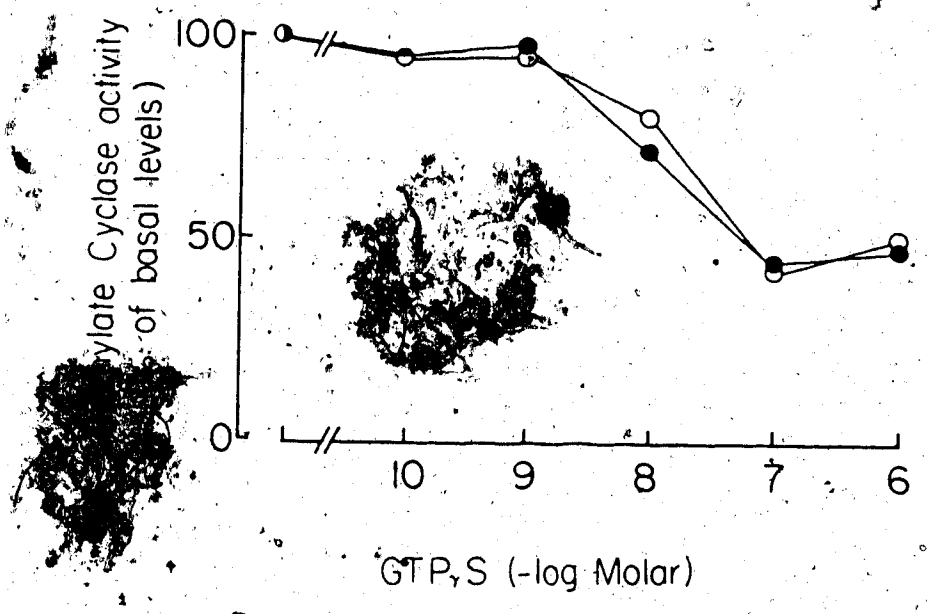


Fig. 57. Effect of GTPγS on cerebellar adenylate cyclase activity at 20°C. The Mg^{2+} concentration was 2 mM. The shaded symbols represent control adenylate cyclase activity. The open symbols represent adenylate cyclase activity of cerebellum treated with supernatant obtained from a lung homogenate (see text for details). The basal activities were 81.2±4.7 and 82.3±1.9 pmoles cyclic AMP/min/mg, respectivley. Each point is the mean of duplicates.

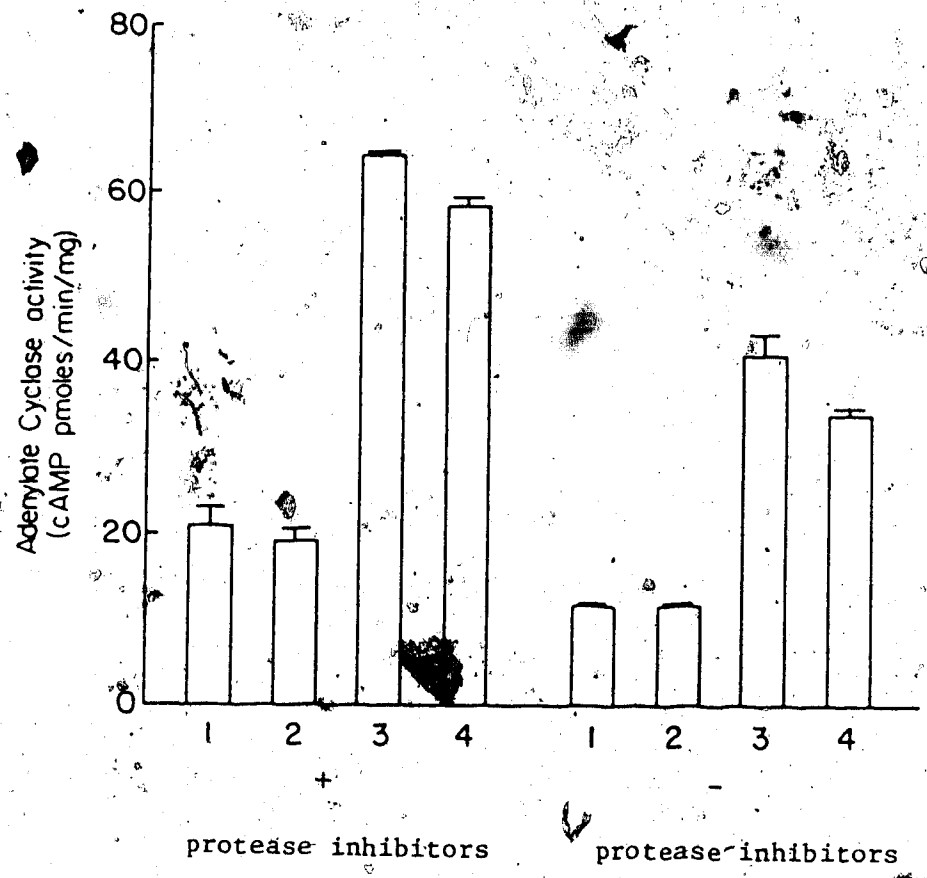


Fig. 58. Effect of protease inhibitors on lung adenylate cyclase activity in the presence of GTPγS (1 mM) at 20°C. The numbers indicate the following: 1 = basal activity; 2 = GTPγS (1 nM); 3 = Forskolin (100 μM); 4 = Forskolin (100 μM) + GTPγS (1 nM). Each bar is the mean±SEM of duplicates.

be related to the low functional status of Ni (as determined with GTPγS) in lung membranes.

Further experiments to determine the effects of a hormonal inhibitor of cerebellar adenylate cyclase (a system where Ni functions are pronounced, see Table 18) were undertaken. This served as a positive control against which the effects of LTD_4 on the lung could be compared. The hormone chosen was GABA (see section 1.2.1.4.7). Furthermore, all subsequent experiments were done at 20°C and 50 μM ATP substrate concentration unless stated otherwise; the former because GTPγS inhibition was greater at lower temperature, and the latter to reduce the production of adenosine in cerebellar adenylate cyclase assays.

4.4.4 Effects of GABA and LTD_4 on cerebellar adenylate cyclase activity.

As shown in Fig. 59 GTPγS-induced inhibition of cerebellar adenylate cyclase activity was not further affected by inclusion of GABA (10 μM) or LTD_4 (10 nM) in the assay. Hence in this system GTPγS cannot substitute for GTP in mediating hormonally-induced inhibition of the enzyme. The crucial role of GTP in mediation of the inhibitory effects of hormones (Rodbell, 1980) is shown in Fig. 60. As shown in Fig. 60, GABA and LTD_4 did not affect cerebellar adenylate cyclase activity in the absence of GTP. However in the presence of GTP, both GABA (10 μM) and LTD_4 (10 nM) inhibited enzyme activity. Maximal

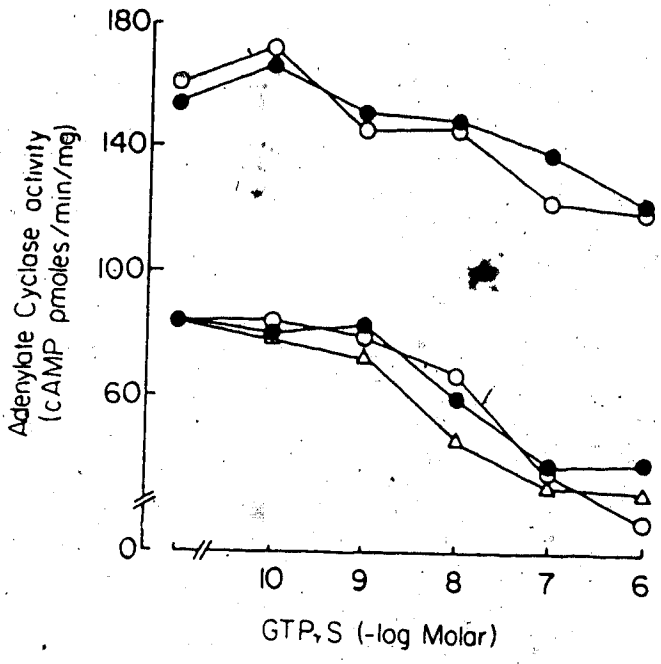


Fig. 59. Effects of GTPγS on cerebellar adenylate cyclase activity in the presence of GABA or LTD_4. The assay was carried out at 20°C in the presence of Mg^{2+} (2 mM). The symbols indicate the following: Basal activity or forskolin (100 μM) stimulated activity (●): LTD_4 (10 nM) (○): or GABA (10 μM) (△). Points are the means of duplicates.

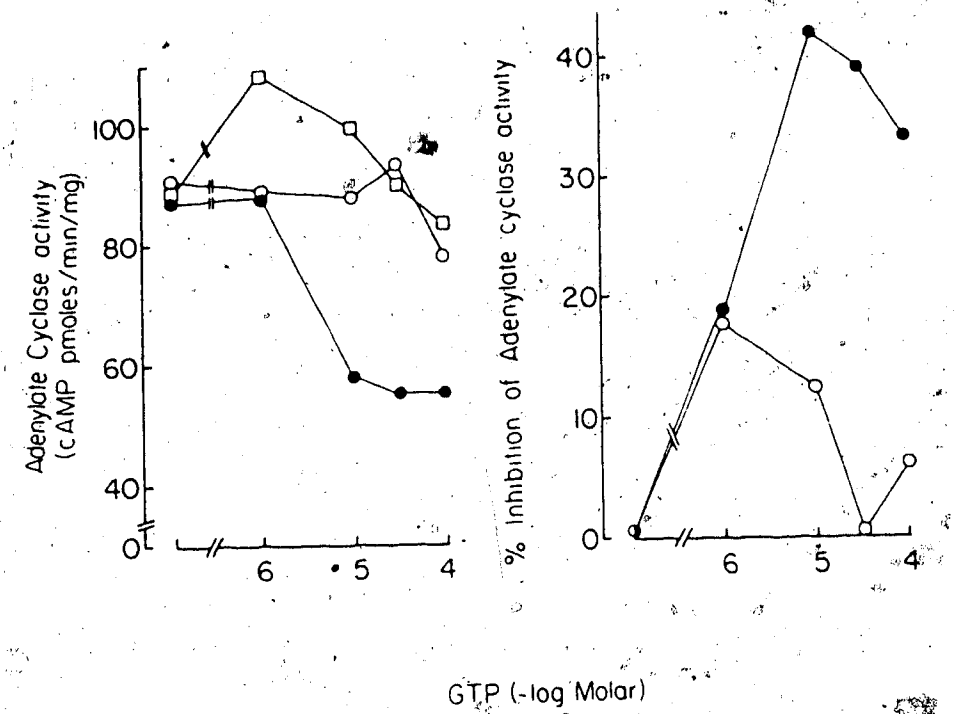


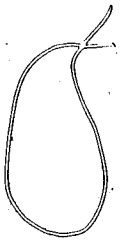
Fig. 60. Effects of LTD_4 and GABA on cerebellar adenylate cyclase activity at 20°C. The effects of GTP are shown in the left panel (□) and of GTP in the presence of LTD_4 (10 nM) (○) or GABA (10 μM) (●). The right panel shows the % inhibition of activity in the presence of GTP alone. Each point is the mean of duplicates.

GABA-induced inhibition of enzyme activity was about 40%.

Inhibition of adenylate cyclase activity occurred maximally at a GTP concentration of 10 μM (Fig. 60). Lower or higher concentrations of GTP tended to reduce the effects of GABA in this and other experiments. LTD_4 (10 nM) also inhibited the enzyme. However, this effect occurred maximally at concentrations of GTP of about 1 μM. Furthermore, the magnitude of inhibition of LTD_4 was small (i.e. less than 20% inhibition of enzyme activity) and as in the lung, problems were encountered with reproducibility of the data between preparations.

The complete concentration-response relationship of GABA in inhibition of cerebellar adenylate cyclase at 20°C, in the presence of GTP (10 μM), is shown in Fig. 61. The maximal effect of GABA (≈35% inhibition) occurred at 10 μM.

In order to demonstrate that the inhibition of adenylate cyclase activity induced by GABA (10 μM) and GTPγS (0.1 μM) was occurring under optimal conditions of temperature and divalent cation concentrations, the experiments shown in Fig. 62 and Table 19 were performed. Maximal inhibition of the enzyme by GABA and GTPγS occurred at 20°C and a low concentration of Mg^{2+} (Fig. 62). Table 19 shows the percentage inhibition of the enzyme by GABA (in the presence of GTP (10 μM) and GTPγS at different concentrations of Mg^{2+} and Mn^{2+} in the assay. Again, maximal inhibition of enzyme activity by GABA (36.3±3.6 n=3) and by GTPγS (57.1±3.0% n=4) occurred at a concentration of 2 mM Mg^{2+}. Higher concentrations of Mg^{2+} and Mn^{2+} reduced GTPγS-induced



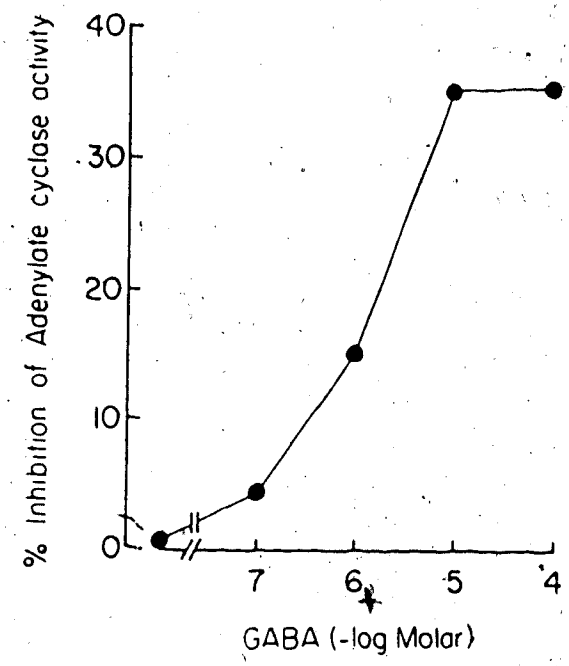


Fig. 61. Inhibition of cerebellar adenylate cyclase activity by GABA at 20°C. GTP (10 μM) was present in all tubes. The Mg^{2+} concentration was 2 mM. Each point is the mean of duplicates.

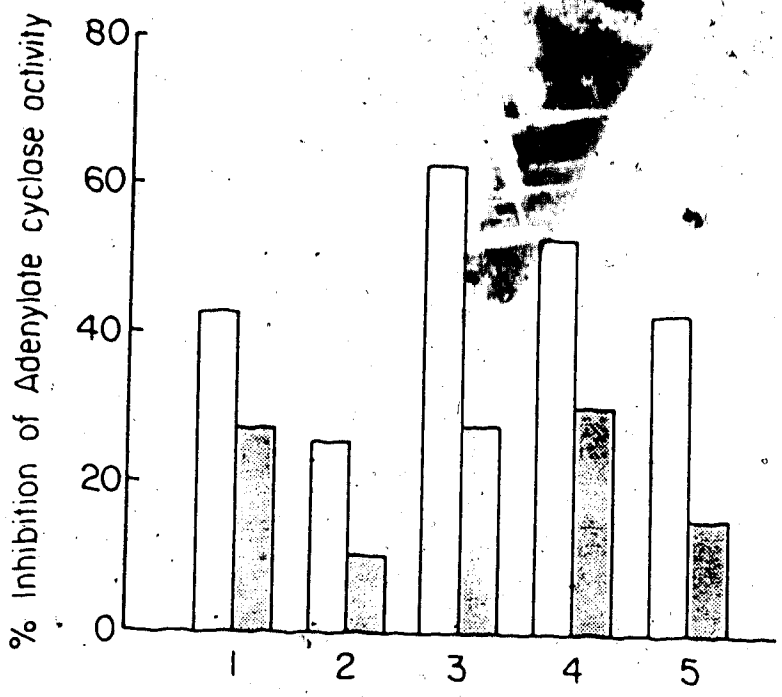


Fig. 62. Inhibition of cerebellar adenylate cyclase activity by GTPγS (clear bars) and GABA (10 μM) in the presence of GTP (10 μM) (shaded bars) under various conditions. The numbers indicate: 1 = Mg^{2+} (2 mM) and 37°C; 2 = Mg^{2+} (10 mM) and 37°C; 3 = Mg^{2+} (0.5 mM) and 20°C; 4 = Mg^{2+} (2 mM) and 20°C; 5 = Mg^{2+} (10 mM) and 20°C. Each bar is the mean of duplicates.

Table 19. % Inhibition of rat cerebellar cyclase basal activity in the presence of Mg^{2+} and Mn^{2+}.

Cation (mM)		GPTγS (0.1 μM)	GABA + GTP (100 μM) (10 μM)
Mg^{2+}	0.5	55.5±10.4%[a]	27.3±0.1%
	2.0	57.1±3.0%	36.3±3.6%
	5.0	62.1±1.2%	17.4±0.1%
	10.0	42.1±0.9%%	15.5±0.3%
Mn^{2+}	1	56.9±0%	10.2±0.4%
	10	37.4±0.9%	Not done

[a] = mean±SEM (n>2).

inhibition (Table 19).

Therefore, the effects of LTD_4 on cerebellar adenylate cyclase activity were investigated at 20°C and Mg^{2+} (2 mM) in the rest of these experiments.

LTD_4 (10 nM) inhibited enzyme activity in the presence of GTP (Fig. 63). Under the same conditions LTC_4 (10 nM) only slightly inhibited enzyme activity whereas GABA (10 μM) inhibited the enzyme. In this experiment LTD_4 inhibited the enzyme to a greater degree than was observed in other experiments (see Fig. 60). Furthermore, this inhibition occurred at a high concentration of GTP (30 μM). A higher concentration of GTP (100 μM) completely abolished LTD_4 induced inhibition and reduced GABA-mediated inhibition.

As shown in Fig. 64 LTD_4 inhibited enzyme activity in the presence of GTP (10 μM) and slightly stimulated activity in the presence of GTP (100 μM). The concentrations of LTD_4 causing maximal inhibition also caused maximal stimulation (Fig. 64).

In summary, LTD_4 slightly reduced or slightly increased adenylate cyclase activity depending on the GTP concentration in the assay. A high GTP concentration tended to enhance the slight stimulatory effect observed in some experiments. It should be mentioned that in some experiments LTD_4 failed to affect enzyme activity (in the presence of GTP) however GABA-induced inhibition was also relatively low in these experiments.

The lack of reproducibility of LTD_4 effects in the lung and in

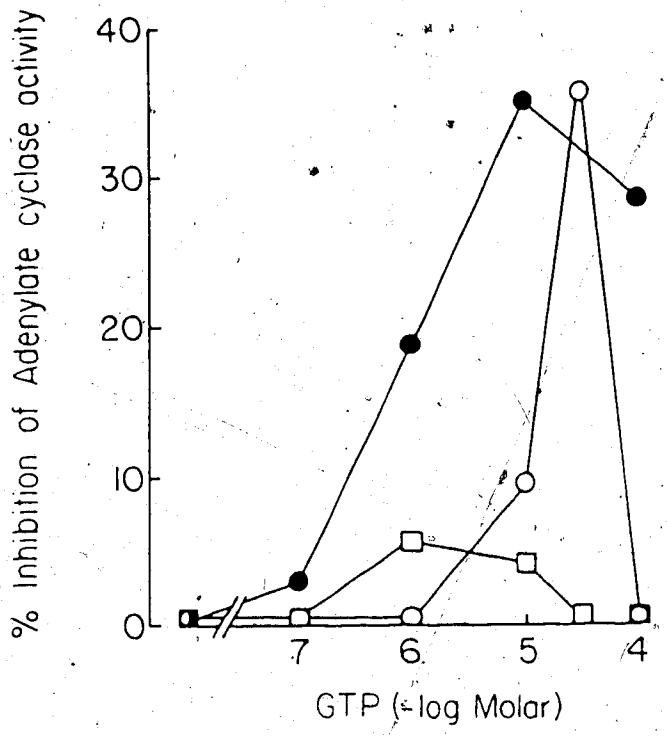


Fig. 63. Percentage inhibition of cerebellar adenylate cyclase activity at 20°C by GABA (10 μM) (●); LTD_4 (10 nM) (○); and LTC_4 (10 nM) (□). The Mg^{2+} concentration was 2 mM. Points are the means of duplicates.

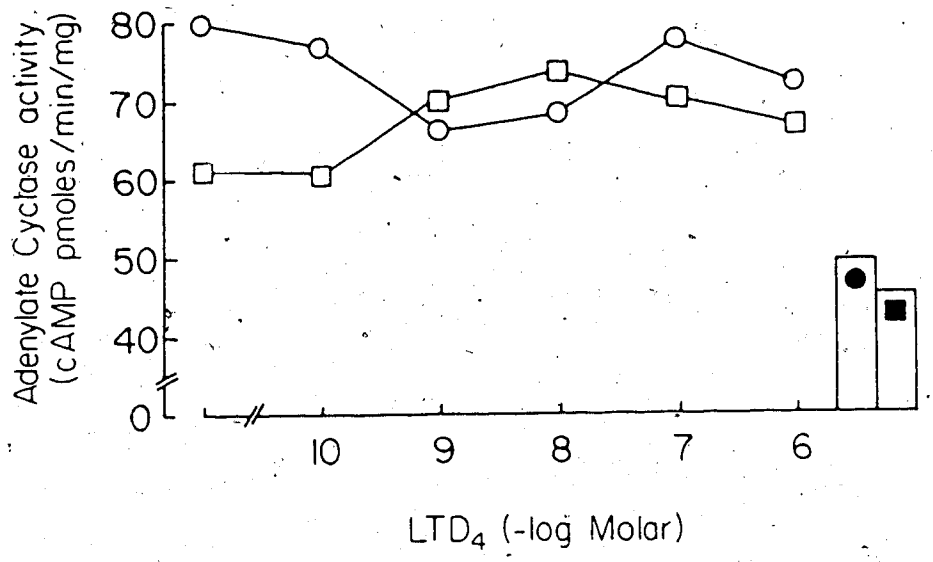


Fig. 64. Effect of LTD_4 on cerebellar adenylate cyclase in the presence of GTP (10 μM) (○) or 100 μM (□). The bars indicate the effect of GABA (10 μM) under both conditions. The assay was carried out at 20°C and Mg^{2+} (2 mM). The points are the mean of duplicates.

cerebellar adenylate cyclase systems was a major problem in these studies. As mentioned earlier, some approaches were undertaken in order to resolve these difficulties. However, the variability persisted and at the end of this work these factors remain unresolved.

5. DISCUSSION

5. Discussion

5.1 Effects of bronchoconstrictors and bronchodilators on normal and sensitized airway tissues:

Challenge of sensitized airway tissues with specific antigen (OA) leads to a prolonged bronchoconstriction in vitro (Schultz-Dale response). The mediators released in this reaction include histamine, LTC_4 and LTD_4 (Fleisch et al. 1982; Section 5.2 of this thesis).

The sensitivity to antigen of sensitized tissues did not result in an enhanced response to the bronchoconstrictor agonists used in this study. Our results indicated that in the absence of inhibitors of AA metabolism there were no appreciable differences between normal and sensitized airways in reactivity and sensitivity to bronchoconstrictors including KCl, histamine, carbachol, LTC_4, LTD_4, U-44069, and A23187. Furthermore, bronchodilator hypoactivity did not exist in sensitized airway tissues since isoproterenol, PGE_2, forskolin, and VIP exhibited similar potency and efficacy on both normal and sensitized airway tissues. Hence in this guinea-pig model of asthma, mere immunological sensitization to OA does not induce bronchoconstrictor hyperreactivity and bronchodilator hypoactivity when these effects are determined in vitro.

It was important to perform the above studies because human bronchial asthma exhibits important characteristics that may differ from animal models of this disease. The results obtained in the guinea pig suggest that mere immunological sensitization per se does

not lead to hyperreactivity and/or hypoactivity and that other factors inherent in human asthma may be deemed to be causatively important. Differences between human asthma and animal models include the following: (1) In human allergic asthma IgE mediates the immediate

hyperreactivity to methacholine and hypoactivity to isoproterenol are IgE producers (Rinard et al. 1979; Antonissen et al. 1980). In guinea pigs sensitized with OA in the presence of Freunds complete adjuvant, hyperreactivity to histamine, 5HT, bradykinin, acetylcholine and $PGF_{2}\alpha$ can be demonstrated (Morcillo et al. 1984). Based on the studies of Andersson (1980), it is possible that IgE may be causative in producing airway hyperreactivity in this guinea-pig model. Evidence that IgE- and IgG-mediated responses are different was obtained by Regal (1984). This author showed that LTs are important mediators of IgG- but not IgE-mediated guinea-pig tracheal contraction. This observation coupled with those of Andersson (1980) and Andersson and Bergstrand (1981) who showed that anti-asthmatic agents differentially affect antigen-induced bronchoconstriction in guinea pigs with high circulating levels of IgE or IgG, seem to indicate that these homocytotropic antibodies may affect airway smooth muscle function and characteristics differentially.

From our results it seems likely that animal models of asthma producing IgG instead of IgE do not exhibit hyperreactivity. This conclusion is also supported by the findings of Brink et al. (1981) who showed that OA sensitized guinea-pig airway tissues do not exhibit hyperreactivity to histamine in vitro.

As mentioned earlier in this discussion, most animal studies of asthmatic hyperreactivity are conducted on isolated tissues. This removes various influences which may be important in the pathogenesis

of airway hyperreactivity. Airway hyperreactivity may arise as a consequence of an enhanced vagal reflex which maybe due to increased sensitivity of irritant receptors in the airways (Leff, 1982; Garland, 1984). This influence is not present if studies are conducted in vitro as are other modulatory influences operating in vivo. Hence their roles cannot be assessed in vitro in order to determine their importance in the pathogenesis of airway hyperreactivity. It is interesting to note that Iwayama et al. (1982) showed airway hyperreactivity to histamine and airway hypoactivity to isoproterenol in OA sensitized guinea pigs (sensitized in a protocol similar to that used in this thesis) in an in vivo study. Furthermore, Brink et al. (1981) showed an increased sensitivity to histamine in guinea pigs after indomethacin pre-treatment. This increased sensitivity was only observed in sensitized animals studied in vivo.

The study by Barnes et al. (1980) demonstrated that in the OA-sensitized and challenged guinea pig there was a decreased β-receptor density and an increased α-receptor density. This animal model exhibited symptoms of asthma and has demonstrable airway hyperreactivity to bronchoconstrictors and hypoactivity to isoproterenol. In this model, the animals had undergone the stress of bronchoconstriction induced by antigen-induced release of mediators in vivo. This may be expected to lead to desensitization of relaxant receptors and/or down regulation (Lefkowitz et al. 1983). Furthermore, modulatory influences in vivo may be operational and with prolonged exposure

to bronchoconstrictors may adapt at lower efficiency of effect. Finally, LTs released in vivo may sensitize airway smooth muscle to the effects of other bronchoconstrictors (section 1.3.3). Therefore, hyperreactivity may arise as a consequence of the stresses of the disease itself (Meurs et al. 1982). Secondly, a major criticism of the hypoactive bronchodilator theory of asthma is that hypoactivity arises as a consequence of long term treatment with bronchodilators such as β-adrenoceptor stimulants (Connolly & Greenacre, 1976). Our results therefore tend to support the above criticisms, since the model we used in this work was immunologically sensitized but unchallenged. This would result in a model that does not exhibit the above characteristics and hence would not exhibit hyperreactivity or hypoactivity of the airways. In other models of asthma, such as naturally allergic canine models used by Rinard et al. (1976), hypoactivity to isoproterenol does exist. However, there may be genetic differences in the canine species which might account for the observed hypoactivity to β-adrenoceptor agonists.

The reduction of tracheal tone by indomethacin (a cyclooxygenase inhibitor), observed in this study, supports and extends other published data (Orehek et al. 1975; Brink et al. 1981). We have also shown that tone can be reduced by a lipoxygenase inhibitor in sensitized and normal tissues. Previous studies have suggested a role for cyclooxygenase products in the maintenance of tracheal tone and in modulation of active contraction of this tissue (Orehek et al. 1975).

These authors proposed that tracheal resting tension is maintained by an intrinsic production of contractile PGs, chiefly $PGF_{2}\alpha$ and PGE_{2}. Contraction of trachea with histamine or other bronchoconstrictors leads to a production of relaxant PGE_{2} which modulates the extent of the contraction. Hence, inhibition of cyclooxygenase would lead to a reduction of tone and to removal of the negative influence of the relaxant PGE_{2}, thus enhancing contraction. Based on the evidence presently obtained in this study, with histamine, carbachol, and the leukotrienes (Figs. 6 & 7), this scheme was upheld. The fact that NDGA, a lipoxygenase inhibitor, had effects to those exerted by similar indomethacin, is difficult to explain, even though NDGA decreased tone to a lesser extent than indomethacin. It is possible that NDGA at the concentration used exerted some cyclooxygenase inhibiting activity. The reduction of responses to low concentrations of histamine, carbachol, and leukotrienes, and the enhancement of the effects of higher concentrations after treatment of trachea with the inhibitors were similar in both normal and sensitized tissues. This implies similar metabolic pathway for AA in both normal and sensitized tissues. This is in contrast to the effects of high concentrations of the endoperoxide analog (U-44069) after treatment of the tissues with indomethacin (Fig. 8). In this case an enhancement was only observed on normal trachea. U-44069 is a stable analog of the short-lived prostaglandin endoperoxides that are intermediates in the synthesis of PGs from AA via the cyclooxygenase enzyme. U-44069 has been reported

to be platelet aggregatory in man (Menzel et al. 1976), is a bronchoconstrictor in dogs (Wasserman, 1976), and contracts rabbit aorta (Loutzenhiser & Van Breeman, 1981). Its actions resemble TxA_2, a known mediator in human anaphylactic reactions. The lack of enhancement of the effect of U-44069 on sensitized trachea after pretreatment with indomethacin was an unexpected finding, since from the evidence with other bronchoconstrictors we would have expected an enhancement as well. However, U-44069 is not a typical agonist. The C-R curves generated on the trachea were not the normal sigmoid shape, but were biphasic, hence implying an effect at more than one receptor. In addition, low concentrations of this agent exerted similar effects in the presence and absence of indomethacin on normal trachea. The effects of low concentrations were not abolished or reduced, unlike other agonists. Thus U-44069 may be acting differently on trachea. This agent may be activating a "different" cyclooxygenase or utilizing a "different" pool of AA when compared with the other bronchoconstrictors. This "different" enzyme and (or) pool may have been affected by sensitization, and hence no enhancement was observed on sensitized trachea. Support for this hypothesis comes from the work of Sun et al. (1977) who suggested different cyclooxygenases in normal and sensitized tissues. Furthermore, it is known that normal trachea is capable of synthesizing sigificantly greater amounts of prostanoids than sensitized trachea (Burka et al. 1981). Clinically, a diversion

mediators has been suggested (Yen and Morris, 1981). Thus, our results, to a certain extent, support the pattern of alterations in the activity of AA-metabolizing enzymes as a result of sensitization.

In this work the importance of studying large and small airways becomes apparent. Lung parenchymal strips are representative of the small airways although vascular components are also present (Clayton et al. 1981). However, parenchymal strips as a whole behave more similarly to airway than to vascular smooth muscle (Songsiridej et al. 1982). The differential effects of histamine and carbachol on the trachea and lung parenchyma (Fig. 5) agree with the findings of Drazen & Schneider (1978). These investigators found that carbachol induced a greater response on the trachea than did histamine at equimolar doses, whereas the reverse was true for parenchyma. Further emphasis on differences between large and small airways is demonstrated by the lack of effect of indomethacin, phenidone, and NDGA on the tone of parenchymal strips and the lack of effect of these inhibitors on the contractile responses of the parenchyma to histamine, carbachol, and U-44069 (Fig. 9, Table 9). Although Brink et al. (1981) demonstrated an inhibition of histamine-induced responses on the parenchyma after treatment with indomethacin, we also demonstrated a reduction of the contractile activity of LTD_4 on normal and sensitized parenchyma following treatment with indomethacin, phenidone, and NDGA. This result is in agreement with the work of Piper and Samhoun (1981) who demonstrated a reduction in the contractile activity of LTC_4 and LTD_4

on parenchyma following inhibition of the cyclooxygenase enzyme. Indomethacin and phenidone would inhibit the synthesis of TxA_2, a mediator released from lung tissue following stimulation with leukotrienes and possible other agonists. Hence our results support the concept that leukotrienes exert an indirect effect on the lung parenchyma which is mediated by TxA_2. Although sensitized lung has been reported to produce more cyclooxygenase products than normal lung (Mathe et al. 1977) we did not observe any differenes in the effects of cyclooxygenase and/or lipoxygenase blockade on the contractile activity of LTD_4 on normal and sensitized lung parenchyma.

Adenylate cyclase is intimately linked with a variety of hormone receptors and is one of the links between receptor occupancy and the biological effect (Lefkowitz & Michel, 1983). It can thus be used as a direct index of bronchodilator receptor activation. In our experiments isoproterenol, PGE_2, VIP and forskolin caused significant stimulation of this enzyme. Again, normal and sensitized lungs responded similarly to these relaxants. Hence we can assume that the receptor linkage with the enzyme, and the enzyme itself, are not affected by immunological sensitization.

Although forskolin caused slightly less relaxation of the airways than isoproterenol, the diterpene caused significantly greater stimulation of pulmonary adenylate cyclase than did isoproterenol. Although it has not been conclusively proven, it is widely accepted that certain types of smooth muscle relaxation are mediated by

increased intracellular levels of cyclic AMP (Scheid et al., 1979). Since forskolin stimulates the synthesis of cyclic AMP to a much greater extent than isoproterenol, we would have expected forskolin to cause significantly greater relaxation of the airways than we demonstrated in this work. A possible explanation of this apparent discrepancy is that in the membrane preparation we used the forskolin receptor, possible the catalytic unit of adenylate cyclase (Schmidt et al., 1984), is much more stable and retains its homogeneity even after the somewhat disruptive procedures needed to prepare membranes from the lung. It is known that hormone receptors are extremely labile and it is entirely possible that during membrane preparation part of the receptor pool for a given relaxant would be inactivated. Forskolin is also not selective in terms of stimulation of adenylate cyclase. In our preparations forskolin would stimulate all cyclase units in all cells, whereas isoproterenol would only stimulate β-adrenoceptor linked cyclases. Furthermore, it is possible that airway smooth muscle relaxation may not be directly related to cyclic AMP accumulation which follows activation of the adenylate cyclase. Drugs like nitroglycerine and sodium nitroprusside can relax vascular smooth muscle (Kukovetz et al., 1979) and concomitantly increase levels of cyclic guanosine monophosphate (cyclic GMP) (Kukovertz et al., 1979; Lewicki et al., 1982). Hence there are other candidates besides cyclic AMP that mediate smooth muscle relaxation.

Similar dissociation between airway smooth muscle relaxation and

cyclic AMP accumulation has been observed by Tipton et al. (1981). These authors used isoproterenol to induce a subsensitivity of guinea-pig airway smooth muscle relaxation. However, during the same time period, cyclic AMP accumulation was normal. Hence our results support a discordance between airway smooth muscle relaxation and cyclic AMP levels.

Another point worth considering is that the lung is a very heterogeneous tissue made up of structural, secretory, and contractile cell types. Hence forskolin may activate the adenylate cyclase of non-smooth muscle cell types, whereas isoproterenol, VIP or PGE_2 may be more selective.

Indomethacin, a cyclooxygenase inhibitor, did not affect tracheal relaxation induced by isoproterenol, VIP, PGE_2 or forskolin but did reduce the magnitude of lung parenchymal relaxations to isoproterenol, PGE_2 and forskolin. The data for VIP were too variable to obtain statistical significance. The reduction of bronchodilator-induced relaxations of the lung strip by indomethacin is hard to explain but different hypotheses may be advanced. Indomethacin, by inhibiting the cyclooxygenase enzyme in the lung, would cause a diversion of endogenous AA metabolism to the lipoxygenase pathway (Piper et al., 1979; section 5.2), resulting in enhanced synthesis of potent bronchoconstrictor LTs which would cause the lung strip to be more resistant to a relaxant influence. Indomethacin might also exert a physicochemical action which may affect receptor activation. Since the EC_{50} of each

bronchodilator in relaxing the lung strip did not change after indomethacin-pretreatment, it was assumed that drug binding to the receptor would not have been affected. Indomethacin has been shown by other workers to completely suppress PGE_2-induced bronchodilation in human subjects (Walters et al. 1982). Furthermore, indomethacin has been shown to inhibit the relaxant effects of PGs (Yamaguchi et al. 1976; Burka & Paterson, 1980).

Many drugs including prostacyclin (MacDermot & Barnes, 1980) and VIP (Robberecht et al. 1981) have been shown to activate adenylate cyclase in lung tissue. We have extended these findings to include forskolin. This agent apparently binds to specific sites located on adenylate cyclase itself (Schmidt et al. 1984). We used it as an agent which might reveal differences in adenylate cyclase of lung from normal and sensitized animals. However, forskolin-induced activation of the lung adenylate cyclase was similar in normal and sensitized animals. Hence, we assumed that the adenylate cyclase systems of normal and sensitized lungs were identical. Furthermore, indomethacin-pretreatment did not significantly affect activation of cyclase induced by forskolin, isoproterenol, PGE_2 or VIP, in normal or sensitized lungs.

Finally, Burka (1983a,b) has shown that isoproterenol, forskolin and PGE_2, in order of potency, can inhibit tracheal contractions induced by OA. Higher concentrations of these agents were needed to inhibit A23187-induced contractions to a similar degree as OA-induced

contractions. Furthermore, low concentrations of these agents enhanced tracheal contraction induced by A23187 in sensitized trachea but not on normal trachea where only inhibition was observed. These agents activate adenylate cyclase and cyclic AMP accumulation which may lead to inhibition of mediator release (Lichtenstein et al., 1979). The differential effects of these bronchodilators on normal and sensitized trachea suggests an alteration of cyclic AMP turnover as a consequence of sensitization.

In summary, the OA sensitized guinea-pig model of asthma does not exhibit easily discernible differences in airway reactivity to bronchoconstrictors or to bronchodilators when studied in vitro.

5.2 AA metabolism in guinea-pig airways. Role of calcium:

In these studies we investigated the nature of the mediators released during stimulation with A23187, OA and AA. We also investigated the effect of Ca^{2+} on AA-induced contraction of the airways. This process depends on initial synthesis of LTs from AA via the lipoxygenase pathway and subsequent contraction of the tissue induced by synthesized LTs. Both synthesis of LTs and contraction of the airways are Ca^{2+} dependent processes. Our aim was to see which of these two processes was more dependent on Ca^{2+}.

Stimulation of AA metabolism by antigen (OA) and A23187, and addition of exogenous AA to indomethacin-pretreated airway tissues, resulted in a prolonged contraction. The contractions were reduced by

NDGA, a lipoxygenase enzyme inhibitor. Furthermore, mepacrine, a phospholipase A_2 inhibitor, did not reduce exogenous AA-induced contractions of the airways but reduced contractions induced by OA and A23187. Finally, analysis of the bath fluid by HPLC after stimulation with OA, A23187, or AA, demonstrated the presence of LTC_4 and LTD_4. The identity of these substances was further substantiated by the biological activity they exhibited on the guinea-pig ileum and lung strip and by UV spectra comparisons with authentic LTC_4 and LTD_4.

Quantitative bioassay of the amounts of LT-like activity (confirmed by radioimmunoassay by Burka, 1985) was conducted on the guinea-pig ileum. The results with indomethacin are interesting. We expected that inhibiting the cyclooxygenase pathway would enhance release since this has been observed earlier in other LT generating system (Piper et al. 1979). On the basis of the contractile response of the trachea to OA and A23187 this would seem to have occurred, since we had enhancement of the contraction after indomethacin pretreatment. However, the actual amounts released, as determined by bioassay could not possibly explain the enhancement of contraction as being due to increased release of the mediator. The observed enhancement of contractions may be due to the removal of the negative influence of relaxant PGE_2 which is continuously synthesized by the trachea (Orehek et al. 1975; Burka et al. 1981). PGE_2 exerts a modulatory influence on contractile stimuli acting on the trachea (Orehek et al. 1975). Indomethacin would reduce the synthesis of this

mediator and hence larger contractions for the same or lesser amounts of LTC_4 (or other stimuli) might ensure (section 5.1). Inhibition of SRS-A release by indomethacin has also been observed in rat peritoneal cells (Burka & Flower, 1978; Bach et al. 1977) but may reflect inhibition of calcium flux by indomethacin (Burka & Flower, 1974). The possibility that indomethacin treatment of the trachea may be stimulating the synthesis of another contractile mediator, which we have not yet identified, must not be discounted. AA did not further affect contractions or release beyond that obtained in the presence of indomethacin alone. This may reflect possible saturation of the system at its normal state (i.e. in the absence of AA). Hence further substrate additions would achieve no further effect. It is also conceivable that exogenous AA is not available to the lipoxygenase enzymes during antigen- and ionophore-induced release. This possibility is unlikely since AA induces contraction of the trachea in the presence of a cyclooxygenase inhibitor and the contractions are abolished by lipoxygenase inhibitors.

However, Kuehl et al. (1984) have reviewed evidence that in some systems, addition of exogenous AA would only result in LT synthesis if a stimulus such as A23187 is also present. In the above systems it is conceivable that exogenous AA may displace endogenous bound AA and thus be available for lipoxygenation.

AA is metabolised by lung tissue into contractile cyclooxygenase products (Hamberg & Samuelsson, 1976). Guinea-pig trachea has been

shown to release PGs and TxA_2 after challenge with OA, A23187 and AA (Burka et al. 1981). AA can also be metabolized to lipoxygenase products by guinea-pig airways (Piper et al. 1979; Mitchell & Denborough, 1980; Yen, 1981). In this work we have demonstrated that AA is metabolized by indomethacin-treated guinea-pig airway tissues to LTC_4 and LTD_4. This establishes the inherent capacity of the airways to produce these potent bronchoconstrictors. It is not essential for a stimulus such as A23187 or OA to be present for AA to be metabolized to the above products.

The magnitude of AA-induced contraction was greater on trachea than on lung parenchyma. This is significant especially because the small airways are particularly sensitive to leukotrienes (Drazen et al. 1980). Lung parenchymal strips in the absence of indomethacin contracted to a level equivalent to that observed on the trachea. Subsequent addition of indomethacin partially inhibited the contraction to AA (results not shown). Furthermore, the LTs exert both direct and indirect contractile effects on the lung strip, the latter action being mediated by TXA_2 (Piper & Samhoun, 1981). In our experiments this latter component was abolished by indomethacin. Our results are in agreement with those of Mitchell and Denborough (1980) and Yen (1981).

The effects of mepacrine support the evidence that exogenous AA is metabolized to LT, since the magnitude of OA- and A23187-induced contractions was reduced whereas exogenous AA was unaffected. In the

experiments conducted on mepacrine-treated tissues, AA probably had an additive effect with antigen or ionophore on the trachea. An additive effect of exogenous AA on the lung strip was not observed even though mepacrine appeared to inhibit phospholipase A_2, based on the criteria that the magnitude of antigen- and ionophore-induced contractions was significantly reduced.

The two inhibitors of lipoxygenase, NDGA and phenidone, both markedly reduced the effects and inhibited release by all three stimuli we used. This supported the observations that contractions induced by AA, OA and A23187 are partly a result of leukotriene synthesis.

The presence of the lipoxygenase enzyme in lung tissue has been confirmed for both guinea-pig lung (Hamberg & Samuelsson, 1974) and human foetal lung (Saeed & Mitchell, 1982). It has also been observed that the basal levels of gluthathione-S-transferase, the enzyme that converts LTA_4 to LTC_4, are higher in normal lung than in sensitized lung (Morris et al. 1982). However, no significant difference in leukotriene production between normal and sensitized tissues, when activated with AA, was observed in the present experiments. This is in contrast to the results of Piper and Seale (1979) who observed a greater production of SRS-A from sensitized lung than from normal lung. The use of fragments stimulated with A23187 by these authors may account for the difference from our results using parenchymal strips stimulated with AA while under tension in organ baths.

Furthermore, our methods of purification of the released mediators differed.

Finally, we have shown that addition of substrate (i.e. AA) to indomethacin-treated guinea-pig airways induced synthesis of similar lipoxygenase metabolites to those produced upon activation with ionophore or antigen. In some cells, such as human polymorphonuclear leukocytes, the AA products formed are dependent on the stimulus. AA stimulation leads to very little lipoxygenase product formation, whereas combination of AA with A23187 leads to the production of significant amounts of 5-hydroeicosatetraenoic acid and LTB_4 (Borgeat & Samuelsson, 1979). This does not appear to be the situation in the indomethacin-treated airways. Mere addition of substrate is an adequate stimulus for activation.

With regards the nature of Ca^{2+} influence on AA-induced contractions of airway tissues, the present experiments demonstrated that AA-induced contractions of guinea-pig airways are calcium-dependent. AA-induced contractions of trachea were reduced after 30 min incubation in Ca^{2+}-free KHS. Similarly, LTC_4-induced contractions were reduced under the same conditions. Since we have earlier shown that AA is metabolized by guinea-pig trachea to LTC_4 and LTD_4, the above results were expected. The 30 min incubation in zero-calcium medium is sufficient to remove all free extracellular calcium ions from the trachea (Creese & Denborough, 1981). In our experiments we observed that lung parenchyma was more resistant to extracellular

calcium removal since the contractions to AA were unchanged in Ca^{2+}-free KHS from those of control. This calcium resistance of lung parenchyma has also been observed by other authors (Burka, 1984a; Weichman et al. 1983; Hedman and Andersson, 1982). The heterogeneity of lung parenchyma, relative to the trachea, probably contributes to this calcium resistance.

Although calcium is important for AA-induced contractions of airways, addition of excess levels of this ion does not further increase the response since we failed to see increased responses in KHS containing four-fold higher levels of calcium.

Addition of lanthanum or EDTA completely abolished contractions to AA and LTC_4 in Ca^{2+}-free KHS. This occurred on both trachea and parenchyma. Lanthanum displaces calcium from superficially bound sites on cell membranes (Pearce & White, 1981; Freeman & Daniel, 1983). This element had no effect in normal KHS which agrees with earlier results where lanthanum had no effect on antigen- or A23187-induced contractions of airways in normal KHS (Burka, 1983 c). Lanthanum eliminated the residual contraction of trachea to AA and LTC_4, and of parenchymal contraction to AA and LTC_4 that was observed in Ca^{2+}-free KHS. This is evidence for loosely-bound membrane pools of calcium that can participate in airway contraction to AA and LTC_4. Lanthanum displaces these pools and thus renders them unavailable for contraction. The results with EDTA also support this concept. EDTA chelates extracellular calcium and can thus completely deprive airway

tissues of all extracellular calcium.

TMB-8, the intracellular calcium antagonist, which acts by stabilization of calcium ions to intracellular binding sites (Malagodi& Chiou, 1974) was very effective in completely inhibiting AA-induced contractions of the airways. This action of TMB-8 occurred both in the presence and absence of extracellular calcium. Weichman et al. (1983) have shown that TMB-8 completely abolishes LTD_4-induced contractions of guinea-pig trachea and parenchyma and Burka (1983c) showed that TMB-8 inhibits OA and A23187-induced contractions of guinea-pig airways. Furthermore, we have shown that trifluoperazine, an intracellular calmodulin antagonist, and EGTA, a calcium chelator, were very effective in inhibiting antigen- and A23187-induced contraction of trachea and parenchyma (Burka, 1984).

The above results indicate that calcium plays a vital intracellular role in AA-induced contractions. Furthermore, calcium from a lanthanum-sensitive extracellular compartment probably contributes to the overall process of AA-induced contraction.

What is the main role of calcium in AA-induced contraction of the airways? To shed light on this question we used two calcium entry blockers, verapamil and nitrendipine. These drugs inhibit the influx of calcium ions through voltage-dependent channels (Triggle, 1983). In our experiments verapamil and nitrendipine inhibited AA-induced contractions of the airways but did not affect LT-induced contractions (Figs 31 & 34; Burka, 1983c; Weichman et al. 1983). Thus, verapamil

and nitrendipine appear to exert their inhibitory effects by preventing the synthesis of LTs from AA and not by affecting smooth muscle contraction induced by LTs. This concept agrees with the work of Patel (1981 a, b) who demonstrated that nifedipine and verapamil inhibited exercise-induced asthma by inhibiting mediator release, since they did not have any effect on bronchoconstriction induced by histamine or methacholine. The 5-lipoxygenase enzyme responsible for the oxidation of AA to 5-HPETE, the precursor for LT synthesis, is calcium-dependent (Jakschik & Lee, 1980; Furukawa et al. 1984) and it is therefore highly probable that this is where calcium is acting in this study.

Nitrendipine was effective in inhibiting the tracheal and parenchymal contractions obtained upon re-addition of calcium to tissues in Ca^{2+}-free KHS plus lanthanum. This effect of nitrendipine further demonstrates the importance of calcium in AA-induced contractions of the airways and provides evidence that calcium is required to cross the cell membrane to an intracellular site of action.

The effects of calcium entry blockers on airway smooth muscle generally occur at much high concentrations than needed for vascular smooth muscle (Triggle, 1983; Lefer et al. 1984). This tissue selectivity is still poorly understood and distracts from the concept that calcium entry blockers may be useful therapeutic agents in asthma since they may significantly affect cardiovascular function at doses

that inhibit asthmatic bronchoconstriction.

5.3 Effects of LTD_4 on lung and cerebellar adenylate cyclase:

As mentioned in the Results, the effects of LTD_4 on adenylate cyclase activity, were complicated by a lack of reproducibility apparently beyond experimental control. Smooth muscle cyclases are notoriously difficult to study because of the variability of measurable hormone-induced effects (Muller, 1985). Hormone-receptor linkage to intracellular effectors, such as adenylate cyclase, is easily perturbed by slight changes in homogenization conditions (Rinard & Jensen, 1981; Muller, 1985).

In this dissertation, we used guinea-pig lung membranes to study the effects of LTD_4 on adenylate cyclase. The enzyme was studied under various conditions. These included low temperature (20°C) and high temperature (37°C), the use of forskolin and isoproterenol to stimulate the enzyme to a higher activity, the use of GTP and GTPγS, the use of various concentrations of divalent cations (Mg^{2+}, Mn^{2+} and Ca^{2+}), and the use of Na^{+} to optimize inhibition. The rationale for the above approaches is described as appropriate in the Results and here in the Discussion.

Guinea-pig lung is a tissue that contains a significant proportion of airway smooth muscle cells (terminal bronchioles) and, to a lesser degree, vascular smooth muscle cells (MacDermot & Barnes, 1980; Songsiridej et al. 1982). In addition, there are other non-contractile cellular elements in the lung. There is no doubt that LTD_4 contracts

lung tissue by an effect ultimately exerted on smooth muscle cells. This contractile action can be characterized as a whole in terms of binding of LTD_4 to specific receptors in the lung (Pong & DeHaven, 1983) and in terms of calcium-dependence of the contractile effect (see section 5.2).

The link between binding of LTD_4 to its receptors and calcium mobilization in the vicinity of contractile proteins is unknown. The hypothesis followed in this dissertation was that LTD_4 receptors are negatively linked to adenylate cyclase and occupation of the LTD_4 receptor ultimately leads to reduced cyclic AMP synthesis with consequent effects on calcium mobilization, leading to contraction.

The above scheme of events is supported by studies of various facets of this system. These include: (1) studies of LTD_4 binding to receptors and regulation of such binding by guanine nucleotides, divalent and monovalent cations (see section 1.2.1.4.6). This implies a negative influence on adenylate cyclase activity (Rodbell, 1980; Jakobs et al. 1984); (2) studies of calcium-dependency of smooth muscle contraction induced by constrictors including LTD_4 (Stull and Sanford, 1981; Middleton, 1984; Rasmussen & Barret, 1984); (3) studies of the link between levels of cyclic AMP and levels of calcium in the intracellular compartment. This is discussed below.

Substantial evidence exists to suggest that cyclic AMP and calcium may function as intermediates in pharmaco-mechanical and stimulus-secretion coupling in various cells. With regards to the

LTs, it is likely that the contractile effect of LTs on the airways is mainly due to release of intracellular Ca^{2+} (Weichman & Tucker, 1984; Raeburn & Rodger, 1984). Furthermore, Ca^{2+} release by subcellular fractions of smooth muscle is increased in parallel with a reduction of cyclic AMP levels (Hedman & Andersson, 1982). Hence this would lead to a contractile effect (Scheid et al. 1979). Furthermore, cyclic nucleotides regulate Ca^{2+} sensitivity of secretory processes. An increase in cyclic AMP inhibits secretion, primarily by reducing intracellular Ca^{2+} concentrations (Knight & Scrutton, 1984). More direct evidence of the involvement of Ns and Ni in regulation of intracellular Ca^{2+} levels was obtained by Molski et al. (1984). These authors showed that inhibition of Ni functions with pertussis toxin leads to a reduction of the LTB_4-induced increase in membrane permeability to Ca^{2+}. Finally, cytoplasmic Ca^{2+} levels in platelets is increased after stimulation with thrombin (Zavoico & Feinstein, 1984). Thrombin inhibits platelet adenylate cyclase (K. Jakobs, personal communication). Hence cyclic AMP serves as an inhibitory second messenger that antagonizes the mobilization of Ca^{2+}. Reduction of cyclic AMP levels by inhibitors of adenylate cyclase would lead to Ca^{2+} release from intracellular stores and/or Ca^{2+} uptake and consequent contractile and secretory events.

Hence, in this work, the aim was to demonstrate or exclude an effect of LTD_4 on adenylate cyclase. Taking the previously mentioned factors into account, it was necessary to demonstrate that LTs can

bind to lung membranes used for cyclase assays. This was demonstrated in all experiments performed with this aim in mind. Thus we were assured that the lack of positive results in a particular experiment was not due to the fact that LTD_4 was not binding to its receptors in the membrane preparation. Other explanations must be sought and amongst these would be a nonfunctional linkage of the receptor with one or more of the components of adenylate cyclase. Furthermore, it was important to demonstrate that the various components of adenylate cyclase are functional. Our preparations responded to various hormones, guanine nucleotides, divalent cations and forskolin. This implied that the hormone receptors, guanine nucleotide binding proteins (Ns and Ni) and the catalytic unit of the enzyme were intact and functional. To a certain degree it also implied that these units could interact with each other in the membrane prepared from whole tissue. However, it did not imply that such an interaction was exactly as found in the intact tissue in vivo.

Adenylate cyclase activity of lung membranes had an 8-fold lower specific activity than cerebellar membranes. This may be reflection of the relative homogeneity of cell types in the cerebellum when compared to the lung. Furthermore, a more important difference between these two tissues was the fact that GTPγS inhibited cerebellar cyclase activity to a much greater degree than lung cyclase activity. The process of inhibition of cyclase activity was probably mediated by Ni. In this study various characteristics (Jakobs et al. 1984) of Ni

mediated inhibition were tested and in all cases the system responded typically. Hence the inhibitory effect was greater at a low temperature (20°C) red to 37°C. Furthermore, inhibition was reduced by high Mg^{2+} and Mn^{2+} concentrations. Since GTPγS inhibits adenylate cyclase via Ni, it was logical to conclude that Ni existed in a quantitatively and/or qualitatively greater degree in the cerebellum than in the lung. This is reflected by the fact that LTD_4-induced inhibition was much greater in the cerebellum than in the lung. Furthermore, adenosine and GABA inhibited cerebellar adenylate cyclase activity markedly. GABA did not affect lung cyclase activity to any significant degree (data not shown). Unfortunately, there is no known hormone that will inhibit lung adenylate cyclase activity in a GTP-dependent fashion. Hence, the effects of LTD_4 on lung cyclase could not be compared to another hormone in this tissue. As mentioned earlier, the lack of consistent data distracts from the significance of these findings. LTD_4 inhibited lung cyclase activity with the following characteristics: (1) The inhibition by LTD_4 was small and between 10-15% of the control level. However, on some occasions relatively greater effects (about 60-80% inhibition of enzyme activity) were obtained. The fact that these greater effects occurred reproducibly, though, as a whole, rarely, is significant. Obviously, an unknown factor is responsible and sometimes this factor was permissive for the greater effect to occur. (2) The inhibition by LTD_4 did not exhibit a classical concentration-response relationship.

This observation is true for both lung and cerebellar cyclase systems. However, it was noted that the maximum effect of LTD_4 occurred at low concentrations about 10-100 nM in the lung and at about 10 nM in the cerebellum. Lower or higher concentrations of LTD_4 did not inhibit the enzyme to the same degree. Furthermore, in some experiments (Fig.45), high concentrations of Na^+ optimized the inhibitory response to LTD_4 in the lung. Na^+ reduces binding of LTD_4 to its receptor (see section 1.2.1.4.6) and has been shown to optimize hormone-induced inhibition in some systems. The fact that this occurred in the lung adds further support to the argument that LTD_4 inhibits lung adenylate cyclase. Na^+ did not affect cerebellar cyclase inhibition by LTD_4, GABA, or GTPγS (data not shown). (3) LTD_4 or GABA did not affect cerebellar adenylate cyclase activity in the absence of GTP or in the presence of GTPγS. Effects were only measured in the presence of GTP, the maximum effect occurring at GTP (10 μM); (4) An important observation was the fact that LTC_4 did not inhibit cerebellar adenylate cyclase under conditions where LTD_4 and GABA inhibited the enzyme in the presence of GTP. Secondly, in the presence of high concentrations of GTP (>50 μM), LTD_4 tended to slightly stimulate cerebellar enzyme activity. Under this condition, the concentrations of LTD_4 that caused the most stimulation were the same that caused the maximum inhibition (about 10 nM).

In the lung, LTD_4 (10 nM) slightly stimulated enzyme activity in the presence of Mn^{2+}. However, in the presence of GTP (10 μM), LTD_4

(10 nM) slightly inhibited the enzyme. The above evidence further indicated that LTD_4 effects cannot be nonspecific. The effects although small, occurred consistently at a given concentration. Similar observations have been made regarding the inhibitory effects of other hormones on other systems. Abramowitz & Campbell (1983) showed that [D-Ala^2, Met^5] enkaphalin amide (Da-ENK) inhibited rabbit luteal adenylate cyclase activity in a GTP dependent fashion. Da-ENK did not inhibit the enzyme in the presence of Gpp(NH)p, a nonhydrolysable analog of GTP. The extent of inhibition by Da-ENK was about 16-24%, which is small, relative to other studies. Our results with LTD_4 in the lung are comparable with regards to the above features.

Webster & Olsson (1981) demonstrated that adenosine inhibited canine cardiac adenylate cyclase activity in the presence of GTP. In the presence of Mg^{2+}, stimulatory effects were measured. However, addition of Mn^{2+} (0.5 mM) allowed adenosine to inhibit the enzyme. Again, qualitatively similar effects were measured in this work using LTD_4 and lung adenylate cyclase.

As mentioned earlier, LTD_4 effects were small. The various approaches tried in this work, to maximize the inhibitory effect were futile. The work with GTPγS demonstrates that Ni is not strongly functional in the lung preparation. It is conceivable that the heterogeneity of lung cells contribute to the low Ni-mediated effects. Ni, if present in physiologically effective concentrations in a given cell type, would be diluted by other cell types not containing Ni.

Hence small effects would result. This hypothesis might be proven if lung cells were dispersed and each cell type studied individually. Hence one of the major conclusions of this work is that LTD_4 effects in the lung should be investigated on homogenous cellular preparations of lung tissue. Furthermore, the biphasic effects of LTD_4 on the cyclase systems studied seem to imply that LTD_4 may bimodally regulate adenylate cyclase activity probably via receptor subtypes. The lack of specific agonists for LT receptors makes it difficult to test such a hypothesis. It is well known in pharmacology that receptor subtypes are a fact for various hormone classes. Receptors that bimodally regulate adenylate cyclase activity include adenosine Ra and Ri receptors, α- and β-adrenoceptors and $GABA_A$ and $GABA_B$ receptors. It is conceivable that such receptors exist for LTs and their clarification awaits the development of specific agonists and antagonists. Recent work from other laboratories lends support to the observations made in this work. Nicosia et al. (unpublished observations) also found that LTD_4 reproducibly inhibits lung adenylate cyclase activity by about 10%. Furthermore, Mong and his coworkers (unpublished observations) have also observed similar effects. These workers also showed that LTD_4 stimulates adenylate cyclase activity in whole lung fragments in the presence of low concentrations of forskolin.

In summary, LTD_4 bimodally regulated adenylate cyclase activity in lung and cerebellum, in a GTP dependent fashion. The preliminary evidence presented here supports the above conclusion. However, a

different approach is needed to substantiate the above claim. Such an approach must utilize homogenous cellular preparations of lung tissue. With regards to airway smooth muscle cells, there are a variety of as yet undefined technological difficulties in obtaining hormone-responsive adenylate cyclase.

The physiological significance of the above findings are as yet unknown. However, it is conceivable that lowering of cyclic AMP levels in smooth muscle cells may lead to a reciprocal effect on calcium levels. Furthermore, the above hypothesis should not distract from a newer viewpoint that calcium mobilizing receptors have been shown to exert effects on yet another intracellular messenger, namely, phosphatidylinositol and its "hormone-receptor interaction" stimulated hydrolysis (Salmon & Honeyman, 1980; Berridge, 1981, 1984; Baron et al. 1984).

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