

PAPERS AND ORIGINALS

Response of Lymphocyte Guanyl Cyclase to Propranolol, Noradrenaline, Thymoxamine, and Acetylcholine in Extrinsic Bronchial Asthma

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Summary

The lymphocyte guanyl cyclase response to α -agonists was studied in 10 normal people and 12 patients with bronchial asthma. In the normal subjects α -adrenergic stimulation with noradrenaline plus propranolol and cholinergic stimulation with acetylcholine evoked significant increases in cyclic guanosine monophosphate formation. In addition the α -receptor blocking drug thymoxamine produced a significant stimulation of this enzyme system, and the effects of thymoxamine and acetylcholine were additive. This suggests that receptors for cholinergic and α -adrenergic agents are independent. In contrast, lymphocyte guanyl cyclase activity did not show a significant response to these agents in patients with acute asthma. In asthmatic patients in remission the responses were partially restored. The significance of these results for control of bronchomotor tone and the relation of guanyl cyclase activity to cyclic adenosine monophosphate in normal subjects and patients with asthma is discussed.

Introduction

Szentivanyi's¹ β -blockade theory of asthma has led to studies of the adrenergic responses to catecholamines in patients with bronchial asthma. Several workers²⁻³ have reported that

asthmatic patients have diminished β -2 adrenergic responses to catecholamines, which become more pronounced in acute asthma.⁴ It is well established that 3'-5' cyclic adenosine monophosphate (cyclic AMP) is the second messenger for β -adrenergic responses,⁵ and with the discovery of adenylyl cyclase activity in the plasma membrane of human peripheral leucocytes⁶ it was suggested that observations on isolated leucocytes from asthmatic patients might be used to study this biochemical defect in asthma.⁷ Recent reports⁷⁻⁹ have shown that the membrane-bound adenylyl cyclase activity in leucocytes shows a diminished response to stimulation with isoprenaline in patients with acute asthma as compared with normal people and patients with asthma in remission. Furthermore, the α -receptor blocking drugs, phentolamine and thymoxamine, have been shown to restore the isoprenaline response to normal in patients with acute asthma.^{9, 10} These observations suggest that an enhanced adrenergic activity may be a factor in the autonomic imbalance in asthma.¹¹

It has been proposed that cyclic guanosine monophosphate (GMP) has an opposing influence to that of cyclic AMP in the regulation of cell function.¹² Guanyl cyclase activity has been shown in a wide variety of tissues, including human lung and lymphocytes.¹³ It is well established that cyclic GMP activates cholinergic responses¹⁴⁻¹⁵ and is activated by α -agonists.¹⁶ In view of the abnormal cyclase responses observed in asthmatic patients the role of cyclic GMP in the autonomic receptor system in asthma has been investigated. We report here the lymphocyte guanyl cyclase response to propranolol, propranolol plus noradrenaline, thymoxamine, thymoxamine plus acetylcholine, and acetylcholine in patients with acute asthma, patients in remission, and normal people.

Patients and Methods

Twelve patients, aged 14-44 years, with reversible airways obstruction due to extrinsic bronchial asthma were studied. All patients were positive on prick tests to inhalant allergens and had blood eosinophil counts of over 500 cells/mm³, and six of the patients had associated atopic diseases such as eczema, allergic rhinitis, or hay fever. Some patients were on daily maintenance therapy with bronchodilators—for example, salbutamol aerosol; seven were on sodium cromoglycate; and one was on 5 mg of oral prednisolone on alternate days. All treatment

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was discontinued for at least 24 hours and prednisolone for 48 hours before the experiments.

Six patients had active asthma as assessed by a history of daily wheezing, breathlessness on moderate exercise, clinical and spirometric evidence of airways obstruction, and the amount of bronchodilator and steroid treatment required for the relief of symptoms. The remaining six asthmatic patients were in remission. Ten healthy adults aged 19-45 years without respiratory or atopic disease were studied as controls. Samples of venous blood were collected between 8.30 a.m. and 9.30 a.m. to avoid circadian variations. The forced expiratory volume in 1 second (FEV₁) was measured with a Garthur Vitalograph spirometer within a few minutes of blood collection.

Preparation of Lymphocytes.—Lymphocytes were prepared from 40 ml of whole blood. This volume of blood was carefully layered over an equal volume of ficoll-hypaque and then centrifuged at 400 g at the interface for 20 minutes.¹⁷ The red cells and granulocytes were spun down and the lymphocytes appeared as a narrow white layer immediately below the supernatant ficoll-hypaque interface. The lymphocyte layer was carefully removed and resuspended in the buffer solution (pH 7.40) as described previously.⁹ After centrifuging this suspension for five minutes at 70 g the supernatant was discarded and the procedure repeated. Finally, the lymphocyte pellet was resuspended in 1.5 ml of buffer before incubation with ³H-guanine at 37°C. This method produced a highly purified preparation of lymphocytes.

Guanyl Cyclase Assay and Analysis of Data.—The procedure for the lymphocyte guanyl cyclase assay was as described previously for the leucocyte adenylyl cyclase assay⁹ except that the cells were incubated with ³H-guanine (1 μCi/5 × 10⁶ cells) instead of ³H-adenine, and at the conclusion of each incubation 0.1 ml of a non-radioactive carrier solution containing 5 mmol/l each of 3'5' cyclic GMP, guanosine triphosphate (GTP), guanosine diphosphate (GDP), 5'-GMP, guanosine, and guanine was added. The cell extracts were separated using the same solvent system. Each chromatogram was developed for 18 hours to achieve effective separation of the guanine nucleotides: GTP R_f 0.03, GDP R_f 0.04, 5'-GMP R_f 0.11, 3'5' cyclic GMP R_f 0.34, guanosine R_f 0.45 and guanine R_f 0.51. The elution of the nucleotide spots and counting procedures and the calculation of the guanyl cyclase activities were as already described.⁹

Results

The detailed results are shown in the table. There was no significant difference in the mean percentage value of ³H-cyclic GMP in the

control (or basal) level in either the active asthmatic group, the asthmatic group in remission, or the controls.

Propranolol alone at 200 μmol/l did not evoke any significant difference in cyclic GMP levels in all three groups. Propranolol in combination with noradrenaline at 100 μmol/l, however, produced a very significant increase in guanyl cyclase activity in controls but not in any of the asthmatic patients. Thymoxamine alone at 200 μmol/l produced a significant increase in the enzyme activity in the normal (P<0.01) and remission (P<0.02) groups but none in the active group. Thymoxamine in combination with acetylcholine 100 μmol/l produced a highly significant increase in the guanyl cyclase activity in the normal group but not in either the remission group or patients with active asthma. Acetylcholine alone at 100 μmol/l produced a significant increase in the lymphocyte guanyl cyclase activity in the normal group but not in the other two groups.

The mean FEV₁ (± S.E. of mean) expressed as the percentage of the predicted value was 53.2 ± 7.11% in the active group and 85.3 ± 6.24% in the remission group—a statistically significant difference (P<0.01).

Discussion

The early work on the distribution and subcellular location of guanyl cyclase indicated that in most tissues studied it occurred mainly in the membrane-free cytoplasm of the cell, in contrast with adenylyl cyclase, which is present mainly in the plasma membrane.^{18, 19} Rudland *et al.*²⁰ have recently shown, however, that stimulation of guanyl cyclase activity by fibroblast growth factor (F.G.F.) in BALP/c3TB cells in tissue culture was due almost entirely to an enzyme located in the plasma-membrane fraction. Since our concern was to explore cell receptor activities a method which measured plasma-membrane guanyl cyclase activity seemed more likely to give significant results. Stimulation of lymphocytes with noradrenaline in the presence of the β-blocking drug propranolol greatly stimulated guanyl cyclase activity, which reached a maximum between 5 and 10 minutes and returned towards baseline by about 15 minutes (fig. 1). This time course activity experiment is comparable in the degree of stimulation and duration of effect to the stimulation of membrane-bound leucocyte adenylyl cyclase activity with isoprenaline.⁸ Furthermore, the addition of theophylline to the medium—that is, a phosphodiesterase inhibitor—increased the basal level of

Lymphocyte Guanyl Cyclase Response to Propranolol, Propranolol and Noradrenaline, Thymoxamine, Thymoxamine and Acetylcholine, and Acetylcholine in Controls, Patients with Active Asthma, and Patients in Remission. Results are Percentages of Basal Level of Incorporation of ³H-Guanine into Cyclic GMP

Case No.	Age and Sex		Basal Level	Propranolol (200 μmol/l)		Thymoxamine (200 μmol/l)		Acetylcholine (100 μmol/l)
				Alone	+ Noradrenaline (100 μmol/l)	Alone	+ Acetylcholine (100 μmol/l)	
<i>Controls</i>								
1	20	M.	1.48	260	360	288	277	184
2	22	M.	4.86	170	247	164	302	126
3	30	M.	1.90	174	261			
4	38	M.	2.63	90	200			
5	21	M.	4.42	67	202	96	204	76
6	35	F.	3.88	115	197	181	224	248
7	19	M.	3.31	104	174	150	152	137
8	28	F.	1.84	67	65	139	135	122
9	24	M.	2.44	83	115	118	144	130
10	32	F.	6.75	94	122	69	222	149
Mean ± S.E. of mean			3.35 ± 0.52	122.4 ± 19.0	194.0 ± 26.5	150.6 ± 23.4	203.8 ± 23.6	146.5 ± 17.8
P				N.S.	<.001	<.01	<.001	<.02
<i>Patients with Active Asthma</i>								
11	14	M.	2.40	46	54	63	96	92
12	20	M.	8.63	98	187	138	105	119
13	44	M.	1.37	30	68	60	31	77
14	16	F.	6.54	192	104	138	102	98
15	25	F.	7.13	94	112	137	78	168
16	35	F.	2.10	58	43	69	43	40
Mean ± S.E. of mean			4.70 ± 1.26	86.3 ± 23.8	94.7 ± 21.6	100.8 ± 16.5	75.8 ± 13.0	99.0 ± 17.5
P			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
<i>Asthmatic Patients in Remission</i>								
17	23	F.	2.27	80	97			
18	20	M.	4.19	182	110			
19	35	M.	5.20	104	117	112	159	179
20	28	M.	3.15	112	262	136	270	242
21	28	M.	7.00	68	115	128	94	93
22	16	F.	8.87	130	110	109	120	121
Mean ± S.E. of mean			5.11 ± 1.01	112.7 ± 16.6	135.2 ± 25.2	121.3 ± 6.44	160.7 ± 38.8	158.8 ± 33.0
P			N.S.	N.S.	N.S.	<.025	N.S.	N.S.

lymphocyte guanyl cyclase activity in both controls and asthmatic patients without altering the overall pattern of response (unpublished results). These observations suggest that the lymphocyte guanyl cyclase activity measured was membrane bound.

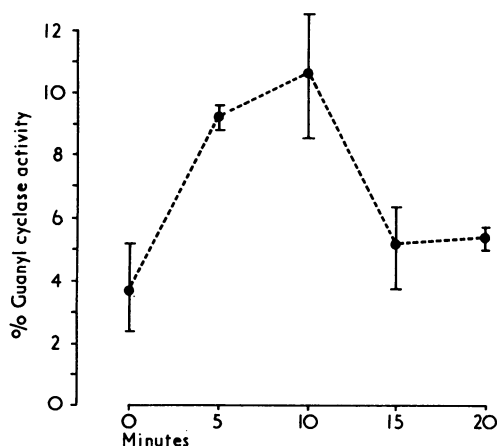


FIG. 1—Noradrenaline (100 $\mu\text{mol/l}$) stimulation of guanyl cyclase in lymphocytes of controls in presence of propranolol (200 $\mu\text{mol/l}$) at various times. Means \pm 2 S.E. of mean are shown.

In controls α -adrenergic stimulation with noradrenaline in the presence of propranolol produced a very significant increase in cyclic GMP formation. Unexpectedly, thymoxamine also produced a significant though smaller increase in cyclic GMP production in the controls, which makes it difficult to explain the action of α -blocking drugs on this basis. Illiano *et al.*,²¹ however, in their studies on isolated fat cells found that atropine (the cholinergic blocking drug) also caused a slight increase in cyclic GMP formation. The stimulation of guanyl cyclase activity with the combination of thymoxamine and acetylcholine seems to be additive, suggesting that independent receptor systems exist for α -adrenergic and cholinergic drugs. In contrast, the only significant stimulatory drug effect on cyclic GMP production in the asthma patients' cells was observed with thymoxamine in the remission group. Our observations are the reverse of what might have been expected as it has been reported with experimental animals that increased lung levels of cyclic GMP are associated with a more severe degree of anaphylaxis.²² Lewis *et al.*,¹⁴ however, have shown that the effect of cyclic GMP on smooth muscle function is dose dependent; low concentrations produce tracheal smooth muscle contraction whereas higher concentrations produce a dose-dependent relaxation. Furthermore, the report of Beavo *et al.*²³ on the influence of cyclic GMP on cyclic AMP phosphodiesterase could explain the relation of cyclic GMP to cyclic AMP. Using particulate preparations of cyclic phosphodiesterase from various tissues it has been shown that low concentrations of cyclic GMP stimulated hydrolysis of cyclic AMP whereas with higher concentrations of cyclic GMP there was an inhibition of cyclic AMP hydrolysis. Similar findings have been reported for rat lymphocytes,²⁴ and the concentration of cyclic GMP required to show this phenomenon is within the physiological range (fig. 2).²³

In normal subjects α -stimulation in the presence of β -blockade gives a significant rise in cyclic GMP. Acetylcholine produces a similar rise, which is enhanced in additive form in the presence of thymoxamine. α -Adrenergic and cholinergic stimulation increase bronchomotor tone and lead to bronchoconstriction, but the highly significant increase in the membrane-bound cyclic GMP in normal subjects reported here may lead to inhibition of cyclic AMP phosphodiesterase,²³ preventing hydrolysis of cyclic AMP and thereby maintaining relaxation of bronchial smooth muscle. On the other hand, in acute asthma

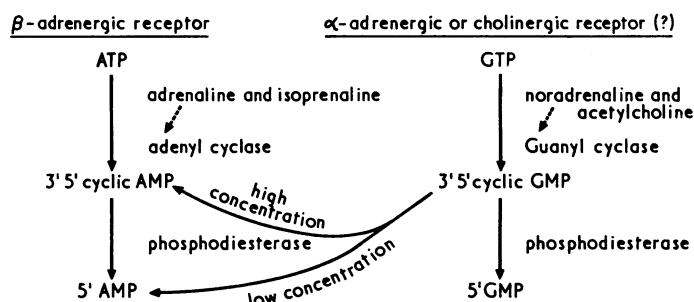


FIG. 2—Proposed relation of cyclic GMP to cyclic AMP in control of bronchomotor tone. Activation of guanyl cyclase by noradrenaline or acetylcholine increases concentration of cyclic GMP. High concentration of cyclic GMP inhibits cyclic AMP phosphodiesterase, preventing the hydrolysis of cyclic AMP to 5' AMP and thus maintaining normal bronchomotor tone. In asthma low levels of cyclic GMP stimulate cyclic AMP phosphodiesterase and promote the hydrolysis of cyclic AMP to 5' AMP, thus increasing bronchomotor tone.

α -stimulation with noradrenaline in the presence of β -blockade, cholinergic stimulation with acetylcholine alone, or α -blockade failed to produce a significant rise in cyclic GMP. Possibly we are observing an increased hydrolysis of cyclic AMP through stimulation of cyclic AMP phosphodiesterase due to a low intracellular concentration of cyclic GMP resulting in increased bronchomotor tone and bronchoconstriction (fig. 2). The high basal levels of adenylyl cyclase activity and the diminished responsiveness to isoprenaline stimulation of adenylyl cyclase in acute asthma already reported^{9, 11} supports this hypothesis. Asthmatic patients in remission show cyclic GMP responses midway between those found in normal subjects and those found in patients with active asthma. It should now be possible to define the place of α -receptor blocking drugs in the management of patients with asthma. The factors depressing cyclic GMP responses in acute asthma and confirmation that cyclic phosphodiesterase activity is increased are being investigated.

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