Prostaglandins and the control of airways responses to histamine in normal and asthmatic subjects

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ABSTRACT Inhalation histamine challenges were performed in groups of normal and asthmatic subjects. On each occasion a regression line for the descending part of the log-cumulative dose-response curve was computed. The dose of histamine causing a 20% fall in specific conductance (sGaw) was taken as an index of "sensitivity." The slope gave the "reactivity". In a double-blind, randomised study the potent inhibitor of prostaglandin synthesis indomethacin (50 mg four times per day for three days) was associated with a small but significant bronchodilatation in the normal but not the asthmatic subjects. Sensitivity to histamine was considerably decreased in the asthmatic patients ($p < 0.005$) but unchanged in the normal subjects. In both groups reactivity was significantly increased ($p < 0.01$). The study was repeated after several weeks of regular salbutamol treatment. In both groups salbutamol caused a decrease in sensitivity ($p < 0.001$) but no change in reactivity. After indomethacin had been reintroduced while salbutamol was continued most of the effects of chronic salbutamol treatment were reversed in the normal subjects, with a similar trend in the asthmatic patients. In both groups the dose-response curves after indomethacin treatment were little affected by pretreatment with salbutamol. Beta-adrenergic stimulation induces changes in the airways that may be dependent on prostaglandin production.

Studies on normal and asthmatic subjects were performed to investigate differences in the response to inhaled histamine and to determine whether prostaglandins play a part in the local control of the airways *in vivo*. The question of whether such relationships may be altered by regular medication with β -adrenergic agonist drugs has also been studied.

In vitro inhibition of prostaglandin synthesis with the non-steroidal anti-inflammatory drug indomethacin was found to decrease the threshold of response (increase sensitivity) of guinea-pig tracheal muscle to vagal stimulation, and to decrease the variability of the response.¹ Under these conditions added prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increased the response to vagal stimulation. Hypersensitivity of the airways to bronchoconstrictor agents has also been demonstrated in normal human subjects after previous inhalation of prostaglandins.^{2,3} It was thought that local production of prostaglandins in pathological conditions such as bronchial asthma might play a part in the increased sensitivity of the airways seen in these clinical situations.

A protective negative feedback role for PGE_2 in

the airways has been proposed.⁴ According to this hypothesis PGE_2 released during induced contraction of the smooth muscle of the airways would antagonise the muscle contraction, and thus reduce the reactivity of the airways—that is, the slope of the agonist dose-response curve.⁵

In the present series of experiments both of these potential modes of prostaglandin action—on sensitivity and reactivity of the airways—have been investigated. Both normal and asthmatic subjects were treated with indomethacin and changes in sensitivity and reactivity to histamine were determined.

Beta-adrenergic stimulation of airways smooth muscle has been shown to generate prostaglandins even after cessation of the direct muscle action.⁶⁻⁸ Furthermore, the direct bronchodilator action of PGE_2 on the airways may be altered after long-term β -adrenergic treatment.⁹ The second part of the present study comprised an examination of airways sensitivity and reactivity to histamine after regular salbutamol treatment in both groups of subjects. The effect of indomethacin on these indices was then reassessed during regular salbutamol treatment.

Methods

STUDIES ON NORMAL SUBJECTS

Eight normal, non-atopic subjects (six men and two

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women) with a mean age of 32 years (range 24–40 years) took part after informed consent had been obtained. None smoked.

In the first part of the study inhalation histamine dose-response curves were constructed on two separate occasions about one week apart. Before one challenge the subjects were treated with 50 mg of oral indomethacin (micronised, Berk Pharmaceuticals) four times a day for two days and then four hourly on the day of treatment. The last capsules were taken about one and a half hours before the test started. Before the other challenge identical placebo capsules were taken. This part of the study was performed in random order and double blind.

All subjects were then started on regular salbutamol inhalations in a dose of 400 μ g four times a day from pressurised aerosols. After four weeks of this treatment a histamine inhalation dose-response study was again performed. The test was preceded by two days of placebo capsules as above, given single blind. No salbutamol was taken for at least 10 hours before histamine challenge. Regular salbutamol was then recommenced and about a week later the final histamine challenge was performed after indomethacin treatment, as above but single blind and at least 10 hours after the last dose of salbutamol.

For construction of histamine dose-response curves 10 breaths of aqueous solutions of histamine diphosphate, increasing in concentration from 1 to 25 mg/ml in six increments, were taken from a Wright's nebuliser driven at 20 lb/in² (137.9 kPa). Breathing was standardised according to a respiratory metronome set at 18 breaths/min. Doses were given at three-minute intervals. Specific airways conductance (sGaw) was measured in a constant-volume whole-body plethysmograph¹⁰ before the start and then from one and a half to three minutes after each dose. Each recorded value of sGaw represented the mean of the last six of seven individual readings. The preinhalation baseline sGaw was taken as the mean of two such sets obtained at five-minute intervals after the subjects had been seated in the laboratory for at least 15 minutes. Resistance and volume angles were measured with the aid of an electronic resolver so that the numerical values recorded were kept out of sight of the operator until the measuring procedure was completed. It was thus hoped to avoid observer bias. Each individual was always studied at the same time of day, having fasted and avoided caffeinated drinks on the day of study.

The regression line of the descending part of each log-cumulative dose-response curve was computed with values for a 15% or greater fall in sGaw.² The dose of histamine which caused a 20% fall in sGaw (D_{20}) was calculated from this and used as the index

of sensitivity. The slope of the regression line gave reactivity. Paired values of baseline sGaw, log D_{20} , and slope were compared with Student's *t* test.

STUDIES ON ASTHMATIC PATIENTS

Initially, 12 patients with mild atopic asthma volunteered to take part in the study. Informed consent and ethical approval were again obtained. At the time of study all were in a stable condition, requiring only occasional inhalations of β -adrenergic agents for control of symptoms. None smoked or gave a history of wheezing attacks induced by non-steroidal anti-inflammatory drugs. All were, however, given a preliminary challenge of 12.5 mg of oral indomethacin and two patients developed wheezing about one hour later. These were excluded from further study.

The 10 asthmatics who took part had a mean age of 32 years (range 25–50 years). The protocol was the same as for normal subjects. Thus histamine challenges were performed on the third day of both placebo and indomethacin treatment, both with and without four weeks of regular salbutamol treatment in a dose of 400 μ g four times a day. In the initial study of indomethacin the active drug and placebo were given in random order and double blind, but after the regular salbutamol the study was single blind and placebo was given first.

The concentrations of histamine diphosphate used for construction of dose-response curves ranged from 0.1 mg/ml to 15 mg/ml in seven approximately doubling increments. Doses were given as for normal subjects, again at three-minute intervals, and sGaw was measured before the start and from one and a half to three minutes after each. No salbutamol was taken for at least 10 hours before histamine challenge.

Results

For both normal and asthmatic subjects, while figures 1 and 2 plot responses as percentage changes from the baseline sGaw, the statistical comparisons that follow use values of slope obtained directly from log-dose-response curves using actual changes in sGaw.

STUDIES ON NORMAL SUBJECTS

The results are summarised in figure 1 and individual details are given in table 1.

Effects of indomethacin alone The mean baseline sGaw for the group was $2.9 \text{ s}^{-1} \text{ kPa}^{-1}$ after placebo treatment and $3.2 \text{ s}^{-1} \text{ kPa}^{-1}$ after indomethacin, a significant increase ($p < 0.05$). There was no significant change in log D_{20} after indomethacin but a highly significant increase in the mean slope, from $-1.03 \text{ s}^{-1} \text{ kPa}^{-1}$ to $-2.01 \text{ s}^{-1} \text{ kPa}^{-1}$ ($p < 0.005$).

Table 1 Results of histamine challenge in normal subjects after placebo and indomethacin treatment with and without regular salbutamol inhalations: values derived from the regression line of the descending part of each log-cumulative dose-response curve

Treatment: Placebo only				Indomethacin only			Salbutamol and placebo			Salbutamol and indomethacin		
Index:	Baseline	D_{20}	Slope	Baseline	D_{20}	Slope	Baseline	D_{20}	Slope	Baseline	D_{20}	Slope
Subject	$sGaw$ ($s^{-1} kPa^{-1}$)	(mg/ml)	($s^{-1} kPa^{-1}$)	$sGaw$ ($s^{-1} kPa^{-1}$)	(mg/ml)	($s^{-1} kPa^{-1}$)	$sGaw$ ($s^{-1} kPa^{-1}$)	(mg/ml)	($s^{-1} kPa^{-1}$)	$sGaw$ ($s^{-1} kPa^{-1}$)	(mg/ml)	($s^{-1} kPa^{-1}$)
1	2.0	3.5	-0.79	2.3	5.0	-1.90	2.6	13.5	-1.49	2.4	4.5	-2.20
2	3.9	13.5	-1.60	4.2	13.8	-2.74	4.4	27.5	-1.05	4.4	17.0	-3.00
3	2.6	3.6	-1.37	2.6	4.1	-2.57	3.0	64.6	-1.75	2.8	5.6	-2.50
4	3.3	1.2	-0.86	4.0	6.2	-3.31	3.8	10.2	-2.12	3.6	6.5	-2.67
5	2.4	2.2	-1.45	2.3	2.3	-1.48	2.7	15.8	-1.30	2.5	3.5	-1.43
6	3.2	8.7	-1.33	4.2	12.3	-2.50	4.2	21.9	-1.89	4.1	17.4	-2.28
7	2.8	8.9	-0.40	3.3	22.9	-1.65	3.4	38.0	-0.59	3.3	32.4	-2.17
8	2.6	9.8	-0.46	2.9	8.1	-1.30	3.0	57.5	-1.10	2.9	9.1	-0.68
Mean	2.9	6.4	-1.03	3.2	9.3	-2.01	3.4	31.1	-1.41	3.25	11.9	-2.12
SD	0.59	4.38	0.46	0.82	6.76	0.78	0.63	20.51	0.50	0.73	9.85	0.74
SEM	0.21	1.55	0.17	0.29	2.39	0.28	0.22	7.35	0.18	0.26	3.48	0.26

Fig 1 Mean cumulative dose-response curves for histamine for eight normal subjects, constructed after placebo and indomethacin medication both before and after regular salbutamol inhalations. Doses are plotted on a logarithmic scale and responses as percentage changes from the baseline $sGaw$

Effects of salbutamol alone After regular salbutamol inhalation there was a 17% increase in mean baseline $sGaw$, to $3.4 s^{-1} kPa^{-1}$ ($p < 0.001$), compared with placebo pretreatment values. There was a significant increase in $\log D_{20}$ ($p < 0.0001$), the mean value of D_{20} increasing from $6.4 mg/ml$ after placebo to $31.1 mg/ml$ after salbutamol. There was no significant difference in slope from the post-placebo values.

Effects of indomethacin after regular salbutamol After regular salbutamol, indomethacin treatment was associated with a small but significant fall in the baseline $sGaw$, to $3.25 s^{-1} kPa^{-1}$ ($p <$

0.01). There was also a significant decrease in $\log D_{20}$ ($p < 0.01$), the mean value of D_{20} falling to $11.9 mg/ml$, but an increase in the mean value of the slope to $-2.12 s^{-1} kPa^{-1}$ ($p < 0.05$). There was no significant difference between the values of baseline $sGaw$, $\log D_{20}$, or slope after the courses of indomethacin given with and without regular salbutamol.

STUDIES ON ASTHMATIC PATIENTS

The changes are summarised in figure 2 and individual values are given in table 2.

Effects of indomethacin alone The mean baseline

Table 2 Results of histamine challenge in asthmatic subjects after placebo and indomethacin treatment with and without regular salbutamol inhalations: values derived from the regression line of the descending part of each log-cumulative dose-response curve

Treatment: Placebo only				Indomethacin only			Salbutamol and placebo			Salbutamol and indomethacin		
Index:	Baseline	D ₂₀	Slope	Baseline	D ₂₀	Slope	Baseline	D ₂₀	Slope	Baseline	D ₂₀	Slope
Subject	sGaw (s ⁻¹ kPa ⁻¹)	(mg/ml)	(s ⁻¹ kPa ⁻¹)	sGaw (s ⁻¹ kPa ⁻¹)	(mg/ml)	(s ⁻¹ kPa ⁻¹)	sGaw (s ⁻¹ kPa ⁻¹)	(mg/ml)	(s ⁻¹ kPa ⁻¹)	sGaw (s ⁻¹ kPa ⁻¹)	(mg/ml)	(s ⁻¹ kPa ⁻¹)
1	2.0	1.4	-0.75	2.5	3.2	-1.30	2.3	4.5	-0.62	2.5	1.3	-0.94
2	2.1	0.5	-0.66	2.5	0.6	-1.80	2.5	2.1	-1.20	2.6	9.6	-1.82
3	2.7	0.3	-1.17	2.6	2.0	-1.66	2.8	5.8	-3.59	2.6	1.4	-1.80
4	1.9	0.4	-0.50	2.2	7.1	-0.93	2.1	4.0	-0.55	2.2	4.3	-1.15
5	1.8	0.2	-0.86	2.1	0.7	-1.13	2.0	0.5	-0.61	2.1	0.7	-1.76
6	2.1	0.4	-0.77	2.1	0.8	-0.60	1.7	2.6	-0.77	2.2	2.7	-0.95
7	3.0	0.3	-1.03	3.3	1.6	-3.16	3.2	0.2	-1.10	3.3	1.4	-3.14
8	3.5	0.3	-0.97	4.0	0.4	-1.86	3.5	2.0	-1.75	2.1	0.3	-0.75
9	1.5	0.4	-0.44	1.7	1.5	-1.21	1.6	0.5	-0.72	1.2	0.6	-0.81
10	2.2	0.3	-0.58	1.8	0.3	-0.81	2.3	1.2	-1.34	1.8	0.4	-0.58
Mean	2.3	0.4	-0.61	2.5	1.8	-1.45	2.4	2.3	-1.23	2.3	2.3	-1.37
SD	0.6	0.34	0.33	0.7	2.06	0.73	0.61	1.89	0.91	0.55	2.85	0.77
SEM	0.19	0.11	0.11	0.22	0.65	0.23	0.20	0.60	0.29	0.18	0.90	0.24

Fig 2 Mean cumulative dose-response curves for histamine for 10 asthmatic patients, constructed after placebo and indomethacin medication both before and after regular salbutamol inhalations. Doses are plotted on a logarithmic scale and responses have again been normalised and plotted as percentage changes from baseline sGaw.

sGaw for the group was 2.3 s⁻¹ kPa⁻¹ after placebo and 2.5 s⁻¹ kPa⁻¹ after indomethacin, but this difference was not significant. There was a significant increase in log D₂₀ (p < 0.005), with an increase in mean D₂₀ from 0.4 mg/ml after placebo to 1.8 mg/ml after indomethacin. At the same time there was a significant increase in the mean value of the slope from -0.61 to -1.45 s⁻¹ kPa⁻¹ (p < 0.01). *Effects of salbutamol alone* After regular salbutamol inhalations the mean baseline sGaw was 2.4 s⁻¹ kPa⁻¹, which was not significantly different from the value obtained after placebo. There was, however, a significant increase in log D₂₀ values over

the control values (p < 0.001), the mean D₂₀ increasing to 2.3 mg/ml. There was no significant change in slope. *Effects of indomethacin after regular salbutamol* After regular salbutamol, indomethacin treatment was not associated with any significant change in any of the indices. The mean baseline sGaw, D₂₀, and slope were 2.3 s⁻¹ kPa⁻¹, 2.3 mg/ml, and -1.37 s⁻¹ kPa⁻¹ respectively. There was again no significant difference between the mean values of these indices obtained after the two periods of indomethacin treatment — that is, intervening salbutamol treatment made no difference.

ASTHMATIC COMPARED WITH NORMAL SUBJECTS

Baseline values of sGaw on control (placebo, before salbutamol) days were significantly lower in the asthmatic patients ($p < 0.05$ by Student's t test for unpaired values). Values of log D_{20} were also significantly lower in the asthmatic group ($p < 0.00001$), but there was no significant difference in the values obtained for the slope in the two groups, although they tended to be lower in asthmatics.

Although there was a significant increase in values of log D_{20} after both indomethacin and salbutamol in the patients with asthma these post-treatment levels remained significantly less than normal control values ($p < 0.005$ and $p < 0.025$ respectively).

RELATIONSHIPS BETWEEN BASELINE SPECIFIC CONDUCTANCE, SENSITIVITY, AND REACTIVITY

Linear regression analyses were performed on all values of baseline sGaw against corresponding values of D_{20} and slope for each study.

In neither subject group was there a significant correlation between baseline sGaw values and D_{20} . Nor was there a significant correlation between Δ baseline sGaw and ΔD_{20} . There was, however, a significant correlation between baseline sGaw values and values for slope in both groups ($r = 0.60$, $p < 0.001$ for asthmatics; $r = 0.45$, $p < 0.05$ for normal subjects). For Δ sGaw against Δ slope there was no significant correlation in either study.

Discussion

The predominant difference between asthmatic and normal subjects in terms of responsiveness to inhaled histamine was found in their sensitivity to the drug. Reactivity, as defined in this study, was not significantly different in the two groups, although in general asthmatics appeared less reactive than normal subjects.

The indomethacin treatment used in the study was of sufficient duration to give a steady-state blood concentration of the drug.¹¹ The dosage used should have caused almost complete inhibition of urinary prostaglandin excretion and prostaglandin synthesis.^{12,13}

Indomethacin caused no significant change in baseline sGaw in asthmatics and only a small change in the normal subjects. Similar results were obtained by Fish *et al.*,¹⁴ supporting the view that prostaglandins have a minimal effect on the resting tone of the airways in vivo. The effect of chronic use of salbutamol on airways calibre was rather greater, at least in the normal subjects. Some of this change may be due to alterations in the synthesis or action of prostaglandins, as indicated by the fall that fol-

lowed indomethacin. Beta-adrenergic stimulation of respiratory muscle in vitro has been shown to generate prostaglandins, probably predominantly PGE_2 .⁸

Fish *et al.*¹⁴ found that indomethacin caused only a small and non-significant increase in the dose of bronchoconstrictor agonist causing a 35% fall from baseline sGaw (D_{35}) in a group of atopic subjects. D_{35} is a complex function incorporating both sensitivity and reactivity, and changes are difficult to interpret. Indeed, in the studies reported in this paper measurement of D_{35} only would have provided little information.

In asthmatic patients indomethacin caused a large and significant decrease in histamine sensitivity, in contrast to the results found in normal subjects. One attractive explanation is that in asthmatics increased background amounts of prostaglandins, although having little effect on baseline airways calibre, may have been having a deleterious "sensitising" effect on the response of airways smooth muscle to histamine.

Overall there was a highly significant linear correlation between values of baseline sGaw and D_{20} . This reflected the greatly increased sensitivity in the asthmatics. Baseline sGaw, however, in itself is probably of limited importance in determining D_{20} , as shown by the lack of correlation within each group separately despite large variations in baseline sGaw. Thus even asthmatic subjects with control baseline sGaw values well within the normal range (for example, subjects 3, 7, and 8) still had values of D_{20} well below normal.

There was a significant decrease in histamine sensitivity in both normal and asthmatic subjects after long-term salbutamol treatment. This effect may well be mediated by a prostaglandin-dependent mechanism as it is largely reversed by indomethacin. Such interrelationships between β -adrenergic function, histamine sensitivity of airways smooth muscle, and prostaglandin synthesis have also been observed in guinea-pigs.¹⁵ Peel and Gibson, however, found an inconsistent effect of four weeks' treatment with inhaled salbutamol on histamine sensitivity in a group of patients with mild asthma.¹⁶ Unfortunately in their study only changes in FEV_1 were monitored, with the inherent uncertainties for airways sensitivity that forced manoeuvres imply.¹⁷ The dose of salbutamol used was also relatively small.

The increase in reactivity that occurred after indomethacin in both groups is consistent with a negative feedback action of prostaglandins released by the contractile process. PGE_2 is the prostaglandin most likely to be concerned if such a homeostatic mechanism exists⁴ and it would be expected to act as an antagonist of induced contraction in both asthmatic patients and normal subjects.⁹

Other explanations are possible for the increase in reactivity seen after indomethacin. Indomethacin might have direct physicochemical effects on smooth muscle,¹⁸⁻²⁰ although it seems unlikely that these would both decrease sensitivity and increase reactivity. By decreasing the activity of the cyclooxygenase enzyme indomethacin may increase the availability of substrate for the lipoxigenase and thereby increase the production of leukotrienes. This would substitute production of bronchoconstrictor slow-reacting substance of anaphylaxis (SRS-A) for bronchodilator PGE₂. Such a mechanism for the increased reactivity of airways muscle after treatment with cyclo-oxygenase inhibitors has been proposed.²¹ This proposition is, however, difficult to reconcile with the suggestion that SRS-A may have a predominantly indirect effect on airways smooth muscle via induction of increased production of prostanoids (PJ Piper, paper presented to the International Symposium on Leukotrienes and other Lipoxigenase Products, Florence, 1981).

Indomethacin has also been described as having a potent inhibitory effect on phosphodiesterase activity.²² This has not been found in lung tissue, but the increase in cyclic nucleotides that might be expected from such an action could be responsible for the baseline changes seen in normal subjects. It should, however, have been even more effective in the asthmatic patients and would be expected to decrease rather than increase reactivity.

Baseline airways calibre seemed to have an influence on the subsequent slopes of the corresponding dose-response curves. Similar results have been reported by others.²³ The relationship found is the inverse of what one might expect if the Poiseuille equation were a dominant influence in the airways, as has been suggested.²⁴ Some of the effect of the baseline calibre on reactivity could also be explained in terms of a negative feedback role for PGE₂, because its relative bronchodilator potency depends on the initial tone of the airways smooth muscle.^{25, 26} Thus when the airways were initially dilated PGE₂ would not as effectively antagonise induced bronchoconstriction, leading to high reactivity. This is unlikely to be the full explanation, however, since the mean slope in the normal group became significantly steeper than in the asthmatic group after inhibition of prostaglandin synthesis. Similarly, within each group of subjects changes in reactivity after drug treatments were not entirely due to the influence of changes in baseline sGaw. Thus after chronic treatment with salbutamol in the normal subjects there was a relatively large increase in baseline sGaw but there was no significant change in slope. This may have reflected the enhanced bronchodilator action of PGE₂ that has been shown to go

with chronic salbutamol treatment,⁹ which might have overcome the influence of baseline sGaw on reactivity in these circumstances.

The current studies suggest that prostaglandins may well be concerned in the control of both sensitivity and reactivity to bronchoconstrictor agents in human airways. Such relationships are potentially complex.

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