Evidence for opioid modulation and generation of prostaglandins in sulphur dioxide (SO)$_2$-induced bronchoconstriction

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Abstract

Background – Inhalation of sulphur dioxide (SO_2) provokes bronchoconstriction in asthmatic subjects. Cholinergic mechanisms contribute, but other mechanisms remain undefined. The effect of morphine, an opioid agonist, on the cholinergic component of SO_2-induced bronchoconstriction was investigated, and the effect of indomethacin, a cyclooxygenase inhibitor, on SO_2-induced bronchoconstriction and tachyphylaxis was studied.

Methods – In the first study 16 asthmatic subjects inhaled either ipratropium bromide or placebo 60 minutes before an SO_2 challenge on days 1 and 2. On day 3 an SO_2 challenge was performed immediately after intravenous morphine. In the second study 15 asthmatic subjects took either placebo or indomethacin for three days before each study day when two SO_2 challenges were performed 30 minutes apart. The response was measured as the cumulative dose causing a 35% fall in specific airways conductance (sGaw; PDsGaw$_{35}$).

Results – Ipratropium bromide significantly inhibited SO_2 responsiveness, reducing PDsGaw$_{35}$ by 0·89 (95% CI 0·46 to 1·31) doubling doses. This effect persisted after correction for bronchodilatation induced by ipratropium bromide. The effect of ipratropium bromide and morphine on SO_2 responsiveness also correlated ($r^2 = 0·71$). In the second study SO_2 tachyphylaxis developed with PDsGaw$_{35}$ on repeated testing, being reduced by 0·62 (95% CI 0·17 to 1·07) doubling doses. Indomethacin attenuated baseline SO_2 responsiveness, increasing PDsGaw$_{35}$ by 0·5 (95% CI 0·06 to 0·93) doubling doses.

Conclusions – These results suggest that opioids modulate the cholinergic component of SO_2 responsiveness and that cyclooxygenase products contribute to the immediate response to SO_2.

(*Thorax* 1996;51:159–163)

Keywords: sulphur dioxide, bronchoconstriction, asthma, mechanisms.

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In asthmatic subjects inhalation of sulphur dioxide (SO_2), a common air pollutant, causes bronchoconstriction.[1] The mechanisms involved in this airway response are not clear, but it appears that activation of cholinergic nerves occurs.[2 3] Parasympathetic nerves are the predominant bronchoconstriction neural pathway in the airways.[4] It has recently been shown that opioids modulate cholinergic neurotransmission in canine airways[5] and in isolated human airway smooth muscle.[6] In addition, exogenous opioids have been found to inhibit the vagally mediated component of the airway response to inhaled water in asthmatic subjects.[7] Opioids could modulate the cholinergic component of the airway response to SO_2. This possibility was evaluated by assessing the effect of the opioid agonist, morphine sulphate, on the airway response to SO_2

Cholinergic antagonists only partially protect against SO_2-induced bronchoconstriction,[2 3 8] indicating that other mechanisms must play a part in the response of the airways to SO_2. It is now clear that many acute challenges to the airways of asthmatic subjects involve the release of mediators including cyclooxygenase products.[9 10] Bronchoconstriction induced by inhalation of sodium metabisulphite, a sulphite preservative which is thought to act via liberated SO_2, involves endogenous prostaglandins.[11] We assessed the role of prostanoids in SO_2-induced bronchoconstriction and tachyphylaxis by studying SO_2 responsiveness after administration of indomethacin, a cyclooxygenase inhibitor.

Methods

SUBJECTS

Thirty one subjects with mild asthma gave their informed consent to take part in these studies which were approved by the Royal North Shore Hospital ethics committee. All had a pre-challenge forced expiratory volume in one second (FEV$_1$) of at least 70% of their predicted value, no subject had had a respiratory tract infection for six weeks before testing, all were non-smokers, and all had previously demonstrated bronchial hyperresponsiveness to SO_2. Inhaled sympathomimetics were withheld for six hours or more before testing, but during testing all other inhaled therapy was maintained at a constant rate.

STUDY PROTOCOLS

Opioid study
This study involved three visits which were completed within four weeks. All tests were performed at the same time of day (12.00 to 16.00 hours), but any two consecutive tests were separated by at least 48 hours.

At visits 1 and 2 an SO_2 challenge was performed 60 minutes after randomised, double blind administration of either nebulised 0·025% (500 µg) ipratropium bromide solution or its placebo (Boehringer Ingelheim, Germany). Two ml of either solution was mixed with 2 ml of 0·9% normal saline and inhaled from an Acorn jet nebuliser attached to a Miser-22 Misthaler, driven with oxygen at 8 l/min. At visit 3 morphine sulphate (Sigma Pharmaceuticals Pty Ltd) was slowly injected intravenously over 15 minutes before an SO_2 challenge. At the end of the challenge, 0·4 mg of intravenous naloxone was given. Blood pressure and pulse rate were monitored before and during administration of each agent.

Indomethacin study

Fifteen subjects were studied at the same time of day on two separate days which were seven days apart. On each day, after measurement of baseline specific airways conductance (sGaw), two SO_2 challenges separated by 30 minutes were performed. The second SO_2 challenge was only performed if sGaw had returned to within 0·5 s^{-1} cm H_2O^{-1} of the baseline value measured before the first SO_2 challenge. Subjects were pretreated with either 50 mg oral indomethacin (Alphapharm, Australia) or placebo, taken twice daily for three days, with the final dose being taken within three hours of the first SO_2 challenge. Treatments were administered in a double blind randomised fashion.

In both studies sGaw was measured before and after administration of an agent, and then immediately before an SO_2 challenge.

SO_2 INHALATION CHALLENGE

Thoracic gas volume and airways resistance were measured in a variable pressure, constant volume body plethysmograph (P K Morgan, UK) and converted to sGaw.[12] The mean of five recordings, measured at 30 second intervals, was used on each occasion.

Subjects were challenged with SO_2 during sequential three minute periods of eucapnic hyperpnoea which were separated by five minutes. After inhalation of the control partially humidified air, subjects inhaled doubling concentrations (0·5, 1·0, 2·0, 4·0 and 8·0 ppm) of SO_2. A challenge was stopped when sGaw decreased by more than 60% of the control response, or the highest concentration was inhaled.

One hundred percent SO_2 was delivered via a Nupro dual double pattern metering valve and 60 µm filter to a stainless steel chamber where it was continually mixed with partially humidified air and fed into a 100 litre Seran bag (Aspec, Ann Arbor, Michigan, USA). End tidal carbon dioxide tension was maintained at normal resting levels during periods of hyperpnoea by adding 4–5% CO_2 to the bag gas mixture. Subjects inhaled the gas mixture using a noseclip via a two way Hans Rudolf valve. The air temperature and humidity of the inspired gas mixture, which were maintained at 65% relative humidity and 27°C, were measured using a Novasina temperature and humidity probe (Novasina, Switzerland) with the probe placed in the inspiratory port of the Hans Rudolf valve. Inspired SO_2 concentration was continuously measured using an electrochemical cell SO_2 analyser (Draeger, Sweden) through a port proximal to the Hans Rudolf valve. A Fleisch No. 3 pneumotachograph and differential pressure transducer (PK Morgan, UK) measured air flow which was digitally integrated to obtain minute ventilation ($\dot{V}E$). A constant $\dot{V}E$ was maintained by instructing the subjects to breathe in time to a metronome and to inhale a constant tidal volume, with each subject being cued by watching their respiration on a visual display unit. Subjects inhaled a constant tidal volume of either 1·0 or 1·5 l, depending on their total lung capacity. Using this method, the mean $\dot{V}E$ in the indomethacin study was 37·48 (0·44) l/min while in the opioid study a mean $\dot{V}E$ of 34·28 (0·52) l/min was achieved.

DATA ANALYSIS

Log dose response curves were constructed and the cumulative dose of SO_2 needed to cause a 35% fall in sGaw was calculated by linear interpolation ($PDsGaw_{35}$). A cumulative dose of SO_2 was used as SO_2 acts cumulatively when administered using the protocol described in this paper. For the purposes of analysis, if sGaw fell by 60% after inhalation of the first dose of SO_2, a $PDsGaw_{35}$ of 0·5 ppm was assigned; if sGaw did not change sufficiently after administration of the highest dose of SO_2, then a value of 15·5 ppm was given. $PDsGaw_{35}$ values were log transformed for analysis and are expressed as geometric mean values with 95% confidence intervals. The effect of treatment on SO_2 responsiveness was calculated by comparing the difference in log $PDsGaw_{35}$ after active and placebo treatments and is expressed in terms of doubling doses. SO_2 $PDsGaw_{35}$ and the difference in $PDsGaw_{35}$ for SO_2 challenges were compared within subjects by the paired t test. Regression analysis was used to examine the relationship between the effect of ipratropium bromide and morphine on SO_2 and to relate the change in sGaw after ipratropium bromide to the change in $PDsGaw_{35}$ after ipratropium bromide. Airway conductance measurements are expressed as means (SD). Analysis of variance was used to assess if there were any differences in sGaw before and after treatments. A p value of <0·05 was considered significant.

Table 1 Mean (SD) airway calibre measured as specific airways conductance (sGaw; s^{-1} cm H_2O^{-1}) before and after treament with placebo, ipratropium bromide (IPB), and morphine

Agent	Before agent	After agent
Placebo	0·18 (0·09)	0·19 (0·08)
IPB	0·19 (0·11)	0·26 (0·07)*
Morphine	0·19 (0·10)	0·18 (0·08)

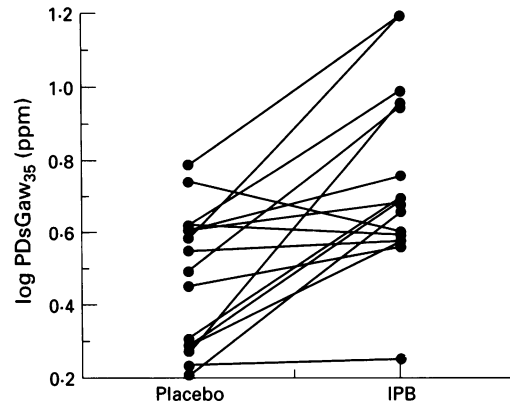* p<0·05 compared with value before IPB.

Figure 1 Individual doses of sulphur dioxide (SO₂) causing a 35% fall in specific airways conductance (PDsGaw₃₅) after placebo and after ipratropium bromide (IPB) in 16 mild asthmatic subjects.

Results

OPIOID STUDY

Sixteen asthmatic subjects (10 men) aged 25–50 years with a mean prechallenge FEV_1 of 87 (4·6)% (range 70–111%) were recruited. Five subjects were taking inhaled steroids regularly (beclomethasone dipropionate, 400–1000 µg/day) and all took an inhaled β_2 agonist as required. No subject developed bronchoconstriction after inhaling the control partially humidified air for three minutes.

The baseline sGaw was similar in each study (table 1) and did not change significantly after administration of placebo and morphine, but after ipratropium bromide there was a significant increase in sGaw ($p<0·05$). However, the degree of bronchodilatation induced by ipratropium bromide and the effect of ipratropium bromide on SO₂ airways responsiveness ($R^2 = 0·29$) were not significantly correlated.

Ipratropium bromide attenuated SO₂ responsiveness. The placebo geometric mean PDsGaw₃₅ of 2·97 ppm increased to 5·50 ppm after ipratropium bromide, a mean difference of 0·89 (95% CI 0·46 to 1·31) doubling doses ($p<0·0005$) (fig 1). The effects of ipratropium bromide and morphine on SO₂ PDsGaw₃₅ were significantly correlated ($R^2 = 0·71$, $p<0·0001$) (fig 2).

INDOMETHACIN STUDY

This study involved six men and nine women with asthma aged 19–60 years with a mean prechallenge FEV_1 of 92·3 (3·2)% (range 73–124%). All subjects were receiving treatment with inhaled β agonists only. No subject developed bronchoconstriction after inhaling the control partially humidified air for three minutes.

Baseline airway calibre before SO₂ challenges on the placebo and indomethacin study days did not differ significantly. On the placebo day the mean sGaw before the first and second SO₂ challenges were 0·14 (0·02) and 0·14 (0·16) $s^{-1}cm$ H_2O^{-1}, respectively. On the indomethacin day the mean sGaw before the first and second SO₂ challenges were both 0·14 (0·02) $s^{-1}cm$ H_2O^{-1}.

SO₂ tachyphylaxis was measured after administration of placebo (table 2). On the placebo day the first and second challenge geometric mean PDsGaw₃₅ values were 2·91 ppm and 4·48 ppm, a mean difference of 0·62 (95% CI 0·17 to 1·07) doubling doses ($p<0·01$). Indomethacin significantly attenuated baseline SO₂ responsiveness. On the indomethacin day the first challenge geometric mean PDsGaw₃₅ of 4·10 ppm differed significantly ($p<0·05$) from the placebo first challenge geometric mean of 2·91 ppm, the mean difference being 0·50 (95% CI 0·06 to 0·93) doubling doses. After indomethacin there was no significant difference between the first and second challenge geometric mean PDsGaw₃₅ values of 4·10 and 4·22 ppm, respectively. There was also no significant difference between the placebo and indomethacin second challenge geometric mean PDsGaw₃₅ values of 4·48 and 4·22 ppm.
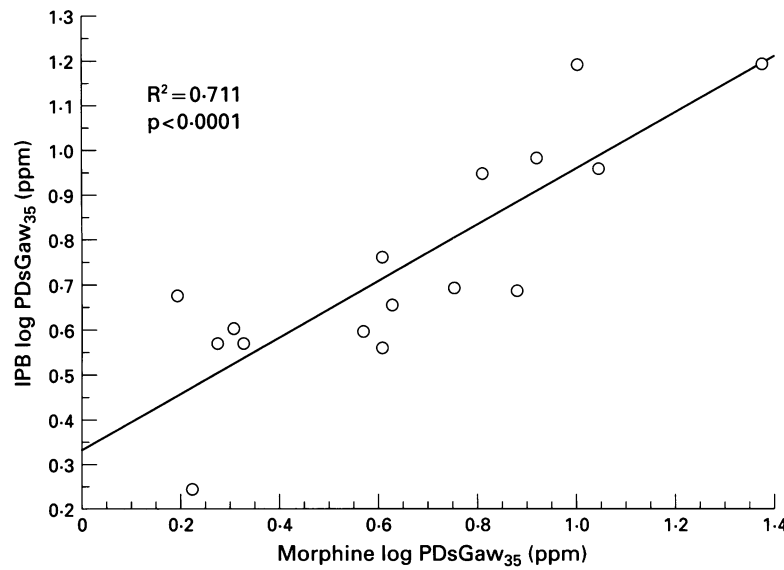

Figure 2 Comparison of the effect of ipratropium bromide (IPB) and the effect of morphine on airway responsiveness to sulphur dioxide (SO₂) in 16 mild asthmatic subjects.

Table 2 Doses of sulphur dioxide (SO₂) provoking a 35% fall in specific airways conductance (PDsGaw₃₅) in ppm for SO₂ challenges on placebo and indomethacin study days

Subject no.	Placebo study day		Indomethacin study day	
	First challenge	Second challenge	First challenge	Second challenge
1	0·50	2·40	1·70	1·65
2	0·80	3·00	1·70	1·55
3	1·25	3·65	3·50	4·00
4	1·05	2·05	3·70	4·60
5	4·50	8·90	5·20	5·40
6	9·80	11·75	10·80	10·80
7	3·55	5·60	3·70	3·50
8	3·90	5·90	6·60	6·00
9	4·80	7·90	8·20	15·50
10	1·80	2·30	2·25	3·80
11	4·75	4·05	4·35	5·00
12	2·80	2·75	4·00	2·50
13	7·80	7·00	4·45	4·20
14	5·80	5·60	4·80	3·90
15	5·50	3·90	4·40	3·70
Geometric mean	2·91	4·48	4·10	4·22
SD	2·42	1·71	1·67	1·82

Discussion

We have reported mechanisms which could contribute to the response of the airways to SO₂ in subjects with asthma. Firstly, it has been

shown that the cholinergic component of SO_2-induced bronchoconstriction can be modulated by exogenous opioids and, secondly, that cyclooxygenase products mediate the immediate bronchoconstrictive response to SO_2. The attenuation of SO_2-induced bronchoconstriction by ipratropium bromide is consistent with results from previous studies[2 3 8] in which muscarinic receptor antagonists have partially inhibited the response of the airways to SO_2. Ipratropium bromide provided a variable protection against inhaled SO_2. Similar variability has been observed in other studies,[2 3 8] with clearcut inhibition being demonstrated in one study[2] and more variable protection in others.[3 8] The bronchodilatation induced by ipratropium bromide could contribute to its variable protective effect. However, in our study there was no relationship between the degree of protection afforded by ipratropium bromide and the degree of bronchodilatation.

The similar degree of inhibition of the airways response to SO_2 by ipratropium bromide and morphine suggests that opioids modulate the cholinergically mediated component of the bronchoconstrictive response to SO_2. There is to our knowledge only one other published study demonstrating opioid inhibition of cholinergically mediated bronchoconstriction in humans in vivo.[7] In that study morphine inhibited the vagally mediated component of water-induced bronchoconstriction via opioid receptors in subjects with asthma. Opioid receptors have been identified throughout the central and peripheral nervous system[13] and are present in lung tissue.[14] The site at which morphine acts to modulate SO_2-induced bronchoconstriction is not known, but opioid receptors have been localised to sensory fibres of the vagus nerves.[15] In in vitro studies of human[6] and canine[5] airways it has been shown that exogenous opioids modulate cholinergic neurotransmission by stimulating prejunctional postganglionic μ opioid receptors which inhibit acetylcholine release.[6] In some animal models, however, cholinergic neurotransmission is enhanced by tachykinins and opioids reduce cholinergic neurotransmission by inhibiting the release of tachykinins from airway sensory nerves.[16] Tachykinins now appear to be involved in the airway responses to inhaled metabisulphite,[17] a sulphite preservative which is thought to act via liberated SO_2 Thus, opioids could modulate the vagally mediated component of SO_2-induced bronchoconstriction by inhibiting the action of endogenously released tachykinins. However, non-cholinergic neural constrictor responses have not been found in in vitro studies of human airways,[6] and tachykinins do not appear to modulate cholinergic neurotransmission.[18]

The attenuation of initial SO_2 responsiveness by indomethacin suggests that the airway response to SO_2 is partially mediated by cyclooxygenase products present in the airways of asthmatic subjects. However, this effect of indomethacin on initial SO_2 responsiveness confounded interpretation of the effect of indomethacin on the development of SO_2 tachyphylaxis. After indomethacin the mean

change in SO_2 responsiveness between the first and second challenge was significantly less than after placebo. This difference may have been due solely to the change in initial SO_2 responsiveness induced by indomethacin, but it may also have reflected an inhibition of the development of SO_2 tachyphylaxis by indomethacin.

The effect of indomethacin in attenuating airways responsiveness to SO_2 was small, indicating that cyclooxygenase products are likely to play a minor role. The dose of indomethacin administered should have provided adequate inhibition of prostanoid production, as similar doses have been effective in inhibiting allergen-induced bronchoconstriction[9] and in attenuating histamine tachyphylaxis[10] in asthmatic subjects. At the dose used in this study the production of prostaglandins is suppressed on average by more than 70%,[19] but between subjects there is considerable variation in plasma levels following a given dose.[19] It is therefore possible that, with the use of a different cyclooxygenase inhibitor, greater inhibition of SO_2-induced bronchoconstriction may have occurred. When different cyclooxygenase inhibitors have been used to study the role of prostanoids in bronchoconstriction induced by inhalation of sodium metabisulphite, disparate results have been obtained. Flurbiprofen caused significant attenuation of the constrictor response to sodium metabisulphite,[11] while indomethacin had no effect.[20]

The results of our study have confirmed that asthmatic subjects can develop tachyphylaxis to SO_2.[21] The underlying mechanisms are not known. A loss of airway smooth muscle responsiveness to released neurotransmitters does not appear to be involved as the bronchoconstrictor response to histamine, a direct airway smooth muscle agonist, is preserved after development of SO_2 tachyphylaxis.[21] Inhibitory prostaglandins may play a part as refractoriness to the bronchoconstrictor response to sodium metabisulphite has been shown to involve inhibitory prostaglandins.[20] Unfortunately, it was not possible in our study to determine whether inhibitory cyclooxygenase products also contributed to the development of SO_2 tachyphylaxis.

The source of cyclooxygenase products that contribute to the bronchoconstrictive response to SO_2 remains undetermined. Prostaglandins with bronchoconstrictor properties such as PGD_2 are generated by mast cells.[22] However, the role of mast cells in SO_2 airway responsiveness is unclear. Sodium cromoglycate which blocks the release of mast cell mediators such as histamine inhibits the airway response to SO_2,[8] but the antihistamine terfenadine[23] does not affect airway responsiveness to sulphites. Another source of bronchoconstricting prostaglandins is airway epithelium.[24] One mechanism by which SO_2 could stimulate generation of epithelial-derived prostaglandins is via bradykinin. In allergic sheep inhalation of metabisulphite solutions promotes the release of lung kinins[25] which could stimulate the generation of prostaglandins via specific receptors present on airway epithelial cells.[24]

In conclusion, the airway response to SO_2 involves the release of inflammatory mediators. In addition, the parasympathetic reflexes which play a part in the SO_2-induced broncho-constriction have the potential to be modulated by opioids.

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