Br. J. Pharmac. (1983), **80**, 287–294

Indomethacin enhances the effect of histamine on airways resistance in the anaesthetized guinea-pig

H.W. Mitchell

Department of Physiology, University of Sheffield, Sheffield S10 2TN

1 In anaesthetized guinea-pigs intravenous histamine caused an increase in airways resistance (R_A) and a fall in dynamic compliance (C_{Dyn}).

2 Indomethacin (1 mg kg^{-1}, i.v.) significantly enhanced the effect of histamine on R_A. Indomethacin also increased the basal R_A and the R_A response to a histamine infusion. The effect of indomethacin on C_{Dyn} was less consistent but here also there was a trend for an increased response to histamine. Sodium carbonate (the vehicle for indomethacin, 0.05 ml 100 mM solution) had no effect on R_A or C_{Dyn} in control experiments.

3 Propranolol (0.1 mg kg^{-1}, i.v.) enhanced the effect of histamine on R_A in animals pretreated with either indomethacin or Na_2CO_3 vehicle, but the effect was more consistent in indomethacin pretreated animals.

4 Indomethacin also tended to enhance the effect of histamine on R_A in animals pretreated with reserpine or BW755c but it had little effect on the C_{Dyn} response to histamine.

5 The results show that indomethacin augments the responsiveness of the airways to histamine in the anaesthetized guinea-pig. The results with propranolol and reserpine suggest that an operational β-adrenergic system is not required for the effect of indomethacin on R_A. No confirmation for lipoxygenase involvement was obtained with the lipoxygenase inhibitor, BW755.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have several effects on bronchial and pulmonary tissues. Because they are cyclo-oxygenase inhibitors they reduce prostaglandin synthesis and release from lung and trachea (Piper & Walker, 1973; Gryglewski, Dembinska-Kiec, Grodzinska & Panczenko, 1976) but under certain conditions they may also enhance the anaphylactic release from pulmonary tissues of histamine and slow reacting substance (leukotrienes C_4/D_4) (Boot, Brockwell, Dawson & Sweatman, 1977; Engineer, Niederhauser, Piper & Sirois, 1978; Hitchcock, 1980).

Indomethacin, which is often used as a 'typical' NSAID, can modify lung function *in vivo* in animals during anaphylactic reactions. For instance in the allergic monkey and in the sensitized guinea-pig, indomethacin increases the response of the airway to antigen (Patterson, Harris & Greenberger, 1978; Hitchcock, 1980; Andersson, 1982) possibly because of its actions outlined above. In man NSAIDs may precipitate symptoms of acute asthma in a small subpopulation of atopic asthmatic individuals and it has been strongly argued that this effect of NSAIDs is due to increased mediator production by mast cells and to the removal of the relaxant action of prostaglandin E_2 on the bronchial smooth muscle (Szczeklik, Gryglewski & Czerniawska-Mysik, 1977). Other asthmatic subjects, in contrast, show little or no change in lung function after taking NSAIDs (e.g. Smith & Dunlop, 1975). Recently however, a careful study by Fish, Ankin, Adkinson & Peterman (1981) has shown that whilst the effect of antigen challenge on lung function in atopic asthmatics is unaffected by indomethacin, in subjects with allergic rhinitis but not asthma, lung function following antigen challenge is depressed by indomethacin.

In addition to modifying allergic responses, indomethacin and other NSAIDs also have a direct effect(s) on airways smooth muscle *in vitro*. In both man and animals, contractions in response to local hormones such as histamine are markedly potentiated. Contracting smooth muscle preparations release prostaglandins of the E series and therefore inhibition of their release might contribute to the effect of indomethacin (Orehek, Douglas & Bouhuys, 1975). The potentiating effect of in-

domethacin has been shown in two laboratories (Adcock & Garland, 1980; Mitchell, 1982a, b) to be prevented when lipoxygenase is blocked, suggesting that excitatory products of a lipoxygenase reaction are involved in the effect of NSAIDs on *in vitro* muscle contraction.

The significance of the *in vitro* findings with NSAIDs and drug-induced bronchial smooth muscle contraction has been obscured by the lack of data on corresponding bronchoconstrictor effects in animals *in vivo*. Indeed Collier and his co-workers have investigated a number of NSAIDs in the guinea-pig and did not observe a modified response to agents such as histamine (Collier, Holgate, Schachter & Shorley, 1960; Collier & Shorley, 1960; Berry & Collier, 1964). However, the technique which was used for measuring bronchoconstriction by overflow (modifications of the Konzett–Rössler technique) may not be sufficiently sensitive. However, if pressure and flow parameters are followed, there has been a suggestion of a more powerful bronchoconstriction to histamine in guinea-pigs pretreated with indomethacin (Peterson, Biggs & Aaron, 1980; Brink, Duncan & Douglas, 1981). The present study using relatively sensitive techniques was designed specifically to ascertain whether a potentiated bronchial response could be observed to histamine in the presence of indomethacin, and if so, to investigate the mechanism of this response.

Methods

Lung mechanics (airways resistance, R_A, and dynamic compliance, C_{Dyn}) were measured in a total of 21 spontaneously breathing guinea-pigs. All were males and they weighed between 350–450 g. The guinea-pigs were anaesthetized with 2.0 g kg^{-1} urethane intraperitoneally. A glass cannula was inserted into the trachea and a jugular vein and a carotid artery were cannulated for injecting drugs and recording the blood pressure. A slightly blunted O gauge serum needle was inserted into the pleural space through the chest wall in the region of the 5th intercostal space. This pleural cannula was secured by a purse string ligature. Body temperature was maintained by means of a heater under the operating table.

R_A and C_{Dyn} were measured by the method of Amdur & Mead (1958) using signals of intrapleural pressure, airflow and tidal volume. Intrapleural pressure was measured by connecting the pleural catheter to one port of a Grass Volumetric Pressure Transducer type PT5A. The other port of the transducer was connected to a side arm of the tracheal cannula. Airflow and tidal volume were recorded with a Spirometer (Mercury, CS5) and a F11 pneumotachograph head. The spirometer integrator was modified to give a continuous record of volume. These signals together with blood pressure (Bell and Howell transducer) were displayed on a 4-channel Washington pen recorder (Bioscience) with rectilinear writing arms. The transducers and amplifiers were all calibrated in the laboratory.

The experimental protocol was to obtain a pair of responses to histamine (i.v., at 10 min intervals) and then to inject indomethacin (1 mg kg^{-1}, i.v.) and repeat the histamine challenges 10 min and 20 min later. The animals were then dosed with propranolol (0.1 mg kg^{-1}, i.v.) and after a further 10 min, histamine was re-injected. The average change in R_A and C_{Dyn} obtained to the two doses of histamine prior to indomethacin or propranolol was taken as the control response. Responses to histamine obtained after indomethacin or propranolol were then compared to the appropriate control and differences were considered to be significant when $P<0.05$ by Student's paired *t* test. Indomethacin was dissolved in 100 mM sodium carbonate just before it was injected (0.05 ml). Histamine and propranolol were dissolved in 0.9% w/v NaCl solution. Control experiments were performed substituting the sodium carbonate vehicle for indomethacin. All drugs were washed-in with the above NaCl solution and total injection volumes never exceeded 0.5 ml. In two additional animals the effect of 0.05 ml indomethacin/sodium carbonate solution on the arterial blood gases was measured. The P_{O_2} before and after indomethacin was 89.7/84.7 mmHg, the P_{CO_2} was 26.3/28.6 mmHg and the pH was 7.18/7.10 respectively.

Some experiments were carried out on animals pretreated with either reserpine 5 mg kg^{-1} (i.p. made up in polyethylene glycol and injected 24 h before the experiment) or BW755c (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) (see Results). In the experiments shown in Figure 4, histamine was infused into the jugular vein (12.5–25 μg min^{-1}) for at least 20 min. Indomethacin (1 mg kg^{-1}) was then injected via a cannula in the other jugular vein whilst the histamine infusion was maintained.

Results

In 21 animals the mean resting airways resistance, R_A, was 0.150 ± 0.016 cmH_2O ml^{-1} s^{-1} (mean ± s.e.mean) and the dynamic compliance, C_{Dyn}, was 1.49 ± 0.11 ml cmH_2O^{-1}. The doses of histamine used (1–6 μg kg^{-1}) were selected to produce approximately 50% reduction of the tidal volume in each animal. These injections increased R_A by about 50–75% and decreased C_{Dyn} by a similar extent. In most cases R_A and C_{Dyn} were calculated for every

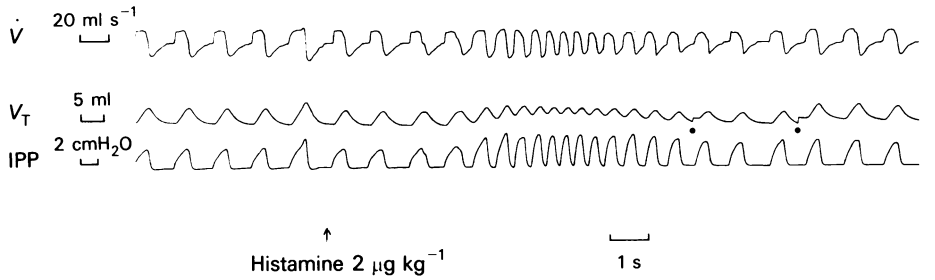

Figure 1 Chart record showing the effect of histamine on respiration in an anaesthetized guinea-pig. The records shown are airflow ($\dot{V}$), tidal volume (V_T) and interapleural pressure (IPP). Following the injection of histamine there is a transient increase in lung volume and an increase in the respiratory frequency. The tidal volume integrator was reset at the points indicated (●).

second or third breath until the values returned close to baseline. The maximum change usually occurred between 5–15 s after histamine injection and the $t_{\frac{1}{2}}$ (time after histamine injection when the R_A had returned half way to baseline) was approximately 18 s. In addition to the above changes in lung mechanics, histamine also caused an increase in lung volume and an increase in breathing frequency (Figure 1, Table 1). The hyperpnoea was only transient and the maximum changes in R_A occurred after the breathing frequency had stabilized. On the other hand, as might be expected the peak change in C_{Dyn} often coincided with the hyperpnoea. However, there were no differences in the pattern of breathing in animals treated either with indomethacin or the sodium carbonate (100 mM) vehicle.

Effect of indomethacin and propranolol on airways resistance and dynamic compliance

The basal values for R_A and C_{Dyn} were not altered by the injection of sodium carbonate in the control experiments. However, indomethacin caused a small but significant ($P<0.01$) increase in R_A ($17.6\pm4.3\%$, $n=7$) but it had no effect on resting C_{Dyn}. After the injection of propranolol R_A further increased by $27.9\pm6.4\%$ ($n=7$, $P<0.01$) in indomethacin-treated animals but there was still no significant effect on C_{Dyn}. In control animals propranolol had no effect on R_A but C_{Dyn} was reduced by $13.8\pm3.9\%$ ($n=5$, $P<0.05$).

The major effect of indomethacin was that it enhanced the effect of histamine on R_A (Figure 2, Table 2). This potentiation was small but it occurred in every animal tested (cf. Figure 2) and it was statistically significant. The fall in C_{Dyn} in response to histamine was also greater after indomethacin but the extent was more variable (Figure 2). Sodium carbonate had no effect on the histamine-induced changes in R_A or C_{Dyn} (Figure 2, Table 2).

The responses obtained to histamine, on R_A, after the injection of propranolol were larger than those before, both in animals pretreated with indomethacin or Na_2CO_3 vehicle. However, this effect reached significance only in the indomethacin pretreatment group (Figure 3, Table 2). On the other hand, when

Table 1 Effect of histamine on lung volume and respiration rate in the anaesthetized guinea-pig

	Volume (ml)*		*Rate* (% increase)[1]	
	Pre	Post	Pre	Post
Controls (Na_2CO_3) (5)	2.8 ± 0.7	2.8 ± 0.7	172 ± 30	210 ± 9
Indomethacin (7)	2.4 ± 0.5	2.3 ± 0.4	180 ± 40	145 ± 25

Results show the mean ± s.e.mean values for the maximal increases in lung volume and breathing frequency following the injection of histamine. Responses to histamine ($1-6\,\mu g\,kg^{-1}$, i.v.) before (pre) and after (post) Na_2CO_3 vehicle or indomethacin ($1\,mg\,kg^{-1}$, i.v.) are shown.

* The increase in lung volume in ml was determined from the tidal volume record.

[1] Percentage increase in breathing rate. Number of animals in parentheses.

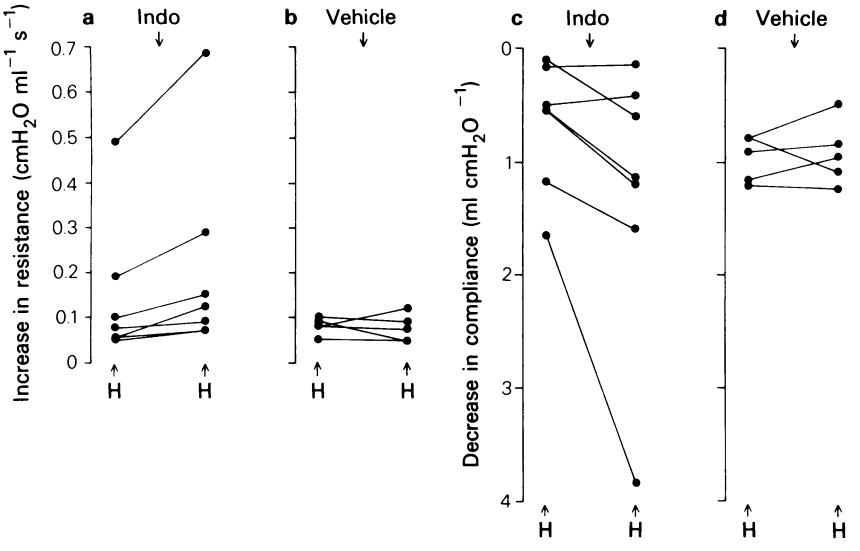

Figure 2 Combined data for the effect of indomethacin (a and c) and Na_2CO_3 vehicle (b and d) on airways resistance and dynamic compliance in anaesthetized guinea-pigs. Each line represents results from one animal. The left hand point in each graph is the increase in resistance or decrease in compliance in response to histamine (H) in that animal. The right hand point then shows the response obtained after the injection of indomethacin (Indo, 1 mg kg^{-1}, i.v.) or Na_2CO_3 vehicle. The increase in resistance seen in the presence of indomethacin (a) was significantly greater than that observed before indomethacin ($P<0.05$).

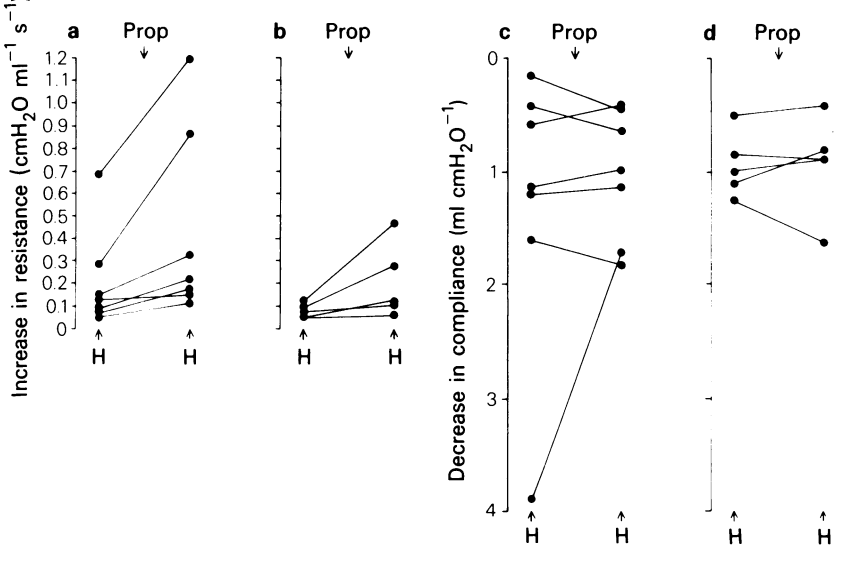

Figure 3 Effect of propranolol (Prop, 0.1 mg kg^{-1}, i.v.) on airways resistance and dynamic compliance in anaesthetized guinea-pigs. Data in (a) and (c) are from animals pretreated with indomethacin (1 mg kg^{-1}, i.v.) and data in (b) and (d) are from animals pretreated with Na_2CO_3 vehicle. Each line represents results from one animal. The left hand point in each graph is the increase in resistance or decrease on compliance in response to histamine (H) in that animal, and it corresponds to the right hand point in each graph in Figure 2. The right hand point then shows the response obtained after the injection of propranolol.

Table 2 The percentage effects of the Na_2CO_3 vehicle or indomethacin on the histamine-induced changes in airways resistance and dynamic compliance in the anaesthetized guinea-pig

	No pretreatment[+]		*Propranolol*[1]		*Reserpine*[2]		*BW755c*[3]	
	R_A	C_{Dyn}	R_A	C_{Dyn}	R_A	C_{Dyn}	R_A	C_{Dyn}
Na_2CO_3 vehicle	+0.2±17.5 (5)	−1.2±14.5 (5)	+131.7±55.8 (5)	−3.8±11.4 (5)	NT	NT	NT	NT
Indomethacin	+49.3±17.4* (7)	+119.6±64.0 (7)	+116.8±24.2** (7)	+21.9±33.4 (7)	+161.9±106.4 (5)	+33.8±19.9 (5)	+103.0±34.2 (4)	−5.4±18.5 (4)

[+] Values show the percentage difference in the histamine-induced increases in R_A and falls in C_{Dyn} after either 0.1 M Na_2CO_3 (0.05 ml i.v.) or indomethacin (1 mg kg^{-1} i.v.).
[1] Histamine-induced responses, in the same animals, were then re-established following injection of propranolol (0.1 mg kg^{-1}, i.v.).
[2,3] Results from separate experiments showing the effect of indomethacin on histamine-induced responses in animals pretreated with either reserpine or BW755c (for doses see Results).
* $P<0.05$, ** $P<0.01$ difference between the pairs of responses obtained before and after injection of indomethacin or propranolol. (Student's paired *t* test, see Methods). Number of animals in parentheses, all values show the mean ± s.e.mean. NT, not tested.

compared to the effect of indomethacin or Na_2CO_3 vehicle alone (i.e. no pre-treatment, Table 2) the propranolol-induced potentiation of the histamine response was statistically significant ($P<0.05$, Student's unpaired *t* test) in both groups of animals (indomethacin or Na_2CO_3 vehicle). Propranolol had no effect on the C_{Dyn} response to histamine in animals pretreated with Na_2CO_3 vehicle, and in the indomethacin pretreated group the effect of propranolol was varied and was not different from that seen after indomethacin alone (Figure 3, Table 2).

Effect of indomethacin on responses to histamine infusions

Initially infusion of histamine caused an increase in R_A and a fall in C_{Dyn}. These changes were then sustained whilst the infusion was maintained although R_A tended to increase further over a 10 min period. Once the new values of R_A and C_{Dyn} had stabilized, indomethacin was administered. A representative tracing from one experiment is shown in Figure 4. In 3 of 3 experiments R_A increased further following the injection of indomethacin and C_{Dyn} fell in 2 of 3 experiments. In the third case C_{Dyn} did not appear to be affected by indomethacin.

Effect of reserpine

Animals pretreated with reserpine (5 mg kg^{-1}, i.p., 24 h previously) required higher doses of histamine (3–8.8 μg kg^{-1}) to produce changes in and C_{Dyn} equivalent to those obtained in unreserpinised animals (see above). In these experiments indomethacin still enhanced the effect of histamine on R_A and to a lesser extent on C_{Dyn} (Table 2). However, these changes were not significant either when compared to the responses before the injection of indomethacin or when they are compared with the responses obtained to histamine after indomethacin in animals which were not pretreated with reserpine (Student's unpaired *t* test).

Effect of BW755c

BW755c (10–20 mg kg^{-1}) was either administered intravenously at 15 min before the injection of histamine or at − 24 h (20–50 mg kg^{-1}, i.p.) followed by a further similar dose intravenously at − 15 min. In every experiment the histamine-induced increases in R_A tended to be greater after the injection of indomethacin and so the results for the two treatment protocols are pooled and shown in Table 2. The effect of histamine on R_A after administration of indomethacin was not significantly different, however, from that before. Nevertheless the differences in these histamine-induced responses before and after

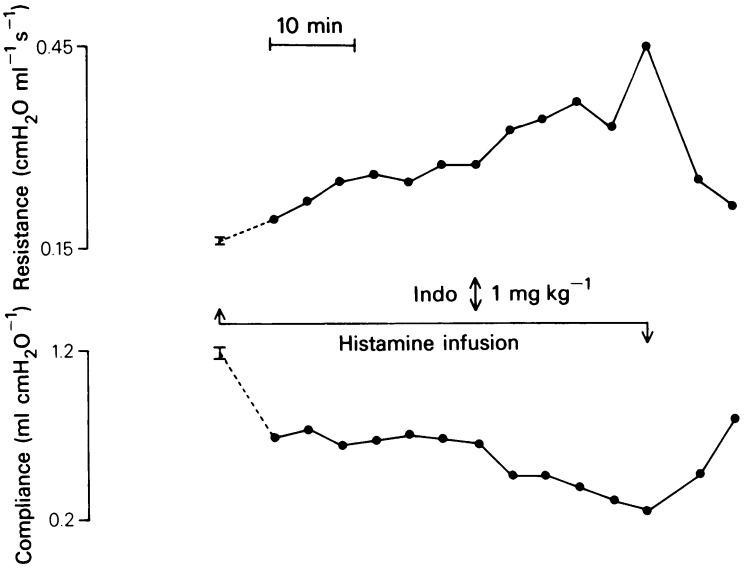

Figure 4 An experiment showing the effect of indomethacin on airways resistance and dynamic compliance in an anaesthetized guinea-pig. The baseline values for lung function were first depressed by an infusion of histamine (25 $\mu g\,min^{-1}$). Whilst maintaining the infusion, indomethacin (Indo) was administered as a bolus injection of 1 $mg\,kg^{-1}$. After a further 20 min the histamine infusion was terminated. The left-hand bars are the average values (± s.e.mean) for R_A and C_{Dyn} obtained before the infusion pump was switched on. Subsequently resistance and compliance were calculated at time-intervals of 4 min. The figure shows results of 1 experiment; similar results were obtained in 2 additional experiments.

the injection of indomethacin were greater ($P<0.05$, Student's unpaired t test) than the corresponding changes obtained after the injection of Na_2CO_3 vehicle (but not indomethacin) in a separate group of animals. No differences were apparent in the effect of histamine on C_{Dyn}.

Discussion

It has been shown repeatedly that smooth muscle from the trachea and bronchi of man and animals contracts more powerfully to histamine in the presence of indomethacin or other NSAIDs (Orehek *et al.*, 1975; Anderson, Krzanowski, Polson & Szentivany, 1979; Krzanowski, Anderson, Polson & Szentivanyi, 1980; Adcock & Garland, 1982; Mitchell, 1982a). This effect of indomethacin has been variously explained by arguments invoking a perturbation of arachidonate metabolism in cell membranes. Moreover, there seems to be a fortuitous parallel between the effects of NSAIDs *in vitro* and 'aspirin-induced asthma' where bronchospasm is induced by NSAIDs, possibly because a protective action of relaxant prostaglandins has been removed (Szczeklik *et al.*, 1977). *In vitro* experiments also suggested that a lipoxygenase is important for this effect of indomethacin in isolated tissues (Adcock & Garland, 1980; 1982; Mitchell, 1982a, b).

A problem in the acceptance of the *in vitro* findings as a reflection of what may actually happen in the intact animal is that NSAIDs do not appear to affect the reactivity of bronchial smooth muscle *in vivo* (Collier *et al.*, 1960; Collier & Shorley, 1960; Berry & Collier, 1964). Results using the Konzett-Rössler technique of measuring pulmonary overflow in artificially ventilated animals (used in the earlier studies) are in effect a measure of the elasticity, or compliance, of the lungs and chest wall (Widdicombe, 1963), and as such they may not be the most sensitive measure of tracheobronchial smooth muscle contraction. However, present results show that indomethacin alters the mechanics of airflow (measured as airways resistance, R_A) and indicate that the calibre of the conducting airways is reduced. The most consistent actions of indomethacin were to elevate basal R_A and to potentiate the effect of histamine on R_A (an increase). This effect of indomethacin on R_A, and perhaps on dynamic compliance (C_{Dyn}) may account for the increased sensitivity of the tidal volume response to histamine which was observed by Brink *et al.*, (1981) in the sensitized guinea-pig. In the present experiments the changes were quite modest (Table 2) and the increased response to histamine might have been affected by the elevated baseline R_A (17.6% increase in baseline after injection of indomethacin). Moreover, histamine also increased lung volume and

this would be expected to modify the histamine response on R_A and C_{Dyn}. Since there were no differences in the lung volume response to histamine in control and test animals (Table 1) this is unlikely to have been a causal factor in the potentiation of the effect of histamine which was only seen in the test animals. Furthermore, there was little difference in the pattern of breathing before and after indomethacin. C_{Dyn} was not significantly altered by indomethacin with or without histamine; however, in 5 of the 7 experiments a potentiated C_{Dyn} response to histamine was observed after administration of indomethacin. The doses of histamine used produced submaximal increases in R_A (results not shown; Drazen & Austen, 1974). The technique of calculating R_A and C_{Dyn} from the chart recorder traces is very time consuming; therefore single doses of histamine and indomethacin were used and it is not known whether comparable results would have been obtained over a wider dose range. It was most interesting to note that indomethacin also appears to elevate a resistance which is already increased by an infusion of histamine. This procedure may produce a situation more like the one found in the asthmatic patient with chronically depressed lung function.

To attempt to elucidate the mechanism of action of indomethacin *in vivo*, the effects of propranolol, reserpine and BW755c on the evoked responses were tested. Propranolol potentiated the bronchial response to histamine. Similar observations have been previously made with histamine (e.g. McCullock, Proctor & Rand, 1967; Colebatch, 1970; Douglas, Dennis, Ridgway & Bouhuys, 1973) and antigen in sensitized animals (Collier & James, 1967). These authors have suggested that the effect of propranolol is due to abolition of the effects of circulating catecholamines and of noradrenaline released from nerve endings. Similar doses of propranolol to those used in the present study, however, have been shown to cause a bronchoconstriction which appears to be unrelated to β-adrenoceptor blockade (Maclagan & Ney, 1979). In the present study propranolol still potentiated histamine-induced increases in R_A in animals pretreated with indomethacin. If propranolol renders the lung more reactive to constrictor stimuli because β-adrenoceptors have been blocked (McCullock *et al.*, 1967; Collier & James, 1967; Colebatch, 1970; Douglas *et al.*, 1973) then the present study suggests that the indomethacin effect is not dependent on the integrity of a β-adrenoceptor system i.e. indomethacin is not itself acting as an adrenoceptor inhibitor. At this stage, however, it is difficult to evaluate any possible interaction between indomethacin and non-specific effects of propranolol (Maclagan & Ney, 1979). Likewise in reserpinized animals, indomethacin usually enhanced the effect of histamine on R_A, a result compatible with that from the propranolol experiment. *In vitro* the effect of indomethacin on smooth muscle contraction is inhibited or reversed by BW755c (Adcock & Garland, 1980; 1982; Mitchell, 1982b) suggesting that a lipoxygenase derived substance is involved. In the guinea-pig *in vivo*, it was not possible to demonstrate a similar abolition of the indomethacin-effect with BW755c because responses to histamine were more varied in BW755c-treated animals. It was found that the animals pretreated with BW755c were in a poor condition and lethargic and this might account for the varied results. Thus, at this stage it is not possible to infer that the effect of indomethacin seen *in vivo* is due to a similar action as that observed in isolated tissues. It will be necessary to test other lipoxygenase inhibitors to examine this point.

As R_A is a function of airway calibre some possible sites of action of indomethacin are the smooth muscle, bronchial epithelium (e.g. oedema) and mucous secreting glands. The smooth muscle can be regulated intrinsically by prostaglandins or lipoxygenase products and by the autonomic nervous system. Fish *et al.*, (1981) have noticed that indomethacin depresses lung function in normal (i.e. non-asthmatic) individuals and they suggested that this effect might be due to inhibition of prostaglandin biosynthesis. Ito & Tajima (1981) found that chronic treatment with indomethacin in dogs caused the excised tracheae to contract spontaneously and there were also changes in excitatory junction potentials. Atropine reversed some of those responses and the authors proposed that prostaglandins are involved in cholinergic transmission to the airway. The effect of indomethacin in the histamine infusion experiment (Figure 4) occurred within minutes which may indicate that the effect of indomethacin in the intact animal is more complex than that seen *in vivo* and may involve the non-adrenergic autonomic nervous system. Thus, the present data show a clear constrictor effect of indomethacin in the guinea-pig lung, the mechanism of which remains to be elucidated.

The expertise of Mrs. T. Cousins in the execution of this study is gratefully acknowledged. BW755c was a generous gift from Wellcome Research Laboratories. The work was supported by the University of Sheffield Medical Research Fund.

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(Recieved March 18, 1983.
Revised May 9, 1983.)