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Vagal mechanisms and the effect of indomethacin on bronchoconstrictor stimuli in the guinea-pig

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1 In urethane-anaesthetized guinea-pigs, under spontaneous respiration, indomethacin (1 mg kg^{-1} i.v., 10–45 min) approximately doubled the bronchoconstrictor effect (increase in airways resistance, R_{aw}) of equieffective doses of histamine and 5-hydroxytryptamine (5-HT), but not that of acetylcholine or leukotriene D_4 (LTD_4).

2 In mechanically ventilated guinea-pigs indomethacin increased R_{aw} responses to histamine as well as increasing the fall in dynamic compliance (C_{dyn}) evoked by this agent.

3 Cooling the cervical vagi, to temperatures shown to block efferent and probably afferent pathways (~9°C), abolished the effect of indomethacin on airways responses. Inhibition of indomethacin-induced hyperreactivity was also observed after vagal section.

4 Electrical stimulation of the peripheral vagus (1–20 Hz, 0.75–5 ms pulses) increased R_{aw} and decreased C_{dyn} but these responses were not markedly altered by indomethacin.

5 It was concluded that the indomethacin-induced hyperreactivity of tracheal smooth muscle, which was demonstrated *in vitro*, may not account for the airways hyperreactivity observed in the present *in vivo* experiments. The hyperreactivity to histamine induced by indomethacin *in vivo* depends on the functional integrity of vagal reflex pathways.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) provoke bronchoconstriction in some asthmatics but they have little detectable effect on basal (i.e. non-provoked) airway function in non-asthmatic subjects. Indomethacin (Mitchell, 1983) and other NSAIDs (Mitchell & Adcock, 1987) have recently been found to cause hyperreactivity to histamine in anaesthetized guinea-pigs, but neither the mechanism of this hyperreactivity not its specificity for histamine has been described.

Cyclo-oxygenase inhibitors increase the reactivity of airways smooth muscle *in vitro* to a variety of spasmogens including histamine, 5-hydroxytryptamine (5-HT) and to a lesser extent acetylcholine. This holds true both for guinea-pig (e.g. Orehek *et al.*, 1975) and, in the case of histamine, human tissue (Adcock & Garland, 1982). It has been suggested that this increased reactivity involves blockade of the synthesis of relaxant prostaglandins (Orehek *et al.*, 1975) and the elaboration of excitatory lipoxygenase products (Adcock & Garland, 1982; Mitchell, 1982). Such an effect on the smooth muscle could account for the hyperreactivity observed in guinea-pigs *in vivo*. Since several chemical agents initiate vagal reflex activity another possible site at which NSAIDs may increase airways reactivity is the autonomic nervous system. Ito & Tajima (1981) demonstrated that indomethacin increased cholinergic excitatory junction potentials in the dog trachealis. Furthermore, if NSAIDs attenuated activity in adrenergic or non-adrenergic inhibitory pathways (McCullock *et al.*, 1967; Coburn & Tomita, 1973) increased airways constriction in response to histamine might be expected.

The experiments in this study were carried out in order to examine the specificity of the reported indomethacin-induced hyperreactivity for different bronchoconstrictor agents and to determine whether indomethacin evokes hyperreactivity by modulating the direct (i.e. on smooth muscle) or indirect (i.e. on vagal reflex pathways) effects of histamine.

Methods

Animal preparation and measurements

The methods and protocols were similar to those

previously used (Mitchell, 1983). Guinea-pigs (300–400 g) were anaesthetized with urethane (1.25–1.5 g kg^{-1} i.p.) and tracheal, pleural and venous cannulae were inserted. The resistance of the tracheal cannula was $\leqslant 0.07$ cmH_2O ml s^{-1}.

In some experiments a cannula was also placed in a carotid artery so that the heart rate could be observed from the blood pressure record. The mean arterial blood pressure was within the range 25–50 mmHg which is typical for urethane-anaesthetized guinea-pig preparations. However, absolute arterial pressure was not routinely monitored in this study. The animals were kept warm (37.5 ± 0.2°C rectal temperature) by means of a thermostatically controlled heated blanket. Airways resistance (R_{aw}) and dynamic compliance (C_{dyn}) were obtained breath-by-breath from signals of intrapleural pressure, airflow and tidal volume using an Apple IIe microcomputer (Mitchell, 1985). R_{aw} was calculated at functional residual capacity (FRC) plus 50% tidal volume. C_{dyn} was calculated at zero airflow over inspiration.

In experiments to examine vagal effects mechanical ventilation was used to prevent the changes in respiratory patterns which occur when the vagi are cooled or sectioned (see Results). A BioSciences small animal ventilator was used with a stroke volume > the tidal volume recorded just before connecting the ventilator to the tracheal cannula and adjusted to deliver 80 strokes min^{-1} so that spontaneous respiratory movements were reduced.

Peak changes in lung mechanics (R_{aw} and C_{dyn}) in response to bronchoconstrictor agents (histamine, 5-hydroxytryptamine (5-HT), acetylcholine, leukotriene D_4 (LTD_4) and vagal stimulation) were recorded in duplicate or triplicate at intervals of 10–30 min. The lung was briefly inflated, between drug responses, by one or two tidal volumes by occluding the respirator outflow. Bronchoconstrictor drugs were administered intravenously in doses chosen to produce 50–100% increases in R_{aw} (see Results). Actual changes in R_{aw} and C_{dyn} in response to histamine (or other constrictors) were determined before and 10–45 min after indomethacin (1 mg kg^{-1} i.v.).

Vagal cooling and section

Reversible cooling of the cervical vagal trunk (incorporating the sympathetic nerves when they were distinguishable) was undertaken using brass thermodes through which cold ethanol was circulated (Franz & Iggo, 1968). The nerve on each thermode was embedded in 4% agar in saline to avoid drying and to dissipate temperature gradients. Stimulating electrodes were placed around one nerve central to the thermode and both the nerve and electrode tip were irrigated with mineral oil to delay drying. Stimulation was used in some experiments to produce bradycardia or bronchoconstriction (5–60 V, 1–20 Hz, 0.75–5 ms square waves). No attempt was made to desheath the vagus. Cooling of the nerve trunk typically took approximately 30 s. During this cooling period the temperature gradient across the nerve was about 2°C (determined using two copper-constantan thermocouples placed on either side of the trunk). However, the temperature gradient was then lost within 10 s of reaching the desired end point. The integrity of vagi thus prepared was demonstrated by the presence of bradycardia on electrical stimulation and slowing of respiratory rate when the vagi were cooled.

The vagi were cooled until such time as conduction blockade could be demonstrated. This was usually done whilst animals were respiring spontaneously (i.e. before being connected to the ventilator). The vagi were rapidly cooled until (a) breathing became slow and deep, (b) histamine-induced respiratory responses (tachypnoea and bronchoconstriction) were abolished or reduced and (c) electrical stimulation through the 'cold block' no longer caused bradycardia. The vagi were subjected to this cooling regime for no more than 2 min at any one time before being rewarmed.

Once the temperature at which vagal blockade occurred was found, the animal was connected to the ventilator and all subsequent bronchoconstrictor responses to histamine (in the absence and presence of indomethacin) were determined during acute vagal cooling. It was usually found necessary to increase the dose of histamine so that responses similar to those observed before cooling (e.g. >50% increase in R_{aw}) could be obtained.

In some experiments the vagi of mechanically respired guinea-pigs were surgically sectioned bilaterally.

Drugs and statistics

Histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), acetylcholine chloride and indomethacin were obtained from Sigma Chemical Company. Leukotriene D_4 (LTD_4) was a gift from Dr J. Rokach, Merck Frosst Canada. Drugs were prepared in 0.9% w/v NaCl solution or distilled water, except for indomethacin which was dissolved in 100 mM Na_2CO_3 5 min before injection. Previous experiments demonstrated that Na_2CO_3 was without effect on the measured parameters (Mitchell, 1983).

Mean ± s.e. mean values were calculated and statistical comparisons were made by Student's t test for paired or unpaired data: $P < 0.05$ was taken as indicating a significant difference between means or between paired bronchoconstrictor responses.

Table 1 Effect of histamine, 5-hydroxytryptamine (5-HT) acetylcholine (ACh) and leukotriene D_4 (LTD_4) on airways resistance (R_{aw}) and dynamic compliance (C_{dyn})

	n	ΔR_{aw}	ΔC_{dyn}
Histamine ($3\,\mu g\,kg^{-1}$ i.v.)	4	73 ± 14	-34 ± 9
5-HT ($9\,\mu g\,kg^{-1}$ i.v.)	4	84 ± 12	-31 ± 8
ACh ($3\,\mu g\,kg^{-1}$ i.v.)	6	48 ± 10	-23 ± 3
LTD_4 ($0.3\,\mu g\,kg^{-1}$ i.v.)	3	47 ± 21	-29 ± 10

Data indicate the mean peak % change in R_{aw} and C_{dyn} in spontaneously respiring guinea-pigs; *n*, number of animals.

Results

Effects of indomethacin on bronchoconstrictor responses to histamine, acetylcholine, 5-HT and LTD_4

In spontaneously respiring guinea-pigs the resting R_{aw} was $0.20 \pm 0.04\,cmH_2O\,ml^{-1}\,s^{-1}$ and the C_{dyn} was $0.40 \pm 0.10\,ml\,cmH_2O^{-1}$. Doses of bronchoconstrictor drugs were selected to produce similar submaximal increases in R_{aw}, typically between 50–100%. The effects of histamine, 5-HT, acetylcholine and LTD_4 on R_{aw} and C_{dyn} are shown in Table 1.

Basal values of R_{aw} and C_{dyn} were unaltered by indomethacin ($1\,mg\,kg^{-1}$ i.v.). However, indomethacin almost doubled the effect of histamine on R_{aw} (Table 2) and the histamine-induced fall in C_{dyn} tended to be increased but the difference from control was not significant. Indomethacin also doubled the effect of 5-HT, but it did not alter responses to acetylcholine and responses to LTD_4 were reduced (Table 2).

Table 2 Effect of indomethacin on histamine, 5-hydroxytryptamine (5-HT), acetylcholine (ACh) and leukotriene D_4 (LTD_4)-induced bronchoconstrictor responses

	n	ΔR_{aw}	ΔC_{dyn}
Histamine ($3\,\mu g\,kg^{-1}$ i.v.)	4	79 ± 21**	23 ± 12
5-HT ($9\,\mu g\,kg^{-1}$ i.v.)	4	100 ± 16**	29 ± 17
ACh ($3\,\mu g\,kg^{-1}$ i.v.)	6	14 ± 12	13 ± 13
LTD_4 ($0.3\,\mu g\,kg^{-1}$ i.v.)	3	-31 ± 21	-35 ± 18

Data show % changes from control bronchoconstrictor responses (airways resistance, R_{aw} and dynamic compliance, C_{dyn}) induced by indomethacin pretreatment ($1\,mg\,kg^{-1}$ i.v.) in spontaneously respiring guinea-pigs; *n*, number of animals.
** $P < 0.02$, mean difference in paired responses (i.e. before and after indomethacin).

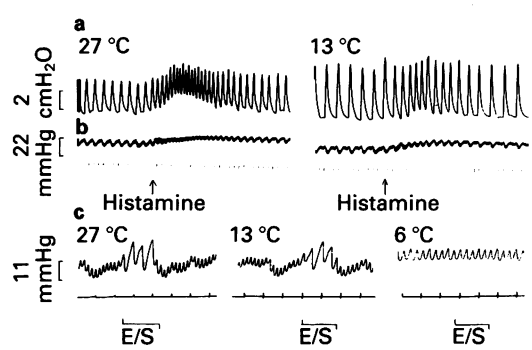

Figure 1 Traces from one experiment to investigate the effect of vagal cooling on respiration. Records are (a) intrapleural pressure, with inspiration shown upwards, (b) arterial blood pressure and (c) arterial blood pressure at increased amplification and chart speed. Timer marks are indicated in s. In both of the blood pressure records zero pressure (i.e. barometric pressure) is shown upwards. The respiratory records indicate the effect of histamine ($6\,\mu g\,kg^{-1}$ i.v.) at vagal temperatures of 27°C and 13°C. Respiratory frequency was reduced, and depth increased at 13°C and histamine-induced tachypnoea was reduced. The lower blood pressure records (c) show the bradycardia evoked by electrical stimulation of the efferent vagus (E/S: 5 V, 1 ms, 10 Hz). Efferent activity was unaffected by cooling to 13°C but it was blocked at temperatures <9°C.

Effect of vagal cooling

When the vagi were cooled to $\leqslant$16°C the breathing pattern altered so that respiration was slower and deeper. An example of the changes in respiration is shown in Figure 1. At the same temperatures the effects of histamine were reduced, noticeably the transient tachypnoea and the increase in R_{aw} (Figure 1). The effect of efferent electrical stimulation on the heart rate was not abolished, however, until lower temperatures were reached (typically 9°C). All parameters returned to normal on rewarming the vagi.

Effects of vagal stimulation, cooling and vagotomy in mechanically ventilated animals

The effect of indomethacin ($1\,mg\,kg^{-1}$ i.v.) on pulmonary responses to histamine ($3–6\,\mu g\,kg^{-1}$ i.v.) in mechanically ventilated animals is shown in Table 3. Indomethacin significantly increased the effect of histamine on both R_{aw} and C_{dyn}. Although the mean increase in histamine responses in mechanically ventilated animals was somewhat less (than the corresponding increase in spontaneously respiring guinea-pigs) this difference was not significant.

Table 3 Effect of indomethacin on histamine-induced bronchoconstrictor responses with intact, cooled or sectioned vagi

	Intact vagi		*Cooled vagi*		*Sectioned vagi*	
	R_{aw}	C_{dyn}	R_{aw}	C_{dyn}	R_{aw}	C_{dyn}
Basal	0.13 ± 0.01 (5)	0.85 ± 0.01 (5)	0.21 ± 0.09 (4)	0.88 ± 0.33 (4)	0.13 ± 0.02 (6)	0.82 ± 0.03 (6)
Δ Histamine response (%)	42 ± 15* (5)	19 ± 6* (5)	−5 ± 22 (4)	−4 ± 32 (4)	4 ± 23 (6)	7 ± 13 (6)

Data indicate basal values of airways resistance (R_{aw}) and dynamic compliance (C_{dyn}) ($cmH_2O\,ml^{-1}\,s^{-1}$ and $ml\,cmH_2O^{-1}$, respectively) and peak % changes in bronchoconstrictor responses to histamine (3–18 $\mu g\,kg^{-1}$ i.v. see Results) after indomethacin (1 $mg\,kg^{-1}$ i.v.). All experiments were performed in mechanically ventilated guinea-pigs. * $P < 0.05$, mean difference in paired responses (i.e. before and after indomethacin). Number of animals is shown in parentheses.

To investigate whether indomethacin interacted with peripheral vagal mechanisms the effect of indomethacin on changes in R_{aw} and C_{dyn} evoked by electrical stimulation was determined.

The right cut vagal trunk (with intact sheath) was placed on a stimulating electrode. A 10 s train of impulses (15 V, 1–20 Hz, 0.75 ms) increased R_{aw} and decreased C_{dyn}. In two experiments these effects of electrical stimulation were abolished by atropine (1 $mg\,kg^{-1}$ i.v.). The frequency-response relationship for vagal stimulation was, however, unaltered by indomethacin (Figure 2).

In a further experiment impulses of longer duration (5 ms) and greater intensity (60 V, 20 Hz) were delivered. There was an average 60% increase in R_{aw} and a 23% fall in C_{dyn} after electrical stimulation. In one experiment this bronchoconstrictor response was blocked by atropine. Paired analysis showed that the bronchoconstrictor responses to 5 ms pulses were little affected by indomethacin (22 ± 22% increase in R_{aw} response and a 5 ± 22% decrease in C_{dyn} response, $n = 4$, not significant).

In separate experiments the vagi were cooled or sectioned. In these experiments higher doses (6–18 $\mu g\,kg^{-1}$) of histamine were used in order to achieve bronchoconstrictor responses equivalent to those observed in animals whose vagi were neither cooled nor sectioned. When vagal conduction was thus interrupted indomethacin no longer had an effect on either R_{aw} or C_{dyn} responses elicited by histamine (Table 3).

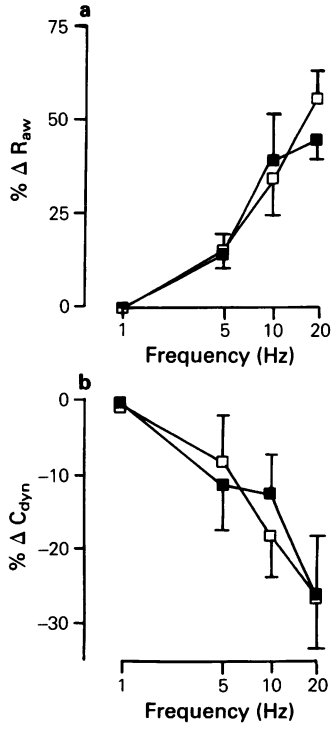

Figure 2 The effect of indomethacin (1 $mg\,kg^{-1}$ i.v.) on (a) the increased airway resistance (R_{aw}) and (b) the decreased dynamic compliance (C_{dyn}) induced by peripheral vagal electrical stimulation (0.75 ms, 15 V, 10 s). Each point is the mean of responses in four mechanically ventilated guinea-pigs, obtained before (□) and more than 10 min after (■) indomethacin. Vertical lines indicate s.e.mean.

Discussion

The purpose of this study was to determine whether the indomethacin-induced pulmonary hyperreactivity, previously described for histamine (increase in R_{aw} or C_{dyn} responses), extends to other spasmogens and whether hyperreactivity to histamine could be related to indirect mechanisms via altered autonomic reflex control of the airways. Hyperreactivity to histamine was confirmed in this

study and, moreover, a hyperreactivity to 5-HT was also shown. In contrast no such effect was apparent using equi-effective doses of acetylcholine or LTD_4. The observed reduction in the effect of LTD_4 was not unexpected. NSAIDs have been shown by several groups to reduce bronchoconstrictor responses to leukotrienes (e.g. Hedqvist *et al.*, 1983; Folco *et al.*, 1983), possibly because part of the bronchoconstrictor effect of these substances is due to thromboxane release. The difference between the results obtained with acetylcholine, compared with histamine and 5-HT, may be due to different physiological mechanisms activated by these drugs at the doses used; for example, in some species histamine and 5-HT are potent stimulators of 'irritant' receptors in the lung whereas acetylcholine may be less effective (Mills *et al.*, 1969; Mills & Widdicombe, 1970; Dixon *et al.*, 1979). Furthermore, non-steroidal anti-inflammatory drugs potentiate smooth muscle contraction to histamine and 5-HT to a greater extent than contraction to acetylcholine, suggesting that mobilization of eicosanoids may differ with these agents (Orehek *et al.*, 1975). Because the effect of indomethacin seen in the present experiments was relatively selective it may be argued that the hyperreactivity to indomethacin is not due to some non-specific effect such as increased pulmonary perfusion.

NSAIDs cause marked increases in tracheal reactivity *in vitro* in the guinea-pig (Orehek *et al.*, 1975). No clear evidence was found, however, for a direct smooth muscle effect of indomethacin (i.e. increased reactivity up to 45 min post-indomethacin) in vagotomized animals or after ablation of vagal activity by cooling to 9°C, conditions where the bronchoconstrictor effects of histamine were largely due to direct effects on the airways smooth muscle; although evidence for a non-vagal innervation has been presented (Lundberg *et al.*, 1983). It was important to ensure that the different effect of indomethacin in animals with intact and interrupted vagi was not due to the differences in respiratory patterns in the two groups of animals. Vagal cooling causes a decline in respiratory frequency and an increase in tidal volume (Figure 1; see Widdicombe, 1974 for review). Moreover, respiratory rate changes associated with drug injection (i.e. histamine) are attenuated by vagotomy which, in turn, might alter the indomethacin effect in this group of animals. For these reasons the experiments were carried out in mechanically ventilated animals so that key respiratory parameters (frequency and volume) could be held constant. The effect of indomethacin tended to be smaller in ventilated animals (with intact vagi), compared to that in the spontaneously respiring animals, possibly because of the increased tidal volume induced when guinea-pigs were placed on the pump. It appears, therefore, that effects of indomethacin on tracheal smooth muscle reactivity seen *in vitro* are less well expressed *in vivo* and that the hyperreactivity consistently observed in the guinea-pig *in vivo* may be of reflex origin.

The data indicate that the acute (10–45 min) hyperreactivity following indomethacin is, at least in part, reflexly mediated. Under the present conditions of anaesthesia the histamine-induced bronchoconstriction was partly reflex (e.g. cholinergic), as evidenced by reduced histamine effects after atropine and after vagotomy or vagal cooling (Mitchell, 1988). Recently, interest has also focused on substance P (Lundberg *et al.*, 1983) and calcitonin gene-related peptide (Palmer *et al.*, 1987) in airway neural regulation. Although these putative neurotransmitters are thought to be principally associated with 'axon reflexes' (i.e. reflexes restricted to the lung in this context), it is possible that their release could be enhanced via some full vagal loop mechanism. Thus there appear to be several potential points of interaction between histamine and indomethacin which could lead to hyperreactivity. No evidence was found in these studies, however, to indicate that indomethacin enhances excitatory cholinergic neurotransmitter mechanisms, as may occur in the dog (Ito & Tajima, 1981). There was no potentiation of cholinergic bronchoconstrictor responses using short pulses, over a range of frequencies of stimulation. Moreover, responses to intravenous acetylcholine were not affected by indomethacin, suggesting that postjunctional changes do not occur. Furthermore, bronchoconstriction following electrical stimulation with 5 ms pulses shown previously to elicit atropine-resistant responses – possibly via tachykinin release (Martling *et al.*, 1984) – was not significantly altered by indomethacin. The atropine-resistance of the response to 5 ms pulses was not readily apparent in this study. Care was taken in these experiments to ensure that vagal and sympathetic trunks were stimulated, in case indomethacin interacted with sympathetic innervation (McCullock *et al.*, 1967; McCaig, 1986) either of smooth muscle or prejunctionally to modify cholinergic activity (Cabezas *et al.*, 1971). The absence of an apparent increase in bronchoconstriction to electrical stimulation does not, however, preclude a possible action of indomethacin to sensitize physiological (i.e. reflexly evoked) release by histamine of excitatory neurotransmitter. For instance it does not exclude a possible effect of indomethacin on afferent receptors resulting in amplified reflex constriction. The possibility that 'aspirin-intolerance' may also be associated with reflex control of the airway could merit consideration, particularly since this condition may be prevented by exposure to sodium cromoglycate (Basomba *et al.*, 1976) in con-

centrations similar to those shown to inhibit vagal activity in animals (Jackson & Richards, 1977; Dixon *et al.*, 1980).

These results suggest that the acute hyperreactivity following indomethacin involves an interaction between indomethacin and vagal mechanisms which are presumably activated by histamine and 5-HT. Further investigations may reveal whether this interaction is at an afferent terminal.

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