Br. J. Pharmac. (1974), 52, 559-565

ANTAGONISM OF TONE AND PROSTAGLANDIN-MEDIATED RESPONSES IN A TRACHEAL PREPARATION BY INDOMETHACIN AND SC-19220

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1 The effects of the prostaglandin synthetase inhibitor, indomethacin and the prostaglandin antagonist SC-19220 (1-acetyl-2-[8-chloro-10,11-dihydrodibenz (b,f) (1,4)oxazepine-10-carbonyl] hydrazine), were examined on the tone of the guinea-pig isolated tracheal preparation and on the responses of the preparation to prostaglandin $F_{2\alpha}$, arachidonic acid and methacholine.

2 Indomethacin (0.05-1.6 μg/ml) produced a long-lasting inhibition of the intrinsic tone of the tracheal preparation and of the contractile responses to arachidonic acid. Much higher concentrations of indomethacin also reduced the responses of the preparation to methacholine. This effect was readily reversible and appeared to be unrelated to the action on tone.

3 The contractile responses of the preparation to prostaglandin $F_{2\alpha}$ were enhanced by low concentrations of indomethacin (1-5 μg/ml) and inhibited by higher concentrations (2.5-80 μg/ml).

4 SC-19220 was shown to inhibit responses of the preparation to prostaglandin $F_{2\alpha}$ in concentrations (0.1-1μg/ml) which had no effect on responses to methacholine. Similar concentrations also inhibited the intrinsic tone of the preparation and the responses to arachidonic acid.

5 The evidence suggests that prostaglandins may be involved in the maintenance of tone of the guinea-pig isolated tracheal preparation.

Introduction

Isolated preparations of guinea-pig trachea exhibit intrinsic tone which is reversibly inhibited by smooth muscle relaxant drugs (Castillo & de Beer, 1947; Foster, 1966). Farmer & Coleman have described an isolated intact tracheal preparation in which tone is increased following periodic relaxation of the preparation with isoprenaline (Farmer & Coleman, 1970; Coleman & Farmer, 1971). In the present study this preparation has been used to investigate the mechanism by which intrinsic tone is maintained in tracheal smooth muscle. Recent studies with isolated gastrointestinal smooth muscle preparations have suggested a possible role of prostaglandins in the production of tone (Ferreira, Herman & Vane, 1972; Willis, Davison & Ramwell, 1974). The effects of a prostaglandin synthetase inhibitor, indomethacin (Vane, 1971), and a prostaglandin antagonist, SC-19220 (1-acetyl-2-[8-chloro-10,11-dihydrodibenz (b,f) (1,4)oxazepine-10-carbonyl] hydrazine) (Sanner, 1969) have therefore been examined on the tracheal preparation with a view to elucidating a possible role of prostaglandins in the maintenance of tone.

A preliminary account of this work was given to the British Pharmacological Society (Farmer, Farrar & Wilson, 1972).

Methods

Guinea-pig tracheal tubes were prepared as described by Farmer & Coleman (1970). The preparations were immersed in a modified Tyrode solution of the following composition (g/litre); NaCl, 8.0; $NaHCO_3$, 1.0; NaH_2PO_4, 0.32; glucose, 1.0; $MgCl_2$, 0.42; KCl, 0.2; $CaCl_2$, 0.1. The solution was maintained at 37° C and gassed with room air. The intraluminal pressure of the preparation was measured with a Statham P23 BB

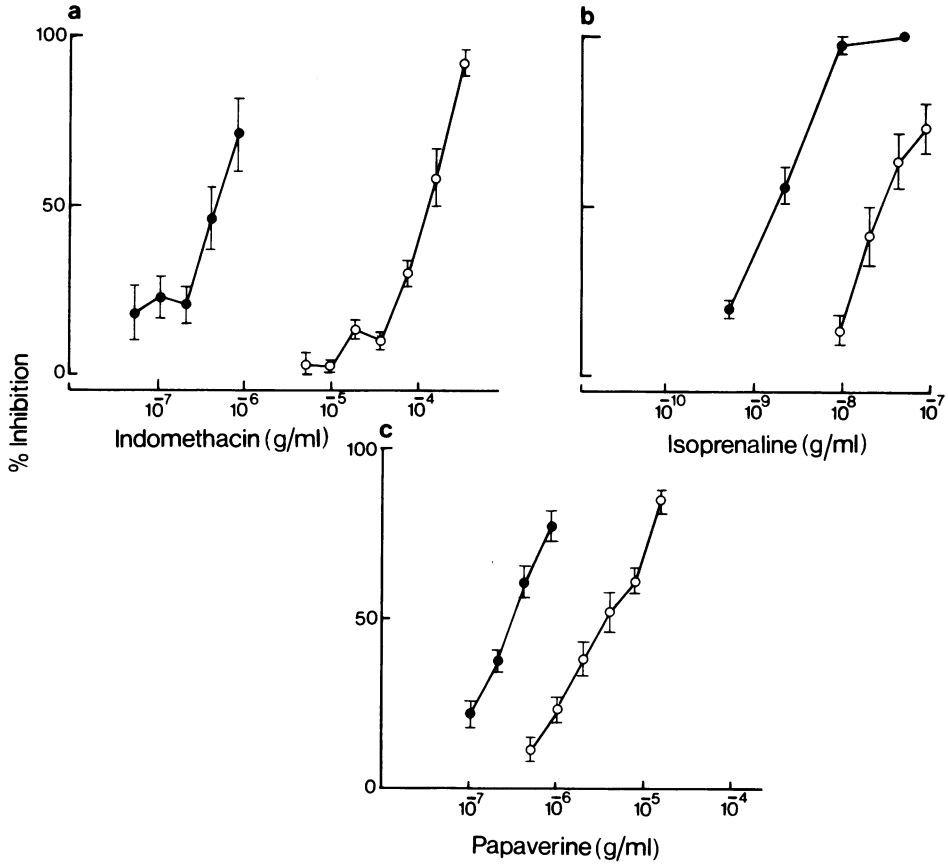

Fig. 1 Inhibition of the intrinsic tone (•) of the guinea-pig tracheal tube and of responses of the preparation to methacholine (○) by (a) indomethacin, (b) isoprenaline and (c) papaverine. The dose of methacholine was adjusted in each preparation to produce responses between 60-80% of the maximum. Each point represents the mean of at least six determinations and the vertical lines indicate s.e.

pressure transducer and recorded on a Devices M19 recorder. Drugs were added to the bathing fluid.

When the effects of relaxant drugs were studied, the tone of the preparation was raised as described by Coleman & Farmer (1971). In this procedure a maximal relaxation of the preparation is produced by the addition of isoprenaline and the lumen of the trachea is then opened to atmospheric pressure. When the lumen is closed off and the isoprenaline removed from the bath, the intraluminal pressure rises to a higher level than before the addition of isoprenaline. By repeating the procedure several times, a resting pressure of between 5 and 25 mmHg (1 mmHg = 1.333 mbar) is obtained. Potencies of smooth muscle relaxants are expressed as the doses required to produce 50% inhibition (IC_{50}) either of the tone of the preparation or of contractile responses to such agonists as methacholine.

Drugs used were 1-acetyl-2-[8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl]-hydrazine, SC-19220 (Searle), arachidonic acid (Sigma), indomethacin (Merck, Sharp & Dohme), isoprenaline hydrochloride (Pharmax), methacholine chloride (Koch-Light), papaverine (BDH) and prostaglandin $F_{2\alpha}$, (Cambrian). Arachidonic acid, indomethacin, prostaglandin $F_{2\alpha}$ and SC-19220 were initially dissolved in ethanol, further dilutions being made in Tyrode solution. Papaverine was dissolved in 0.1 N HCl solution and adjusted to pH 6.5 with NaOH. Solutions of isoprenaline were diluted with 0.9% w/v NaCl solution (saline) containing 0.1% sodium metabisulphite. Other drugs were dissolved in Tyrode solution.

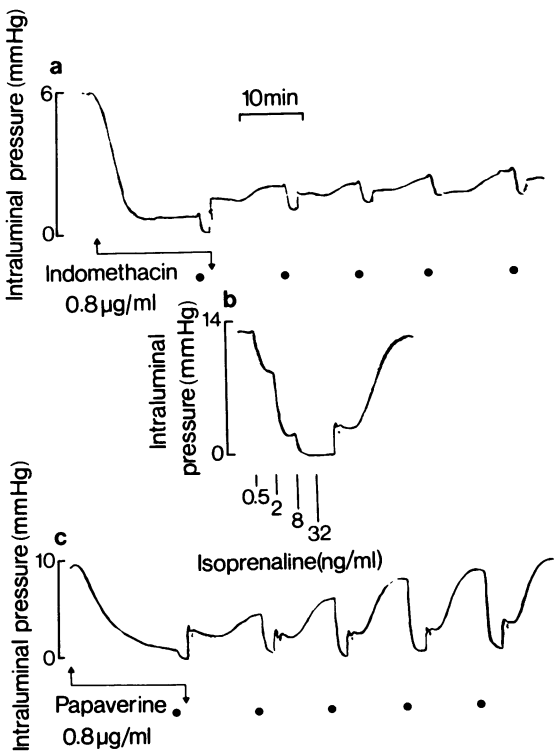

Fig. 2 The effects of (a) indomethacin, (b) isoprenaline and (c) papaverine on the intrinsic tone of the guinea-pig tracheal tube preparation. Isoprenaline, 20 ng/ml (•) was added periodically to obtain maximal relaxation of the preparation.

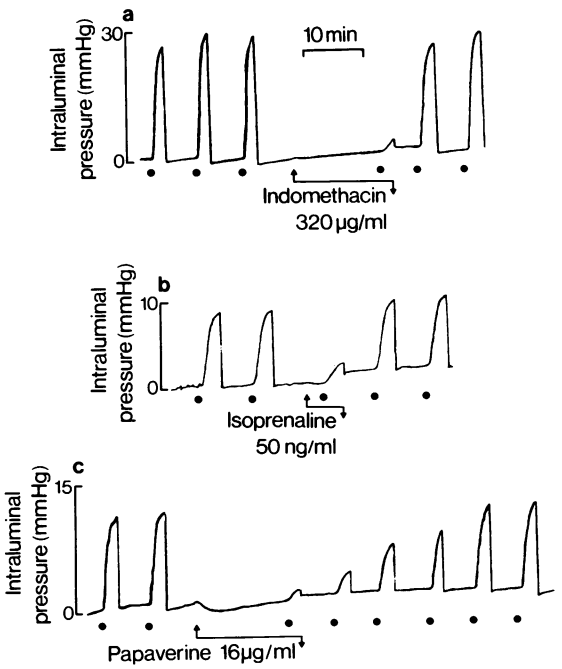

Fig. 3 The effects of (a) indomethacin, (b) isoprenaline and (c) papaverine on the response of the guinea-pig tracheal tube preparation to methacholine (•). The dose of methacholine was adjusted in each preparation to produce responses between 60-80% of the maximum.

Results

Effects of indomethacin on tracheal tone

Indomethacin (0.05-1.6 μg/ml) produced a dose-dependent relaxation of the guinea-pig tracheal tube preparation (Figure 1a). The effect was very persistent, and could not be reversed by frequent changes of bath fluid over a period of several hours (Figure 2a). The specificity of this action was examined by comparing it with the effect of indomethacin on contractions of the preparation produced by methacholine (Figures 3a & 4). At 10 μg/ml indomethacin had no effect on the dose-response curve to this agonist. However, at 100 μg/ml, indomethacin shifted the dose-response curve to the right and depressed the maximum (Figure 4). The IC_{50} of indomethacin against methacholine was 121 μg/ml, this being approximately 270 times greater that the IC_{50} for the inhibition of tracheal tone (Figure 1a). In contrast to the persistent effects of the drug on the tone of the preparation, the inhibitory effect of indomethacin on contractions of the preparation to methacholine was readily reversed by washing (Figure 3a). These effects of indomethacin were compared with those of two compounds known to relax tracheal smooth muscle, isoprenaline and papaverine. These drugs inhibited the tone of the preparation (Fig. 2) and inhibited methacholine-induced contractions of the preparation (Figure 3). As with indomethacin, they were more potent as inhibitors of the tone of the preparation. However, these drugs were only approximately 15 times more active against tone, than they were against contractions produced by methacholine, compared with the 270-fold difference seen with indomethacin, (Figure 1). The inhibitory effects of papaverine were more persistent than those of isoprenaline, but these effects could be reversed by washing over a period of about one hour

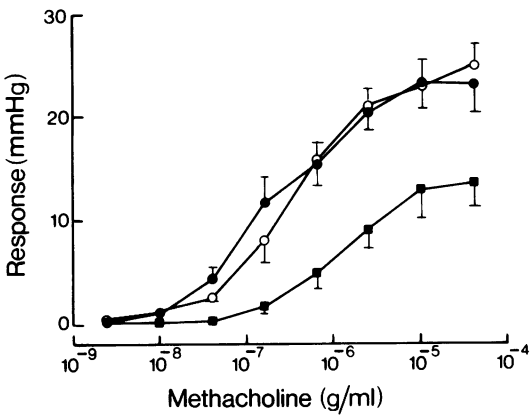

Fig. 4 The effects of indomethacin on dose-response curves to methacholine obtained on the guinea-pig tracheal tube preparation. (●—●) Control; (○—○) indomethacin 10 μg/ml; (■—■) indomethacin 100 μg/ml. Each point represents the mean of eight determinations and the vertical lines indicate s.e.

Effects of indomethacin on responses to prostaglandin $F_{2\alpha}$ and arachidonic acid

Prostaglandin $F_{2\alpha}$ (0.1-3.2 μg/ml) produced a contraction of the preparation. With repeated administration of a dose of prostaglandin $F_{2\alpha}$, the response declined and this tachyphylaxis could not be prevented by prolonging the interval between doses, or by repeatedly changing the bathing fluid between responses. However, after approximately eight doses the responses became constant and the effect of indomethacin on this residual response was studied. Indomethacin (1-5 μg/ml) when first given to a preparation caused a persistent potentiation of the response to prostaglandin $F_{2\alpha}$ (Figure 5a). A similar, but less pronounced potentiation was observed when methacholine was used as the agonist (Figure 5b). Further doses of indomethacin (1-5 μg/ml) either had no effect or caused inhibition of the response to prostaglandin $F_{2\alpha}$. The inhibition was dose-related over the range 2.5-80 μg/ml, with a mean IC_{50} of 13 μg/ml and was easily reversed by washing (Figure 5c).

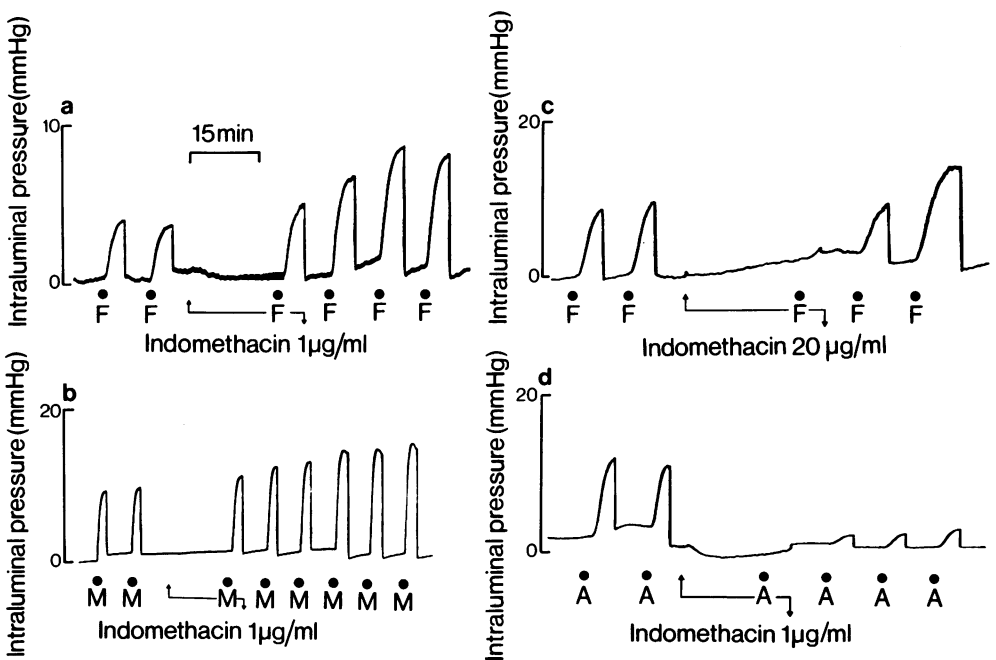

Fig. 5 The potentiating effect of indomethacin (1 μg/ml) on the responses of the guinea-pig tracheal tube preparation to (a) prostaglandin $F_{2\alpha}$, (1 μg/ml) (F) and (b) methacholine, (0.2 μg/ml) (M) and the inhibitory effects of indomethacin on responses to (c) prostaglandin $F_{2\alpha}$, (1 μg/ml) (F) and (d) arachidonic acid (1 μg/ml) (A).

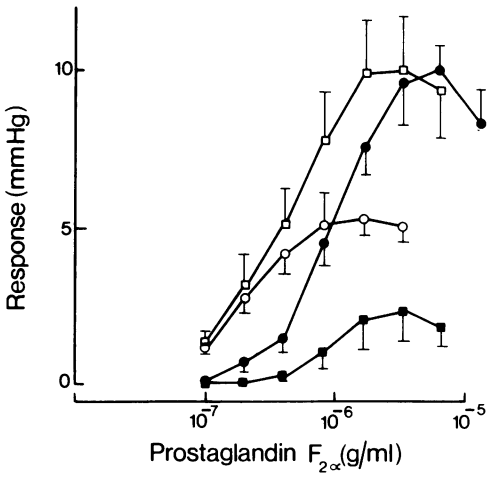

Fig. 6 The effects of indomethacin on the dose-response curve to prostaglandin $F_{2\alpha}$ obtained on the guinea-pig tracheal tube. Control (○—○); indomethacin 1 μg/ml (□—□); 10 μg/ml (●—●); 100 μg/ml (■—■). Each point represents the mean of twelve determinations and the vertical lines indicate s.e.

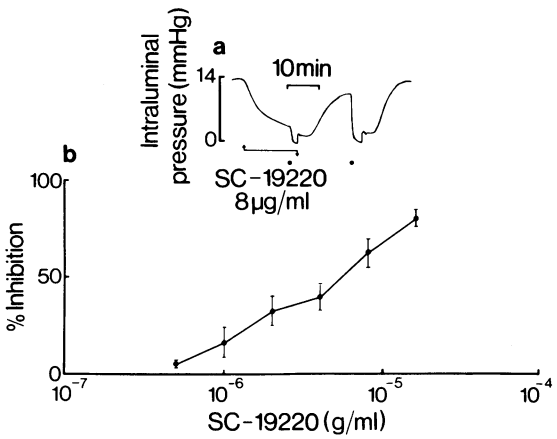

Fig. 7 The effect of SC-19220 on the intrinsic tone of the guinea-pig tracheal tube preparation. (a) Experimental record showing the response to SC-19220, 8 μg/ml. Isoprenaline 20 ng/ml (●) was added periodically (b) Dose-response curve to SC-19220. Each point represents the mean of eight determinations and the vertical lines indicate s.e.

Dose-response curves to prostaglandin $F_{2\alpha}$ (0.1-3.2 μg/ml) in the absence of indomethacin and in the presence of 1, 10 and 100 μg/ml of indomethacin are shown in Figure 6. At 1 μg/ml, indomethacin had no effect on the lower doses of prostaglandin $F_{2\alpha}$ (0.1-0.4 μg/ml), while doses greater than 0.4 μg/ml were significantly potentiated, the maximal response being increased by approximately 100%. In the presence of 10 μg/ml indomethacin, responses to prostaglandin $F_{2\alpha}$ in doses up to 0.8 μg/ml were significantly inhibited, whereas responses to doses greater than 0.8 μg/ml were significantly potentiated. Indomethacin, 100 μg/ml significantly inhibited responses to all doses of prostaglandin $F_{2\alpha}$ and caused a significant depression of the maximal response.

Arachidonic acid (0.1-10 μg/ml) produced contractions of the tracheal tube. Figure 5d shows the inhibitory effect of indomethacin (1 μg/ml) on responses to arachidonic acid (1 μg/ml). In six preparations the responses were reduced by 82.1 ± 4.8% (mean ± s.e.) and this effect persisted for some hours after the removal of indomethacin from the bath fluid.

Effects of SC-19220 on the tone of the preparation and on the responses to prostaglandin $F_{2\alpha}$, arachidonic acid and methacholine

SC-19220 (0.5-16 μg/ml) caused a gradual relaxation of the preparation (Fig. 7a) which could be reversed by washing. This effect was dose-dependent (Fig. 7b), with a mean IC_{50} of 5.4 μg/ml. Inhibition of tracheal tone by SC-19220 was compared with its activity as a prostaglandin antagonist. SC-19220 (0.1 and 1 μg/ml) caused a significant parallel shift of the dose-response curve to prostaglandin $F_{2\alpha}$ to the right (Figure 8a). There was no change in the maximal response to prostaglandin $F_{2\alpha}$. The effect of SC-19220 on responses of the preparation to arachidonic acid was also examined. SC-19220 (1 μg/ml) significantly inhibited the response to arachidonic acid (Fig. 8b), although the shift to the right caused by this dose was not as great as that observed on responses to prostaglandin $F_{2\alpha}$. A greater inhibitory effect was obtained with 10 μg/ml SC-19220 and in the presence of this concentration, arachidonic acid (0.1 μg/ml) produced relaxations in five out of eight preparations. The specificity of these effects was examined by comparing them with the effect of SC-19220 on the dose-response curve to methacholine (Figure 8c). At 0.1-1 μg/ml, SC-19220 had no significant effect on the responses to methacholine although at 10 μg/ml there was a significant shift to the right in the dose-response curve to methacholine.

Discussion

Indomethacin, in a concentration range of 0.05 to 1.6 μg/ml produced a dose-related inhibition of

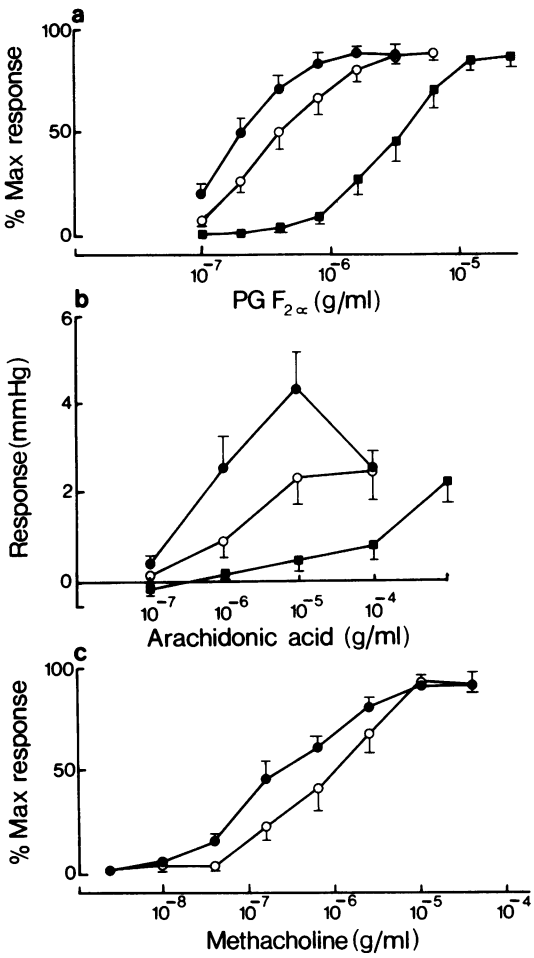

Fig. 8 The effects of SC-19220 on responses of the guinea-pig tracheal tube preparation to (a) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (b) arachidonic acid and (c) methacholine. (a) Control (•—•); SC-19220 0.1 μg/ml (○—○); 1.0 μg/ml (▪—▪). (b) Control (•—•); SC-19220 1 μg/ml (○—○); 10 μg/ml (▪—▪). (c) Control (•—•); SC-19220 10 μg/ml (○—○). Each point represents the mean of at least eight determinations and the vertical lines indicate s.e.

the tone of the guinea-pig isolated trachea. This concentration range was very similar to that shown by Vane (1971) to inhibit the synthesis of prostaglandins in guinea-pig lung homogenate. This suggests that the tone in the tracheal tube may be generated by the release of prostaglandins and that indomethacin is relaxing the preparation by inhibition of their synthesis. However, Northover (1967) reported a non-specific smooth muscle inhibitory action of indomethacin. The effects of indomethacin on the preparation were therefore compared with those of two other drugs which inhibit tracheal smooth muscle, isoprenaline and papaverine. These drugs have differing modes of action, but in each case the inhibitory effect on responses to spasmogens, such as methacholine, is directly related to their smooth muscle relaxant activity. For each of the drugs, the concentration necessary to inhibit responses to methacholine was almost 15 times higher than to produce a relaxation of the preparation. However, the concentration of indomethacin required to inhibit methacholine-induced contractions was some 270 times greater than that which inhibited intrinsic tone. The experiments also demonstrated a qualitative difference between these two actions of indomethacin. Whereas the inhibition of tracheal tone by indomethacin was almost irreversible, the inhibition of responses to methacholine was readily reversed on changing the bathing fluid. It thus seems unlikely that the reduction of tone by indomethacin could be explained by its non-specific inhibitory effect.

Arachidonic acid produced contractions of the tracheal tube which were markedly inhibited by indomethacin. It has been shown that the major products after incubation of arachidonic acid with homogenates of guinea-pig lung were prostaglandins $F_{2\alpha}$ and E_2 (Änggård & Samuelsson, 1965). Other intermediate products from the action of prostaglandin synthetase on arachidonic acid have also been described (Nugteren & Hazelhof, 1973). The predominant effect of prostaglandin E_2 on this preparation is a relaxation (dose range 1-100 ng/ml), although the relaxation is often preceded by a transient contraction (Farrar, unpublished observation). It therefore seems likely that the contraction observed with arachidonic acid is not due to prostaglandin E_2, but to $F_{2\alpha}$, which we have shown to cause a sustained contraction of the preparation. The concentrations of indomethacin which blocked the contractions to arachidonic acid were below the lowest concentration required to inhibit responses to both methacholine and prostaglandin $F_{2\alpha}$. It would appear, therefore, that the effect of indomethacin at this low concentration is a specific inhibitory effect on prostaglandin synthetase.

Further evidence that the tone of the tracheal tube is generated by the release of prostaglandins was obtained with the prostaglandin antagonist SC-19220. This compound has been shown to produce a specific inhibition of contractile responses to prostaglandins E_2 and $F_{2\alpha}$ in a variety of smooth muscle preparations (Sanner, 1969; Bennett & Posner, 1971). SC-19220 reduced the responses of the tracheal tube to prostaglandin $F_{2\alpha}$

in a concentration range which had no effect on responses to methacholine. SC-19220 also inhibited responses of the preparation to arachidonic acid, although it was less potent in this respect than as an antagonist of exogenous prostaglandins. The tone of the preparation was abolished by SC-19220 in concentrations similar to those required to inhibit responses to arachidonic acid.

In a concentration range of 2.5 to 80.0 μg/ml indomethacin also inhibited responses to prostaglandin $F_{2\alpha}$ and as with the inhibition of methacholine-induced responses, this effect was rapidly reversed by changing the bath-fluid. Indomethacin appeared to show some specificity against prostaglandin $F_{2\alpha}$, being about ten times more potent than against methacholine. Sorrentino, Capasso & Di Rosa (1972), have shown that, on the rat uterus and guinea-pig ileum, indomethacin was more active in inhibiting the contractile response to prostaglandin E_2 than to histamine, 5-hydroxytryptamine or bradykinin. The concentrations of indomethacin necessary to reduce responses of the trachea to prostaglandin $F_{2\alpha}$ were higher than those required to inhibit the tone of the preparation and there is unlikely to be a causal relationship between the two effects. On the contrary, doses of indomethacin which specifically inhibited tracheal tone appeared to potentiate both submaximal and maximal responses to prostaglandin $F_{2\alpha}$. Similar enhanced responses to prostaglandin $F_{2\alpha}$ following indomethacin have recently been reported by Puglisi (1973). The mechanism of this potentiation is uncertain, but a possible mechanism suggested by Puglisi was an effect on Ca^{++} movements within the cell. Evidence for an interaction between Ca^{++} and indomethacin was put forward by Northover (1971) and there could be a common mechanism linking the various actions of indomethacin.

We are grateful to G.D. Searle & Co. Ltd., for a sample of SC-19220 and to Professor G.D.H. Leach for helpful discussion of the work.

References

ÄNGGÅRD, E. & SAMUELSSON, B. (1965). Biosynthesis of prostaglandins from arachidonic acid in guinea-pig lung. *J. Biol. Chem.*, **240**, 3518-3521.

BENNETT, A. & POSNER, J. (1971). Studies on prostaglandin antagonists. *Br. J. Pharmac.*, **42**, 584-594.

CASTILLO, J.C. & DE BEER, E.J. (1947). The tracheal chain. 1. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharmac. exp. Ther.*, **90**, 104-109.

COLEMAN, R.A. & FARMER, J.B. (1971). The inducement of tone and its inhibition in isolated tracheal muscle. *J. Pharm. Pharmac.*, **23**, 220-222.

FARMER, J.B. & COLEMAN, R.A. (1970). A new preparation of the isolated intact trachea of the guinea-pig. *J. Pharm. Pharmac.*, **22**, 46-50.

FARMER, J.B., FARRAR, D.G. & WILSON, J. (1972). The effect of indomethacin on the tracheal smooth muscle of the guinea-pig. *Br. J. Pharmac.*, **46**, 536-537P.

FERREIRA, S.H., HERMAN, A. & VANE, J.R. (1972). Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. *Br. J. Pharmac.*, **46**, 328-330P.

FOSTER, R.W. (1966). The nature of the adrenergic receptors of the trachea of the guinea-pig. *J. Pharm. Pharmac.*, **18**, 1-12.

NORTHOVER, B.J. (1967). The effect of anti-inflammatory drugs on vascular smooth muscle. *Br. J. Pharmac. Chemother.*, **31**, 483-493.

NORTHOVER, B.J. (1971). Mechanism of the inhibitory action of indomethacin on smooth muscle. *Br. J. Pharmac.*, **41**, 540-551.

NUGTEREN, D.H. & HAZELHOF, E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim. biophys. Acta*, **326**, 448-461.

PUGLISI, L. (1973). Opposite effects of prostaglandin E and F on tracheal smooth muscle and their interaction with calcium ions. In: *Advances in the Biosciences*, Vol. 9, International Conference on Prostaglandins, Vienna, 1972, ed. Bergstrom, S. pp. 219-227. Oxford: Pergamon Press.

SANNER, J.H. (1969). Antagonism of prostaglandin E_2 by 1-acetyl-2-[8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl] hydrazine (SC-19220). *Archs. int. pharmacodyn. Ther.*, **180**, 46-56.

SORRENTINO, L., CAPASSO, F. & DI ROSA, M. (1972). Indomethacin and prostaglandins. *Eur. J. Pharmac.*, **17**, 306-308.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232-235.

WILLIS, A.L., DAVISON, P. & RAMWELL, P.W. (1974). Inhibition of intestinal tone, motility and prostaglandin biosynthesis by 5, 8, 11, 14-eicosatetraynoic acid. *Prostaglandins*, **5**, 355-368.

(Received May 22, 1974)