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Cyclo-oxygenase inhibitors antagonize indirectly evoked contractions of the guinea-pig isolated ileum by inhibiting acetylcholine release

David T. Okpako & Yetunde O.O. Taiwo

Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria

1 The effects of indomethacin, sodium meclofenamate and ketoprofen on the contractile responses of the guinea-pig isolated ileum to directly and indirectly evoked stimuli were investigated. The effects of the cyclo-oxygenase inhibitors on acetylcholine (ACh) release from plexus containing longitudinal muscle strips were also studied.

2 The cyclo-oxygenase inhibitors reduced contractile responses to transmural stimulation (TMS) and nicotine at concentrations which had no effect on ACh-induced contractions.

3 In whole ileum preparations (WIP) indomethacin and ketoprofen ($40\,\mu g\,ml^{-1}$) reduced TMS responses by $17 \pm 1.8\%$ and $12 \pm 1.8\%$ ($n = 6$), respectively (30 min incubation). In longitudinal muscle strips (LMS) in which Auerbach's plexus is exposed, indomethacin and ketoprofen ($1\,\mu g\,ml^{-1}$) reduced TMS responses by $28 \pm 2.3\%$ and $34 \pm 2.7\%$ ($n = 6$), respectively (10 min incubation). Thus the cyclo-oxygenase inhibitors were up to 80 times more effective in LMS than in WIP. The drugs were similarly more effective in blocking nicotine contractions in LMS than in WIP.

4 The cyclo-oxygenase inhibitors reduced basal and stimulated ACh release from LMS. For example, indomethacin ($1\,\mu g\,ml^{-1}$) reduced stimulated ACh release by 35% after 10 min incubation. The percentage inhibition increased to 79% after 40 min incubation ($n = 6$).

5 Prostaglandin E_2 (PGE_2) ($0.1–2.5\,ng\,ml^{-1}$) restored the contractile responses and ACh release depressed by the cyclo-oxygenase inhibitors but not the contractile responses depressed by atropine. $PGF_{2\alpha}$ had no effect on mechanical responses or ACh release depressed by the cyclo-oxygenase inhibitors.

6 It is concluded that the cyclo-oxygenase inhibitors studied reduced responses to transmural stimulation and nicotine by inhibiting ACh release. The site of action is the postganglionic parasympathetic nerve.

7 It is suggested that the reason why previous investigators needed to use high doses of cyclo-oxygenase inhibitor in the ileum is because the action of the inhibitor is limited by diffusion barriers. There was no evidence to support the view that there is more than one pool of cyclo-oxygenase in guinea-pig gut.

Introduction

Wennmalm & Hedqvist (1971) showed that prostaglandin E_1 (PGE_1) antagonized the negative chronotropic responses to vagus nerve stimulation in rabbit hearts. PGE_1 had no effect on responses to infused acetylcholine (ACh). This and other observations led the authors to conclude that prostaglandins of the E series were involved in a negative feed back regulation of parasympathetic nerve transmitter release, in a manner similar to that proposed for the prostaglandins in sympathetic nerves (Euler & Hedqvist, 1969). However, results of subsequent experiments on the effects of indomethacin and other cyclo-oxygenase inhibitors on indirectly evoked contractions of isolated guinea-pig ileum have not upheld the hypothesis that prostaglandins inhibit transmitter release from parasympathetic nerves. Ehrenpreis *et al.* (1973) found that indomethacin ($40\,\mu g\,ml^{-1}$) and aspirin ($200\,\mu g\,ml^{-1}$) inhibited electrically evoked contractions of the guinea-pig isolated ileum. Prostaglandin E_1 restored the contractions to electrical stimulation. The authors concluded that prostaglandins facilitate cholinergic transmission by a mechan-

ism involving their ability to couple nerve terminal excitation to a transmitter release mechanism. Other studies have generally indicated a facilitatory, rather than inhibitory role for prostaglandins in cholinergic transmission in the guinea-pig isolated ileum. Thus various studies show that indirectly evoked contractions of the guinea-pig ileum such as those induced by nicotine, angiotensin or transmural stimulation are inhibited by cyclo-oxygenase inhibitors and the inhibited responses are restored by low concentrations of prostaglandins (Chong & Downing, 1973; Bennett *et al.*, 1975a; Sokunbi, 1979).

It is difficult to attribute a physiological role to prostaglandins as regulators of transmitter release on the basis of these experiments. Firstly, the concentrations of the cyclo-oxygenase inhibitors used were usually high so that pharmacological effects other than cyclo-oxygenase inhibition were possible (Kadlec *et al.*, 1974). Furthermore, where results have suggested a facilitatory function, it was not certain whether the effect of the prostaglandin in restoring responses depressed by indomethacin was exerted postjunctionally on the smooth muscle or prejunctionally on ACh release.

To explain the need for high concentrations of inhibitor, Bennett *et al.* (1975b) proposed the existence of two pools of cyclo-oxygenase with different susceptibilities to blockade by indomethacin: an extraneuronal pool sensitive to low concentrations of indomethacin which mediates maintenance of muscle tone, and a cholinergic neuronal pool inhibited only by relatively high concentrations of indomethacin and which regulates transmitter release. An alternative view which is explored in these experiments is that the effectiveness of the inhibitors on 'neuronal' cyclo-oxygenase is limited by diffusion barriers. Therefore, in this study we have used whole ileum preparations and longitudinal muscle strips in which Auerbach's plexus is exposed, for studies on the effect of cyclo-oxygenase inhibitors on indirectly evoked contractions and ACh release. Some of the results of these studies have been presented to the British Pharmacological Society (Sokunbi, 1979; 1980).

Methods

Whole ileum preparations (WIP)

Guinea-pigs of either sex weighing 300–400 g were stunned by a blow on the head and exsanguinated. A length of ileum was removed, the region 5–10 cm nearest the ileocaecal junction being discarded. The lumen of the ileum was flushed with 25 ml of physiological salt solution. Pieces of ileum, 2–3 cm long were then set up in 15 ml organ baths containing either Tyrode solution of the following composition (mM): NaCl 138, Na_2CO_3 1.5, KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $MgCl_2$ 1.1 and glucose 5.6 or Krebs solution as modified by Kosterlitz *et al.* (1970) of the following composition (mM): NaCl 4.75, Na_2CO_3 25, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.2 and glucose 11. Tyrode solution was gassed with air; Krebs solution was gassed with 5% CO_2 in oxygen. The solution was maintained at 37°C. For transmural stimulation, the ileum was set up between pairs of perspex-shielded platinum ring electrodes connected to a Grass stimulator model S44. Isometric contractions were recorded with Bell & Howell strain gauges on a Devices recorder (Model MX 212P Ormed Ltd) from a baseline load of 1 g. Tissues were stimulated with supramaximal voltage of approximately 40 V and 0.5 ms pulse width at varying frequencies.

Longitudinal muscle strip preparations (LMS)

Guinea-pigs of either sex weighing 400–500 g were used. Plexus-containing strips of ileum were obtained according to the method described by Paton & Zar (1968). The longitudinal muscle strips were set up in 4 ml baths containing modified Krebs solution gassed with 5% CO_2 in oxygen at 37°C. For determination of ACh release, two ends of a strip were tied together to give a double strip approximately 5 cm long. The Krebs solution contained choline chloride (20 $\mu g\,ml^{-1}$) and physostigmine (2 $\mu g\,ml^{-1}$). The preparation was allowed 90 min to equilibrate in physostigmine before the start of the experiment. Samples for assay were removed every 10 min.

Acetylcholine assay

ACh was assayed as described by Paton & Zar (1968) on guinea-pig isolated ileum. The ileum was kept in eserinized Krebs solution for 90 min at 4°C before setting it up. This increased ileal sensitivity to ACh. The assay solution contained 10 $\mu g\,ml^{-1}$ morphine hydrochloride (to minimize spontaneous contractions) and 5 $ng\,ml^{-1}$ physostigmine. Whenever a cyclo-oxygenase inhibitor or prostaglandin was used in the experiment, the assay solution contained the same concentration of inhibitor or prostaglandin.

Statistical analysis

Results are expressed as mean ± standard error of the mean (s.e.mean); n represents the number of observations. Tests of significance between groups of data were made using Student's t test and a level of $P < 0.05$ was considered to be significant.

Drugs used

Acetylcholine chloride (BDH), atropine sulphate (BDH), choline chloride (BDH), hexamethonium bromide (Sigma), indomethacin (Merck, Sharp & Dohme), ketoprofen (May & Baker), nicotine hydrogen tartarate (BDH), mecamylamine (M & B), physostigmine salicylate (BDH), prostaglandins E_2 and $F_{2\alpha}$ (Upjohn, Kalamazoo), sodium meclofenamate (Parke Davis), tetrodotoxin (Sigma).

Stock solutions of indomethacin, sodium meclofenamate and ketoprofen were made fresh in 2% (w/v) sodium carbonate solution and diluted in the appropriate physiological salt solution before use. Stock solutions of prostaglandins in 95% ethanol were stored at −20°C and diluted fresh before use. Stock solutions of acetylcholine or physostigmine containing 1 mg ml^{-1} of ascorbic acid and stored at −20°C until required.

Results

Whole ileum preparation (WIP) responses to acetylcholine, nicotine and transmural stimulation

These experiments were done in Tyrode solution at 37°C. Hexamethonium, 10 μg ml^{-1} abolished contractions induced by nicotine in the dose range (0.6–38.4 μg ml^{-1}) without effecting equivalent responses to ACh ($n = 3$). In the same experiments hexamethonium reduced responses evoked by transmural stimulation (0.1 Hz, 0.5 ms 40 V) by less than 10%. Tetrodotoxin (100 ng ml^{-1}) abolished both nicotine and transmural stimulation-induced contractions within 15 min of contact with the tissue without affecting responses to ACh. Atropine 100 ng ml^{-1} abolished responses to all three stimuli. Thus in the range of concentrations studied, nicotine-induced contractions were due to stimulation of intrinsic ganglia. The results are also consistent with the view that at low frequencies of transmural stimulation, ACh is released predominantly from postganglionic parasympathetic nerve endings (Paton & Zar, 1968).

Effects of cyclo-oxygenase inhibitors on responses of WIP and LMS to nicotine and transmural stimulation

The effects of cyclo-oxygenase inhibitors on nicotine contractions were investigated by constructing dose-response curves to nicotine before and in the presence of the inhibitors. Two consecutively matching dose-response curves served as controls. In WIP, indomethacin (10 μg ml^{-1}), ketoprofen (10 μg ml^{-1}) and sodium meclofenamate (5 μg ml^{-1}) significantly ($P < 0.001$) reduced contractions to nicotine but not to transmural stimulation or ACh. The effect developed slowly, the maximum inhibitory effect being achieved in 30–45 min. In LMS, on the other hand, indomethacin (1 μg ml^{-1}), ketoprofen (1 μg ml^{-1}) and sodium meclofenamate (0.5 μg ml^{-1}) produced even greater reductions in nicotine contractions than the higher concentrations did in WIP. In LMS, the inhibitory effects took a shorter time (5–10 min) to develop. In both preparations, the inhibition of nicotine contractions, once established, could not be overcome by repeated washing in inhibitor-free Tyrode. However, PGE_2 (0.1–2.5 ng ml^{-1}), but not $PGF_{2\alpha}$, completely restored responses to nicotine in the presence of inhibitor. The effect of indomethacin illustrated in Figure 1 is characteristic of the relative effectiveness of the cyclo-oxygenase inhibitors in WIP and LMS. Figure 2 shows the effects of ketoprofen (1 μg ml^{-1}) and sodium meclofenamate (0.5 μg ml^{-1}) on nicotine and transmural stimulation

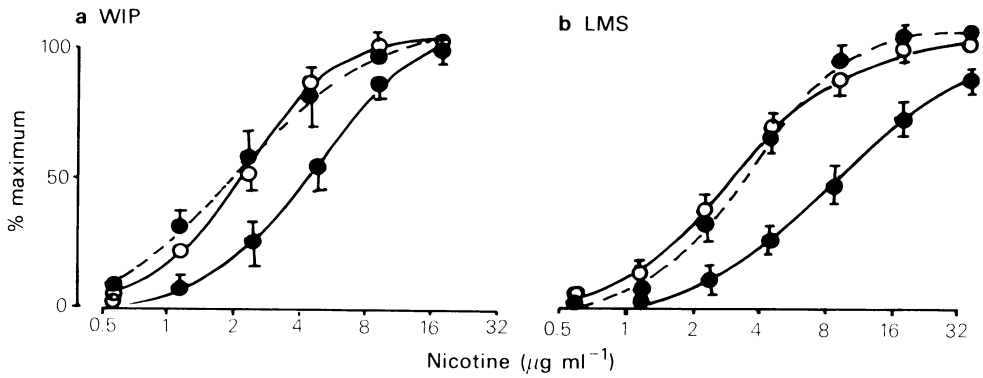

Figure 1 Effect of indomethacin on nicotine-evoked contractions of guinea-pig isolated ileum. (a) Whole ileum preparations, (b) longitudinal muscle strips. (○——○) Control responses; (●——●) responses after incubation with indomethacin, (a) 10 μg ml^{-1}, (b) 1 μg ml^{-1}, for 30 min; (●- - - - -●) responses in indomethacin plus prostaglandin E_2 0.5 ng ml^{-1}. Each point represents the mean of at least 6 measurements. Vertical lines show s.e.mean.

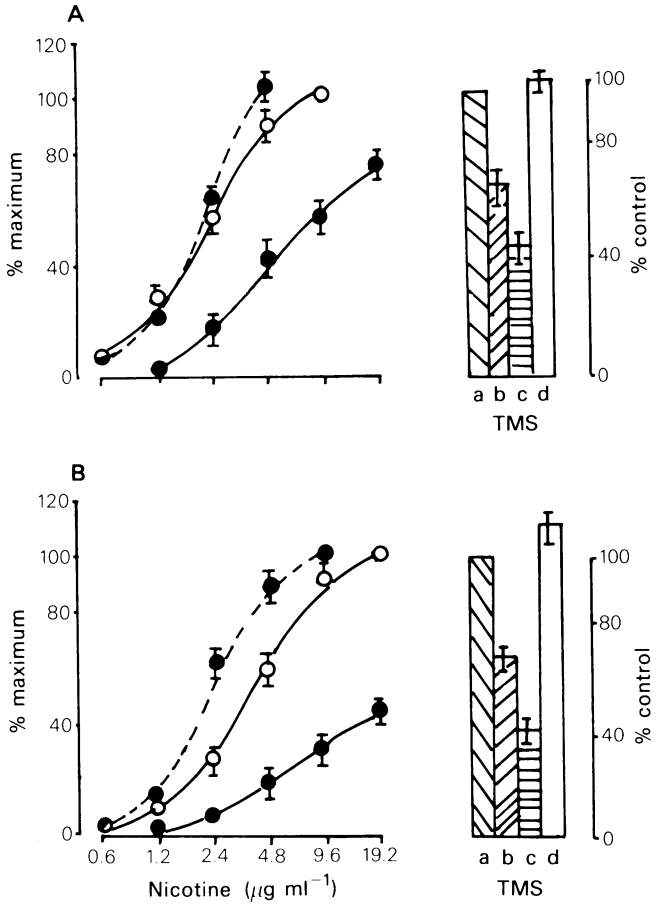

Figure 2 Effects of sodium meclofenamate (A) and ketoprofen (B) on responses of longitudinal muscle strips to nicotine and transmural stimulation (TMS). (○——○) Control responses to nicotine; (●——●) responses after incubating with inhibitor for 30 min: sodium meclofenamate 0.5 $\mu g\,ml^{-1}$ (A) and ketoprofen 1 $\mu g\,ml^{-1}$ (B); (●-----●) responses in the presence of inhibitor plus prostaglandin E_2 (PGE_2) 0.5 $ng\,ml^{-1}$. TMS: in (A) and (B), column (a) represents responses to stimulation at 0.1 Hz. In (A), columns (b and c) are responses to TMS in the presence of sodium meclofenamate, 0.5 and 5 $\mu g\,ml^{-1}$, respectively. In (B), columns (b and c) are responses in the presence of ketoprofen 1 and 10 $\mu g\,ml^{-1}$. In (A) and (B) column (d) represents responses to TMS in the presence of the higher dose of inhibitor and PGE_2, 0.5 $ng\,ml^{-1}$. Each point is a mean of 6 measurements. Vertical lines represent s.e.mean.

induced contractions of LMS. These concentrations of inhibitors had no effect on the ACh dose-response curves.

In WIP, the cyclo-oxygenase inhibitors had weak effects on responses to transmural stimulation. Indomethacin and ketoprofen (40 $\mu g\,ml^{-1}$) each reduced transmural stimulation responses by 17% and 12%, respectively ($n=6$). In LMS, on the other hand, indomethacin (1 $\mu g\,ml^{-1}$), ketoprofen (1 $\mu g\,ml^{-1}$) and sodium meclofenamate (0.5 $\mu g\,ml^{-1}$) reduced transmural stimulation responses by 28, 34 and 35%, respectively ($n=6$). Inhibitory effects were established within 5–10 min in LMS whereas maximum effects required 30–45 min to develop in WIP. PGE_2 but not $PGF_{2\alpha}$ completely restored contractions to transmural stimulation in the presence of inhibitor. Thus, cyclo-oxygenase inhibitors were more effective in blocking indirectly evoked contractions in LMS than in WIP. In both preparations nicotine-induced contractions were more easily blocked than the contractions to transmural stimulation.

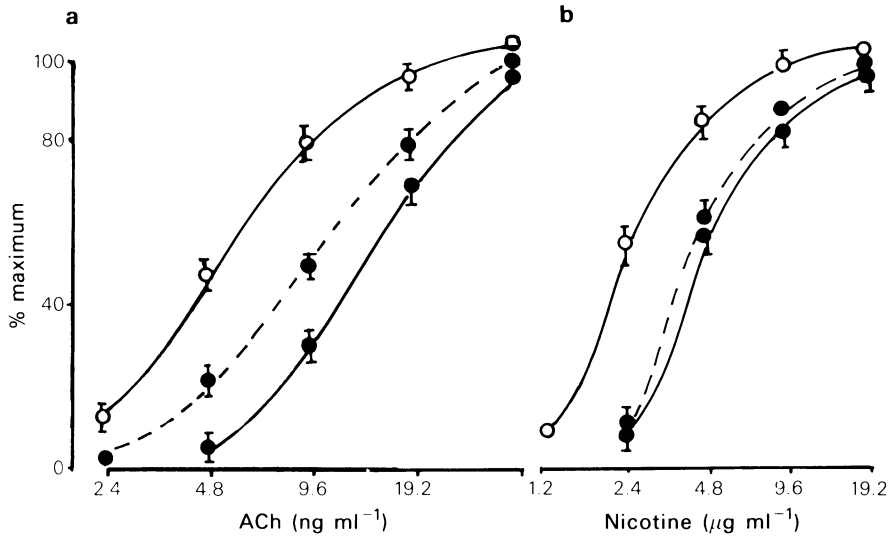

Figure 3 Effect of prostaglandin E_2 (PGE_2) on the antagonism of (a) acetylcholine and (b) nicotine by atropine in the guinea-pig isolated ileum. (○——○) Responses before atropine; (●——●) responses in the presence of atropine 0.5 ng ml^{-1}; (●-----●) responses in the presence of atropine plus PGE_2 0.5 ng ml^{-1}. Each point is the mean of 6 measurements. The vertical lines represent s.e.mean.

Site of action of cyclo-oxygenase inhibitors and PGE_2

(a) *Intrinsic ganglia* The finding that cyclo-oxygenase inhibitors are more effective in blocking nicotine-induced contractions than those to transmural stimulation could be explained if the inhibitors, *inter alia*, blocked intrinsic ganglia as has been suggested by Famey *et al.* (1977). We have compared the characteristics of the block of nicotine-induced contractions, caused by hexamethonium and mecamylamine, with the block caused by cyclo-oxygenase inhibitors in WIP. Mecamylamine (5 ng ml^{-1}) and hexamethonium (200 ng ml^{-1}) caused comparable reductions in nicotine contractions to those induced by cyclo-oxygenase inhibitors. PGE_2 failed to restore responses during ganglion blockade. The maximum effect of the ganglion blockers was achieved in 10 min. Responses to nicotine were fully restored within 30 min of repeated washing in inhibitor-free Tyrode solution.

(b) *Postsynaptic membrane* The cyclo-oxygenase inhibitors reduce indirectly induced contractions at concentrations in which contractions evoked directly by ACh are not affected. It thus seems clear that the inhibitory action is exerted, not on the postsynaptic contractile mechanism, but most probably on processes concerned with transmitter release. PGE_2, but not $PGF_{2\alpha}$ restores these responses. PGE_2 may do this by: (i) enhancing the contractile action of released ACh, (ii) increasing the amount of ACh released in the presence of inhibitor, or (iii) a combination of both actions. We sought to determine the site of action of PGE_2 by testing its effect on ACh and nicotine responses depressed by atropine. Atropine (0.5 ng ml^{-1}) shifted ACh and nicotine dose-response curves to the right. PGE_2 partially restored responses to acetylcholine in the presence of atropine, but had little effect on nicotine responses (Figure 3).

(c) *ACh release* Longitudinal muscle strips prepared as in Methods were allowed to equilibrate in modified Krebs solution at 37°C for 90 min during which time the solution was replaced every 10 min. Basal release of ACh was thereafter determined by draining the bath fluid and assaying the effluent on a piece of whole ileum preparation as already described. Sample contractions were bracketed between contractions to standard doses of ACh. The bath was drained every 10 min and samples assayed immediately or stored at −20°C for at the most 24 h before assay. In 6 experiments, basal ACh release was 25 ± 0.7 pg mg^{-1} min^{-1}. When stimulated at 0.1 Hz ACh release increased to 43 ± 1.0 pg mg^{-1} min^{-1}. Indomethacin and ketoprofen dose-dependently reduced basal or stimulated ACh release. The reduction in stimulated ACh release also increased with prolongation of the time of incubation of the tissue with the inhibitor. Thus, after 10 min of incubation with indomethacin 1 μg ml^{-1} and 10 μg ml^{-1}, stimulated ACh release was

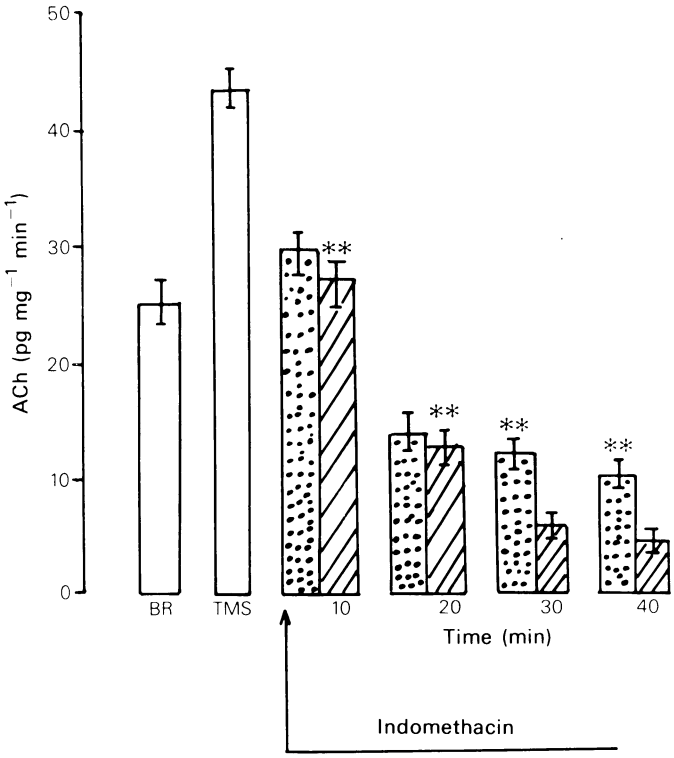

Figure 4 The effect of indomethacin on acetylcholine (ACh) release during transmural (TMS) of longitudinal muscle strips from guinea-pig ileum. BR: basal release; TMS: control release during stimulation at 0.1 Hz ($n = 12$). Dotted and hatched columns represent stimulated release from strips treated with indomethacin 1 $\mu g\,ml^{-1}$ and 10 $\mu g\,ml^{-1}$, respectively ($n = 6$ each column). The figures under the columns represent times (min) of incubation with the inhibitor. Vertical lines represent s.e.mean. $^{**}P < 0.001$ compared to control stimulated release (TMS).

$28 \pm 2.0\,pg\,mg^{-1}\,min^{-1}$ (35% reduction) and $26 \pm 3.0\,pg\,mg^{-1}\,min^{-1}$ (40% reduction), respectively. After 40 min incubation indomethacin 1 $\mu g\,ml^{-1}$ and 10 $\mu g\,ml^{-1}$ reduced ACh release to $9 \pm 1.0\,pg\,mg^{-1}\,min^{-1}$ (79% reduction) and $3 \pm 0.7\,pg\,mg^{-1}\,min^{-1}$ (93% reduction), respectively. The results for indomethacin are presented in Figure 4. It can be seen that although at 10 and 20 min the inhibitory effects of indomethacin on ACh release were already marked, there was no dose-related difference. At 30 and 40 min however, the effect of 10 $\mu g\,ml^{-1}$ was significantly greater ($P < 0.001$) than that of 1 $\mu g\,ml^{-1}$. PGE_2 completely restored ACh release to control levels, but $PGF_{2\alpha}$ was without effect. Ketoprofen (1 and 10 $\mu g\,ml^{-1}$) and sodium meclofenamate (0.5 and 5 $\mu g\,ml^{-1}$) had similar effects to indomethacin (Figure 5).

Discussion

The results show that the cyclo-oxygenase inhibitors, indomethacin, ketoprofen and sodium meclofenamate inhibit contractions of the guinea-pig isolated ileum induced by nicotine and transmural stimulation at concentrations which had no effects on contractions caused by ACh. The results are in general agreement with those previously described (see Introduction for references).

We found no evidence in these studies to support the suggestion by Famey *et al.* (1977) that cyclo-oxygenase inhibitors may have a ganglion blocking action. The characteristics of blockade of nicotine contractions by two ganglion blockers, hexamethonium and mecamylamine, were quite different from the effects of the cyclo-oxygenase inhibitors: responses to nicotine were restored after washing out the ganglion blocker, but not by addition of PGE_2. Moreover, the ganglion blockers unlike the cyclo-oxygenase inhibitors, had minimal effects on responses to transmural stimulation. Our results are consistent with previous observations that transmural stimulation response is due predominantly to ACh released from parasympathetic nerve endings (Paton & Zar, 1968).

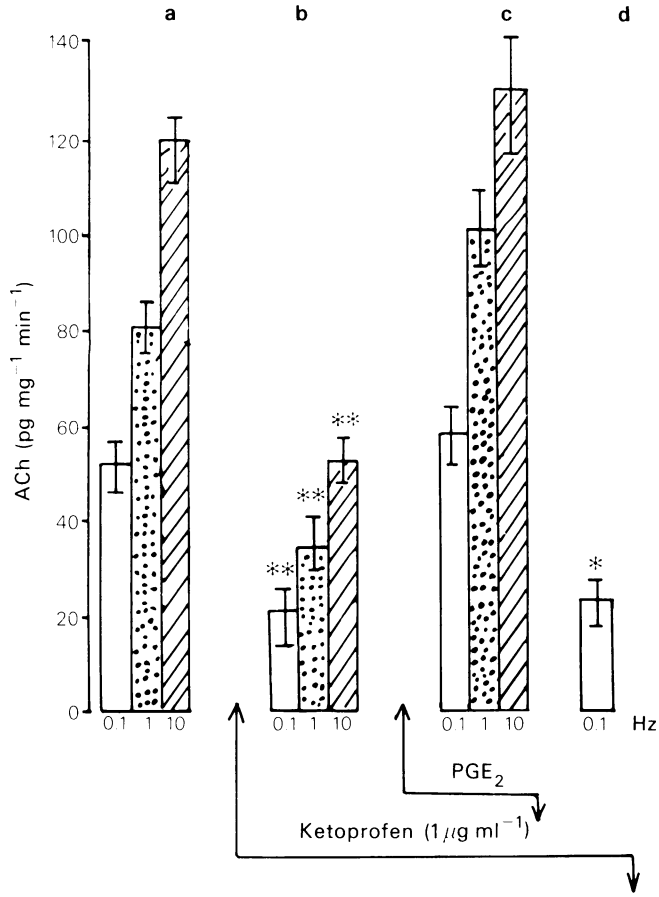

Figure 5 The effect of ketoprofen (1 μg ml^{-1}) on ACh release during transmural stimulation of longitudinal muscle strips from guinea-pig ileum at 0.1 Hz (open columns), 1 Hz (dotted columns) and 10 Hz (hatched columns). A different preparation was used at each frequency. Columns under (a) represent release before inhibitor was introduced. Columns under (b) represent release after the strips had been incubated in inhibitor-containing Krebs for 30 min, (c) restoration of release by prostaglandin E_2 (PGE_2) 0.5 ng ml^{-1}. (d) Persistence of the inhibitor effect 30 min after removal of PGE_2. Each column represents the mean from six preparations and vertical bars show s.e.mean. Significantly different from control release, $*P<0.005$, $**P<0.001$.

The results of the present investigation suggest that the cyclo-oxygenase inhibitors reduce indirectly evoked contractions of the guinea-pig ileum by reducing ACh release. Restoration of the response by PGE_2 also seems likely to be due, at least in part, to increased ACh release in the presence of the inhibitor since (a) the time courses of effect for inhibition of indirect contractions and ACh release were similar in the LMS and (b) PGE_2, but not $PGF_{2\alpha}$, restored contractions and ACh release in the presence of inhibitor. Yagasaki *et al.* (1981) have also shown that PGE_1 increases resting ACh release from guinea-pig isolated ileum. Enhancement of the contractile power of the transmitter at the postsynaptic membrane cannot be ruled out as an additional factor in the action of PGE_2. Prostaglandins of the E series are known to potentiate a variety of agonists in smooth muscles (Eagling *et al.*, 1972; Adeagbo & Okpako, 1980; Figure 3, this study). The action of prostaglandins would appear to vary with species. Nakata *et al.* (1980) have recently observed that PGE_1 depressed transmural stimulation contractions more than ACh contractions of circular, but not of longitudinal muscle of canine small intestine. The authors suggest that prostaglandins may exert a negative feedback control of excitatory transmission in canine circular, but not in longitudinal, muscle.

The effects of the cyclo-oxygenase inhibitors on the intramural nerve network appear to be markedly influenced by diffusion barriers; thus the inhibitors

were 40–80 times more effective in blocking transmural stimulation contractions in longitudinal muscle strips, in which the diffusion barrier to Auerbach's plexus is minimal, than in whole ileum preparations (see Figure 1). Such diffusion factors may explain previous observations (Bennett *et al.*, 1975a; Kadlec *et al.*, 1974) showing that the inhibitory effect of indomethacin on indirectly evoked contractions of the guinea-pig ileum depended on the physiological salt solution in which the tissue was bathed. Bennett *et al.* (1975a) found that indomethacin was more effective in blocking nicotine-induced contractions in modified Krebs solution than in Tyrode solution. In the present series of experiments, we also found that indomethacin was about 10 times more active in blocking contractions to transmural stimulation when whole ileum preparations were suspended in Krebs solution gassed with 5% CO_2 in oxygen (pH 7.4) than when bathed in Tyrode solution gassed with air (pH 8.0). In Krebs solution, indomethacin ($10\,\mu g\,ml^{-1}$) reduced transmural stimulation contractions by $30 \pm 1.8\%$ ($n = 6$) compared to a $17 \pm 1.8\%$ reduction caused by $40\,\mu g\,ml^{-1}$ in Tyrode solution. Indomethacin is a weak acid (pK_a, 4.5). Its greater ionization in Tyrode solution would limit its diffusibility in whole ileum preparations and so account for its apparent greater potency in Krebs solution. Another important factor could be that the inhibitor is bound to protein in its path of diffusion through thick muscle. Diffusion related problems may thus explain why only high concentrations of inhibitor are effective in whole ileum preparations and the often observed long latent period in the action of these drugs (Ferreira *et al.*, 1972; this study). Therefore, there is no evidence from this study to suppose that different pools of enzyme (extraneuronal and neuronal) exist with different sensitivities to blockade by cyclo-oxygenase inhibitors, as suggested by Bennett *et al.* (1975b). It is tempting to speculate that, in therapeutic use, a greater diffusibility of cyclo-oxygenase inhibitors into inflamed areas, is the reason why these drugs exhibit fewer side effects than would be predicted from some important physiological roles attributed to the prostanoids (Hedqvist, 1974).

The mechanism by which cyclo-oxygenase inhibitors and PGE_2 affect basal and stimulated ACh release from the myenteric plexus of the guinea-pig isolated ileum is not known. Calcium ions play an essential role in the coupling of the nerve impulse to the release mechanism (Birks & Cohen, 1968) and Ehrenpreis *et al.* (1973) have suggested a model in which a prostaglandin is thought to be involved in making calcium available for the ACh release process. Reed & Knapp (1978) have speculated that the oxygens of two or more prostaglandin molecules could form a hydrophilic cavity around a dehydrated calcium ion, thus acting as an intracellular ionophore. Indeed, there is evidence that PGE_2 but not $PGF_{2\alpha}$ facilitates Ca^{2+} fluxes in smooth muscle (Eagling *et al.*, 1972; Adeagbo & Okpako, 1980). Since PGE_2-like material is released from the myenteric plexus during coaxial stimulation of the guinea-pig isolated ileum (Coceani *et al.*, 1967; Botting & Salzmann, 1974), it may be argued that the action of cyclo-oxygenase inhibitors on ACh release is due to removal of a prostaglandin which normally amplifies ACh release. Thus if a prostaglandin plays a role at all in the ACh release process, that role would be facilitatory. On the other hand, since indomethacin is known to chelate calcium (Northover, 1972), it could be that the observed effects are a reflection of a pharmacological interaction between agents having opposite effects at a common step in the ACh release mechanism.

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