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EFFECT OF INDOMETHACIN AND PROSTAGLANDIN ON THE SMOOTH MUSCLE CONTRACTING ACTIVITY OF ANGIOTENSIN AND OTHER AGONISTS

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1 Indomethacin had an equal inhibitory effect on the response of the guinea-pig isolated ileum to angiotensin II (angiotensin), bradykinin, histamine and acetylcholine. This effect did not seem to result from inhibition of prostaglandin synthesis, as it did not depend on the time of treatment with indomethacin.

2 Prostaglandin E_2 (prostaglandin) potentiated the responses of the guinea-pig ileum to angiotensin, bradykinin, histamine and acetylcholine without significant differences in the effects observed.

3 In the rabbit isolated mesenteric and coeliac arteries, indomethacin had an equal potentiating effect on the responses to angiotensin and to adrenaline. In these organs pre-incubation with indomethacin was necessary for the effect to be observed, and this effect lasted for 2 h or more after that drug was removed from the medium.

4 No cross-tachyphylaxis between angiotensin and adrenaline was observed in the rabbit mesenteric and coeliac arteries.

5 It is concluded that the effects of indomethacin and prostaglandin on the response of the guinea-pig ileum to the four agonists result from an action on the smooth muscle contractile mechanism *per se* rather than from an inhibitory action on the release of endogenous prostaglandin produced by the four agonists.

6 The results with the rabbit isolated arteries indicate that tachyphylaxis to angiotensin in these organs is not caused by prostaglandin release.

Introduction

It has been postulated that prostaglandin release participates in the smooth muscle contraction induced by a number of agonists. Vane & Williams (1972) suggested that intramural generation of a prostaglandin contributes to the contractions induced by oxytocin in the rat isolated uterus.

Indomethacin, an inhibitor of prostaglandin biosynthesis (Vane, 1971), depresses responses of the rat uterus to bradykinin (Barabé, Park & Regoli, 1975) and angiotensin II (Baudouin-Legros, Meyer & Worcel, 1974). This has been interpreted as evidence that prostaglandin release is involved in the contraction of uterine smooth muscle by these peptides, although under oestrogen domination the uterus is more sensitive to these agonists, but less sensitive to prostaglandins (Weeks, 1972).

In vascular smooth muscles, in which prostaglandins usually produce relaxation (Weeks, 1972), prostaglandin release has been implicated in tachyphylaxis to angiotensin because this phenomenon was partly supressed by indomethacin (Aiken, 1974). However, in the atropinized guinea-pig isolated ileum neither the onset of tachyphylaxis to angiotensin nor recovery from it were affected by indomethacin (Paiva, Mendes & Paiva, 1976). On the other hand, Chong & Downing (1973, 1974) described a selective inhibition by indomethacin of angiotensin-induced contractions of the guinea-pig ileum, which was reversed by prostaglandin. They interpreted this as evidence that prostaglandin mediates angiotensin's action. We have not been able to confirm the selective nature of this inhibition, but have obtained evidence for a non-selective action of indomethacin in both guinea-pig ileum and rabbit arteries.

Methods

Guinea-pigs of either sex weighing 150–200 g were deprived of food for 24 h and killed by decapitation. A 15 cm segment of terminal ileum was removed, and washed with Tyrode solution at room temperature. A

4 cm portion was then suspended in a 5 ml chamber containing Tyrode solution at 37°C, bubbled with air. Under these conditions the pH of the medium was 8.0. Isotonic contractions of longitudinal muscle were recorded on a smoked drum by a frontal writing lever under 1 g load and six-fold magnification.

Male albino rabbits of approximately 5 kg body weight were stunned and killed by exsanguination, the coeliac and mesenteric arteries were removed immediately, and cut into helical strips 2–3 mm wide and approximately 2 (coeliac) or 4 (mesenteric) cm long. The strips were suspended in Tyrode solution at 37°C under the same conditions used for the guinea-pig ileum, except that 95% O_2 and 5% CO_2 were bubbled through the solution, resulting in a pH of 7.4.

Solution and drugs

The composition of the Tyrode solution was (mM): NaCl 140, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.53, $NaHCO_3$ 12, NaH_2PO_4 0.36 and glucose 5.6.

[5-Isoleucine]-angiotensin II (angiotensin) (Paiva, Goissis, Juliano, Miyamoto & Paiva, 1974) and bradykinin (Tominaga, Stewart, Paiva & Paiva, 1975) were synthetic products made in this laboratory. Other drugs used were: histamine dihydrochloride (California Corporation for Biochemical Research), acetylcholine chloride (Sigma), adrenaline bitartrate (Fluka), indomethacin (Merck, Sharp & Dohme) and prostaglandin E_2 (Upjohn). Indomethacin solutions were prepared just before use by dissolving the drug in a small volume of saturated aqueous sodium carbonate, diluting to the desired volume with Tyrode solution, and adjusting to pH 8.0. Concentrated prostaglandin solutions (1 mg/ml) in 25% ethanol were stored frozen and diluted with Tyrode solution immediately before use. The vehicles used for the administration of indomethacin and prostaglandin had no detectable effect on the response to the isolated organs to agonists.

Results

Guinea-pig ileum

The effect of indomethacin or prostaglandin on the response to angiotensin, bradykinin, histamine and acetylcholine, was studied by determining dose-response curves to the agonists before and after treatment with the two drugs. Independent experiments were performed for each agonist, using preparations responding constantly to a submaximally effective concentration administered at 5 min intervals. The responses to 4 concentrations of agonist (producing 20%–80% maximum contraction) were then recorded. After treatment with 56 μM indomethacin for 45 min, and in the continuing presence of that drug, new dose-response curves were obtained. The ratio of the concentrations that produced 50% of maximum response after and before treatment with indomethacin was determined by interpolation (Table 1).

To study the effect of indomethacin on the maximum contractions produced by each agonist, independent experiments were conducted with fresh tissues responding constantly to small, submaximally effective, doses of agonist administered at 5 min intervals and with a 90 s contact time. The organ was treated once with a dose of the agonist 15 times higher than that producing 50% of maximum response. This supramaximal dose was 0.09 μM for bradykinin, 1 μM for histamine, 0.03 μM for angiotensin and 1.2 μM for acetylcholine. It was followed by incubation with 56 μM indomethacin for 45 min, and another administration of the same supramaximal dose. This procedure was adopted to avoid tachyphylaxis to high doses of angiotensin. The reduction in the response to the second supramaximal dose was expressed as a percentage of the pre-indomethacin maximum response (Table 1). In similar experiments, done with 8.4 μM indomethacin, no significant

Table 1 Effect of indomethacin (56 μM) on the isotonic response of the guinea-pig isolated ileum to four agonists

Agonist	*Dose-ratio**	*% Inhibition of maximum response*
Angiotensin	2.03 ± 0.34 (7)	17.8 ± 3.6 (8)
Bradykinin	2.35 ± 0.39 (6)	13.4 ± 2.8 (6)
Histamine	3.31 ± 1.59 (6)	12.3 ± 2.7 (7)
Acetylcholine	2.45 ± 0.58 (7)	16.5 ± 4.4 (8)

* Ratio of the concentrations producing 50% of maximum response after and before treatment with indomethacin. Mean ± s.d. (*n*).

Tsble 2 Effect of prostaglandin (28 nM) on the isotonic response of the guinea-pig isolated ileum to four agonists

Agonist	*Dose-ratio**	*% Potentiation of maximum response*
Angiotensin	0.54 ± 0.09 (5)	6.2 ± 2.5 (4)
Bradykinin	0.31 ± 0.06 (5)	9.9 ± 2.3 (4)
Histamine	0.44 ± 0.08 (4)	5.4 ± 1.3 (4)
Acetylcholine	0.37 ± 0.09 (4)	10.3 ± 3.2 (4)

* Ratio of the concentrations producing 50% of maximum response after and before treatment with prostaglandin. Mean ± s.d. (*n*).

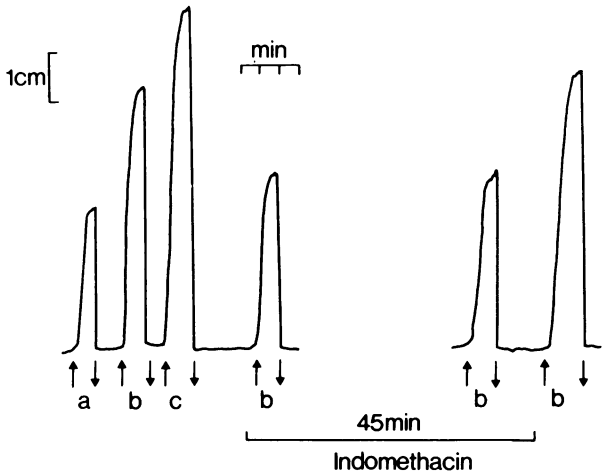

Figure 1 Responses of the guinea-pig isolated ileum to angiotensin: a, 0.4 nM; b, 0.8 nM; c, 1.6 nM. Upward arrows indicate additions and downward arrows indicate washout and interruption of kymograph movement for 3 minutes. Normal Tyrode solution was replaced by one containing 56 μM indomethacin 30 s before addition of the fourth dose and restored 30 s before last dose.

inhibition of any of the four agonists was detected.

Each experiment to obtain the dose-ratio or percentage inhibition of maximum response was paired with a control without indomethacin. These showed no significant changes.

The effect of prostaglandin E_2 on the response of the guinea-pig ileum was studied using the same protocol (Table 2).

The inhibition of the response to the four agonists studied did not depend on the time of incubation with indomethacin, in the range 30 s to 45 minutes. The normal response of the isolated organ to these agonists was rapidly recovered after removal of indomethacin. This was reproducibly observed in at least three experiments with each agonist and is illustrated in Figure 1, for angiotensin.

Rabbit mesenteric and coeliac arteries

Angiotensin administered repeatedly, at 45 min intervals, to either the mesenteric or the coeliac artery, caused tachyphylaxis (Figure 2). This did not occur with adrenaline. However, treatment with 8.4 μM indomethacin for 30 min potentiated the responses of both agonists in both arteries. Figures 3 and 4 are representative of at least 6 experiments in each case. The potentiation was long-lasting, and was found to be present in some degree for up to 2 h after removal of indomethacin. Furthermore, in contrast to the guinea-pig ileum, previous incubation with indomethacin for 30 min was necessary for inhibition to occur.

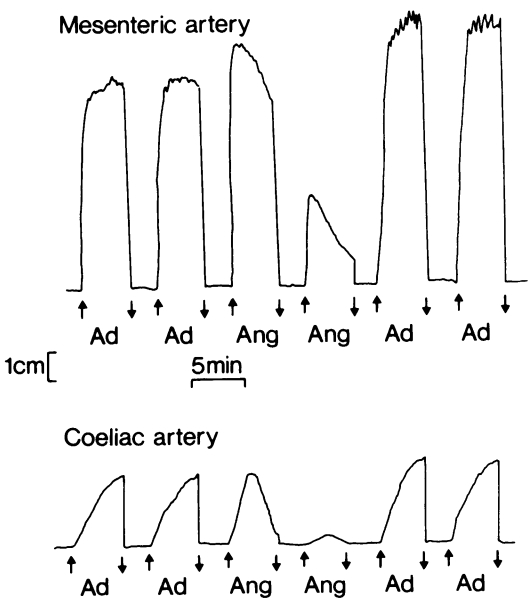

Figure 2 Responses of the rabbit's mesenteric and coeliac arteries to repeated administration of adrenaline (Ad) and angiotensin (Ang). Upward arrows indicate additions and downward arrows indicate washout and interruption of kymograph movement for 3 minutes. Intervals between additions were 10 minutes.

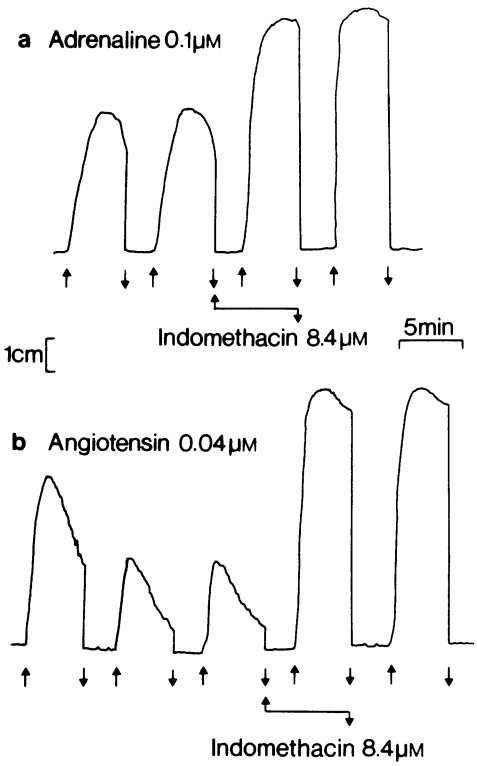

Figure 3 Responses of the rabbit isolated mesenteric artery to sub-maximal doses of adrenaline and angiotensin administered at 45 min intervals. Upward arrows indicate additions and downward arrows indicate washout and interruption of kymograph movement for 38 minutes. Normal Tyrode solution was replaced by one containing 8.4 μM indomethacin 30 min before third (a) or fourth (b) dose, and restored 45 min before last dose.

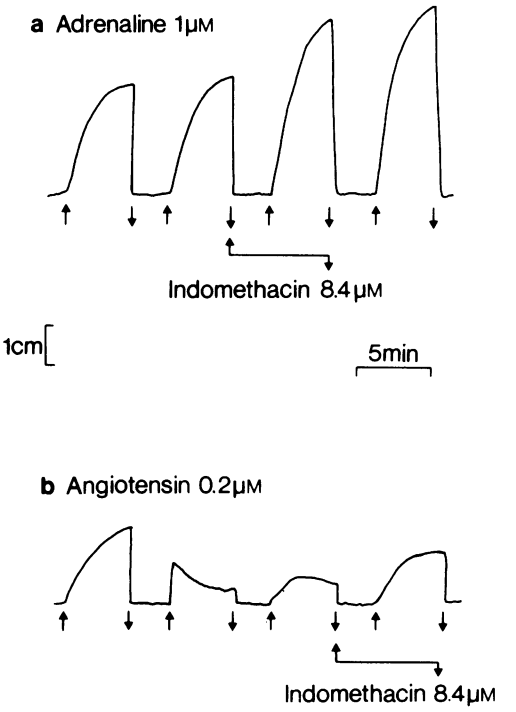

Figure 4 Responses of the rabbit isolated coeliac artery to sub-maximal doses of adrenaline and angiotensin. Experimental details as in Figure 2.

Discussion

Guinea-pig ileum

Treatment of the guinea-pig isolated ileum with indomethacin inhibited contractions to angiotensin, but this effect was less intense than that described by Chong & Downing (1973) and was not selective for angiotensin. The dose-ratios and the inhibition of maximum response (Table 1) were not significantly different for the four agonists that were studied. Similarly, the potentiation by prostaglandin was non-selective, as shown in Table 1.

These results may indicate either that prostaglandin release mediates the action of the four agonists studied, probably at a common step in the excitation-contraction chain of events, or that prostaglandin and indomethacin have opposite effects on the contractile properties of the guinea-pig ileum, which affect the response to all agonists.

We believe that the experiment illustrated in Figure 1 favours the second hypothesis, since the effect of indomethacin occurred too quickly (30 s) to result from inhibition of prostaglandin synthesis. In particular the fast (30 s) return to normal response after the removal of indomethacin argues against this, since indomethacin inhibition appears to be at an early step of the synthetic pathway (Smith & Lands, 1971). It must also be pointed out that the concentrations of indomethacin needed to depress the response to the agonists were higher than those thought necessary to inhibit prostaglandin biosynthesis. Our finding that 8.4 μM indomethacin did not cause significant angiotensin inhibition is not in agreement with the report, by Kadlec, Masek & Seferna (1974), that 1 μM indomethacin depressed the response of the guinea-pig ileum to that agonist. However these authors do not state whether adequate care was taken to avoid tachyphylaxis to angiotensin, which might be enhanced by the lower pH of the Krebs solution that they used instead of the usual Tyrode (Paiva, Juliano,

Nouailhetas & Paiva, 1974). It should be noted that they also report more than 100-fold loss of sensitivity to angiotensin in the presence of 0.4 μM atropine, while, under the usual conditions, 1 μM atropine only reduces the effect of angiotensin by 40–70% (Khairallah & Page, 1961; Robertson & Rubin, 1962).

Further evidence against prostaglandin mediation is the finding that guinea-pig ileum, in which response to prostaglandins was reduced by a dibenzoxazepine hydrazide derivative (SC-19220), responded normally to bradykinin and acetylcholine (Sanner, 1969) as well as to histamine (Ambache, Verney & Zar, 1970).

The available evidence thus indicates that the inhibitory action of indomethacin is probably due to an effect on one of the steps of the excitation-contraction coupling that is common to different stimuli. A similar conclusion was reached by Northover (1971), who worked with the guinea-pig isolated stomach; he observed that as widely different stimuli as electrical stimulation and histamine were inhibited by very large concentrations of indomethacin. This author also presented evidence linking indomethacin inhibition to diminished calcium uptake by the target cells.

The fact that prostaglandin potentiated the responses to the different agonists (Table 2) also points to an effect at a common link in the excitation-contraction chain of events. This might involve a calcium displacement mechanism, as postulated for similar effects in other smooth muscles (Clegg, Hall & Pickles, 1966; Strong & Bohr, 1967; Eagling, Lovell & Pickles, 1972; Villani, Chiarra, Cristalli & Piccinini, 1974) and for prostaglandin actions in other tissues (Ramwell & Shaw, 1970).

Rabbit arteries

The experiments illustrated in Figures 3 and 4, carried out under the same conditions as those described by Aiken (1974), confirm his finding that indomethacin reverses angiotensin tachyphylaxis. However, this effect appears to be due to a non-selective potentiation since adrenaline, which shows no tachyphylaxis, was also potentiated. The possibility must be considered that prostaglandin release may occur with both agonists and that potentiation by indomethacin may be the result of inhibition of prostaglandin synthesis. This hypothesis is based on the non-specific relaxation of the muscle by prostaglandin (Aiken, 1974), and depends on the possibility of the presence of released prostaglandins for relatively long periods in the organ's biophase. If this were the case, however, one might expect that an organ rendered tachyphylactic to angiotensin would not respond fully to adrenaline. This was not observed in the experiments illustrated in Figure 2, where it is also shown that the isolated arteries respond normally to angiotensin after being challenged with adrenaline. Furthermore, we have confirmed Aiken's (1974) observation that tachyphylaxis to angiotensin in the rabbit mesenteric and coeliac arteries occurs even in the presence of indomethacin, although not as severely as in its absence.

Our results indicate that, in the systems that we have examined, tachyphylaxis to angiotensin is not caused by prostaglandin release. We believe that this is also supported by Aiken's (1974) observation that tachyphylaxis produced by repeated administration of angiotensin at shorter intervals (5–10 min) was not affected by inhibitors of prostaglandin synthesis. He interpreted this finding as indicative of a dual mechanism for tachyphylaxis, a short-lasting and a long-lasting one, of which only the latter would be dependent on prostaglandin release. However, it seems unlikely that the prostaglandin released by angiotensin would inhibit the response of a subsequent dose administered 45 min later but not one administered 10 min later. We conclude therefore that prostaglandin does not participate in the mechanism of angiotensin tachyphylaxis in rabbit arteries. We have reached a similar conclusion for the guinea-pig ileum (Paiva *et al.*, 1976).

The need for an incubation period with indomethacin and the persistence of its effect after washout suggests that, in the rabbit mesenteric and coeliac arteries, this drug might act through inhibition of prostaglandin synthesis. A conclusion about the role of prostaglandin and the mechanism of indomethacin's effects on vascular smooth muscle contraction is difficult, because of the reported differences in behaviour towards different prostaglandins, complicated by species variations (Weeks, 1972), and by differences among large and small arteries from the same species (Strong & Bohr, 1967; Aiken, 1974). However, prostaglandins and indomethacin might affect the response of vascular smooth muscle through opposite effects on calcium translocation mechanisms, as proposed for the guinea-pig ileum. The available evidence indicates that calcium transport in vascular smooth muscles is directly affected by indomethacin (Northover, 1971) and prostaglandins (Greenberg, Kadowitz, Diecke & Long, 1973).

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