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Effects of sodium and calcium concentrations on the potentiation by indomethacin of the responses of rabbit mesenteric and coeliac arteries to vasoconstrictor agonists

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1 The contractile responses of the rabbit isolated coeliac and mesenteric arteries to five agonists (angiotensin, adrenaline, histamine, acetylcholine and 5-hydroxytryptamine), but not to K^+, were potentiated by indomethacin (8.4 μM)

2 The potentiation was similar whether indomethacin was added 1 h before or during the response to the agonist.

3 The agonists that were more potentiated by indomethacin were also more dependent on the Ca^{2+} concentration in the medium, for their contractile action.

4 Prostaglandin E_2 in low concentrations (micromolar) did not affect the resting tone but relaxed the agonist-contracted arteries both in normal and in Ca^{2+}-free medium.

5 No prostaglandin E (PGE)-like substances were detected in the perfusate of arteries contracted by angiotensin.

6 Reduction of the external Na^+ concentration to 80 mM resulted in potentiation of the responses to agonists (angiotensin and adrenaline), but not to K^+, and in this Na^+-deficient medium potentiation by indomethacin was greatly reduced.

7 These results suggest that potentiation by indomethacin of the arteries' responses to vasoactive substances may result from that drug's inhibitory action on sodium influx and consequent increase in calcium entry through receptor-operated channels.

Introduction

One of the tools that has been used to study the different roles of prostaglandins in the regulation of vascular smooth muscle tone is indomethacin. Coupar & McLennan (1978) showed that indomethacin depresses the effect of noradrenaline on the rat perfused mesenteric blood vessels but in the rabbit mesenteric and renal vascular beds, indomethacin was shown to potentiate the responses to noradrenaline (Grodzinska, Panczenko & Grylewski, 1976) and to angiotensin (Needleman, Kauffman, Douglas, Johnson & Marshall, 1973; Needleman, Marshall & Douglas, 1973). These effects of indomethacin have been attributed to its inhibitory action on prostaglandin synthesis (Vane, 1971), leading to the conclusion that the vasoconstrictor agonists may induce the release of prostaglandins, which would potentiate (in the rat mesenteric bed) or depress (in the rabbit vascular beds) their contractile effect.

The findings that angiotensin and noradrenaline may promote the release of prostaglandin E-like substances in the rabbit kidney (Needleman *et al.*, 1973a) and mesenteric (Needleman *et al.*, 1973b; Grodzinska *et al.*, 1976) vascular beds have been interpreted as evidence of an intrinsic mechanism for local modulation of blood vessel tone. According to Blumberg, Denny, Marshall, & Needleman (1977), the vasoconstrictor effect of angiotensin in the perfused rabbit mesenteric blood vessels is counteracted by its property to release prostaglandins which decrease vascular resistance.

Aiken (1974) found that indomethacin prevents angiotensin tachyphylaxis in rabbit isolated mesenteric and coeliac artery strips, and this was interpreted as indication that prostaglandin synthesis in the arterial wall is capable of modifying angiotensin vasoconstriction (Blumberg *et al.*, 1977). However, Aboulafia, Mendes, Miyamoto, Paiva & Paiva (1976) suggested that potentiation of the arterial response to angiotensin by indomethacin may be due to an unspecific effect, since indomethacin, besides its inhibitory action on prostaglandin synthesis, is

also known to affect calcium and sodium transport directly in smooth muscles (Northover, 1971; 1972). We have investigated this possibility by studying the effect of indomethacin and of prostaglandin E_2 on the responses of rabbit isolated coeliac and mesenteric arteries to five different agonists (angiotensin, adrenaline, histamine, acetylcholine and 5-hydroxytryptamine) and to potassium ions, in the presence and in the absence of calcium ions as well as in a sodium-deficient medium.

Methods

Male albino rabbits of approximately 4 kg body weight were killed by a sharp blow to the head, the coeliac and mesenteric arteries were removed immediately, and cut into helical strips 2–3 mm wide and 2–3 cm long. The strips were suspended in a 5 ml chamber containing gassed (95% O_2/5% CO_2) Tyrode solution at 37°C. Isometric contractions, under 1 g tension, were recorded with a Sanborn force transducer through a Hewlett-Packard model 8805B amplifier and an ECB model RB 102 potentiometric recorder.

Chicks were killed by decapitation and the rectum was immediately removed and washed with Tyrode solution at room temperature. The organ was suspended in a chamber of 5 ml capacity, containing Tyrode solution maintained at 37°C and its isotonic contractions, under 1 g load, were recorded by means of a frontal lever, on a smoked kymograph drum. The system of superfusion in cascade consisted of two chambers of 5 ml capacity, maintained at 37°C, mounted in series as described by Gilmore, Vane & Willie (1968). A period of equilibrium of 30 min for the chick rectum and of 60 min for the arteries was allowed before starting the assays.

The composition of the Tyrode solution was (mM): NaCl 138, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.49, $NaHCO_3$ 12, NaH_2PO_4 0.36 and D-glucose 5.6. The sodium-deficient (80 mM Na^+) solution was obtained by reducing the NaCl concentration to 68 mM, and 140 mM sucrose was added. The 'calcium-free' medium had the same composition as the Tyrode solution, with the exception that $CaCl_2$ was omitted, without the addition of chelating agents. Atomic absorption photometry of several samples of this medium indicated some contamination by calcium, which, however, was always less than 0.01 mM.

[5-Isoleucine]-angiotensin II (angiotensin) was a synthetic product made in this laboratory (Paiva, Goissis, Juliano, Miyamoto & Paiva, 1974). Other drugs used were: histamine dihydrochloride (California Corporation for Biochemical Research), acetylcholine (Sigma), adrenaline bitartrate (Fluka), 5-hydroxytryptamine (Sigma Chemical Company), indomethacin (Merck Sharp & Dohme) and prostaglandin E_2 (PGE_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 7.4. Concentrated PGE_2 solutions (1 mg ml^{-1}) in 25% ethanol were stored frozen and diluted with Tyrode solution immediately before use. Adrenaline stock solutions were prepared in 0.01 N HCl and diluted just before use by dissolving in 0.9% NaCl solution containing 0.5% ascorbic acid. The vehicles used for the administration of indomethacin, PGE_2 and adrenaline had no detectable effect on the responses of the isolated organs to agonists.

Results

Potentiation of the contractile responses of coeliac and mesenteric arteries by indomethacin

Agonist doses that produced contractions in the lower third of the linear portion of the dose-response curve were selected. The doses used and the corresponding mean tensions obtained are shown in Table 1, where the relative maximum responses (intrinsic activities) to the different drugs are also given.

Table 1 Agonist concentrations that were employed and corresponding responses

		Coeliac artery		*Mesenteric artery*	
Drug	*Concentration*	Tension (g)	Intrinsic activity	Tension (g)	Intrinsic activity
Adrenaline	1.0 μM	0.8±0.2	1.00	1.1±0.3	1.00
Histamine	5.0 μM	1.1±0.2	0.94±0.05	1.1±0.2	0.91±0.05
Angiotensin	0.2 μM	1.1±0.2	0.55±0.09	1.2±0.2	0.60±0.05
5-Hydroxytryptamine	3.7 μM	0.6±0.1	0.47±0.05	1.5±0.2	0.62±0.06
Acetylcholine	0.5 mM	0.5±0.2	0.32±0.04	0.6±0.1	0.35±0.05
KCl	10.0 mM	0.6±0.1	0.60±0.05	0.7±0.1	0.63±0.09

Values are means ± s.e.mean of 5–8 experiments. Intrinsic activities (see text) were calculated in relation to the effect of adrenaline at 15 μM.

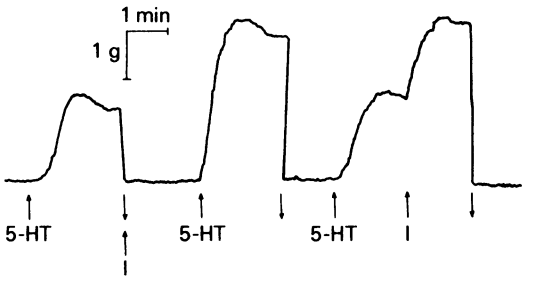

Figure 1 Potentiation of the responses of the rabbit isolated mesenteric artery to 3.7 μM 5-hydroxytryptamine (5-HT) by 8.4 μM indomethacin (I). Downward arrows indicate washing of the preparation and interruption of chart movement for 1 h. The first addition of indomethacin was made immediately after washing, resulting in a pre-incubation of 1 h before the next addition of 5-hydroxytryptamine.

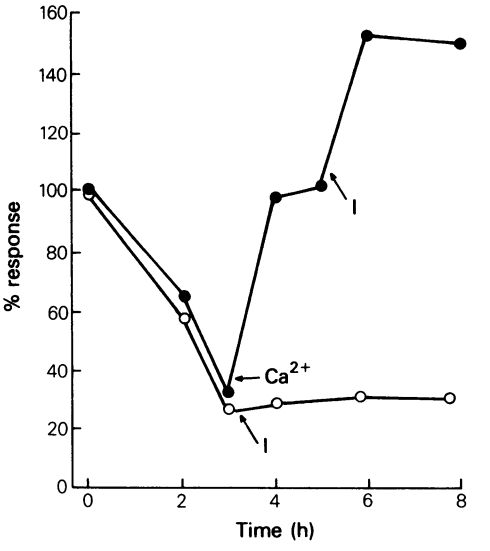

Figure 2 Responses of two helical strips of rabbit mesenteric artery to repeated administrations of adrenaline (1 μM) in calcium-free medium. The arrow marked Ca^{2+} indicates that the calcium concentration in the medium was raised to 1.36 mM and maintained at that level for the rest of the experiment. The arrows marked I indicate that 8.4 μM indomethacin was present in the medium thereafter.

The responses of the mesenteric arteries to 5-hydroxytryptamine were potentiated by indomethacin, whether it was added 1 h before or during the exposure of the tissues to the agonists (Figure 1). Similarly, indomethacin potentiated the responses of both arteries (coeliac and mesenteric) to the other four agonists studied, though to different degrees (see Table 2). In contrast, the contractions produced by increasing the K^+ concentration in the medium to 10 mM were not potentiated by indomethacin. ED_{50} values for K^+ in the absence and in the presence of 8.4 μM indomethacin were, respectively, 45 ± 5 mM and 44 ± 4 mM in the coeliac artery, and 48 ± 9 mM and 36 ± 5 mM in the mesenteric artery ($n = 7$).

Effect of calcium-free medium

When the arteries, after being equilibrated with the normal medium, were exposed to the calcium-free solution, there was an immediate reduction of the contractile responses, followed by a slower decrease in reactivity which reached a stable level, lasting from the third hour after the calcium removal until the end of the experiments, at the eighth hour. If, at any time during the experiment the calcium concentration was raised by exposing the preparations to the normal Tyrode solution, the reactivity returned to its original level. Figure 2 illustrates these observations, for adrenaline in the mesenteric artery. Similar results were obtained with the coeliac artery and with the other agonists. Indomethacin did not potentiate the re-

Table 2 Calcium-dependence and potentiation by indomethacin of the responses of the rabbit coeliac and mesenteric arteries to vasoconstrictor drugs

	Coeliac artery		*Mesenteric artery*	
Drug	% calcium dependence*	% potentiation by indomethacin**	% calcium dependence*	% potentiation by indomethacin**
Histamine	32 ± 1	9 ± 14	27 ± 11	30 ± 4
Adrenaline	31 ± 9	10 ± 4	32 ± 4	10 ± 7
Angiotensin	50 ± 6	19 ± 6	52 ± 6	66 ± 36
5-Hydroxytriptamine	54 ± 9	30 ± 8	36 ± 9	55 ± 5
Acetylcholine	81 ± 13	61 ± 23	52 ± 13	113 ± 40

Average ± s.e.mean from five independent experiments.
*Measured by the response in Ca-free medium relative to that in normal medium in the same preparation.
**Measured by the % increase in response in the presence of 8.4 μM indomethacin.

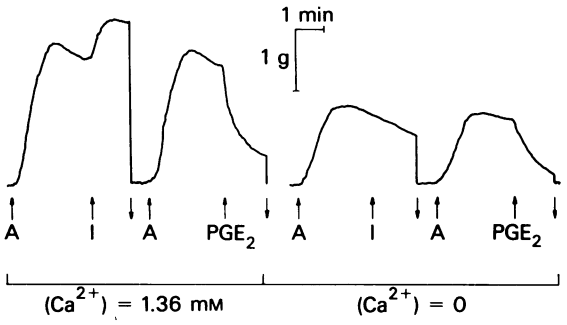

Figure 3 Effect of adding 8.4 μM indomethacin (I) or 2.8 μM prostaglandin E_2 (PGE_2) on the response of the isolated mesenteric artery to 0.3 μM angiotensin (A) in normal Tyrode solution (1.36 mM Ca^{2+}) and in calcium-free medium. Downward arrows indicate washing of the preparation with fresh medium and interruption of chart movement for 1 h.

sponse of the arteries in calcium-free medium to any of the agonists that were studied.

The responses to the various agonists were affected differently by the calcium-free medium, indicating different degrees of dependence on external calcium, which could be estimated by the relative decrease in response observed just after exposure of the arteries to the calcium-free medium. Table 2 shows that, for both the mesenteric and coeliac arteries, the agonists that are more dependent on external calcium for their activity, are also more potentiated by indomethacin. In the coeliac artery there was a linear correlation (correlation coefficient, $r = 0.93$) between the potentiation by indomethacin in normal Tyrode solution and the calcium-dependence of the responses (Table 2). In the mesenteric artery, where there was a narrower range of calcium dependence among the five drugs, at significant linear correlation was also observed ($r = 0.84$).

The effect of the calcium in the medium on the relaxing effect of prostaglandin was studied, using PGE_2. Low concentrations of this compound (in the micromolar range) do not induce measurable changes in the tone of resting isolated arteries, but produce relaxation of arteries that are contracted in response to K^+ or to agonists. This relaxation, by PGE_2 of mesenteric and coeliac arteries contracted by K^+ or by any of the five agonists studied, was observed both in normal and in calcium-free medium (Figure 3).

Effect of sodium-deficient medium

A dose of angiotensin, adrenaline or potassium ions that produced a response in the lower third of the respective dose-response curve was administered, first in presence of normal Tyrode solution (150 mM Na^+) and again after this solution had been replaced by a medium containing 80 mM Na^+ (equilibrated for 60 min). In the low sodium medium, the responses to angiotensin and to adrenaline were increased, while no significant differences were observed in the responses to K^+ (Table 3). The potentiation of the mesenteric artery's responses to angiotensin by indomethacin, which was 51 ± 6% in normal medium, was reduced to 9 ± 3% in low sodium medium (mean ± s.e. mean of 5 experiments). For adrenaline, a similar reduction of the indomethacin potentiation from 66 ± 11% to 10 ± 3% was observed (mean ± s.e., $n = 4$) in low sodium.

Lack of prostaglandin release by angiotensin

We have attempted to detect prostaglandin release by angiotensin in the isolated mesenteric artery using the cascade method and taking advantage of the fact that the chick rectum is particularly sensitive to prostaglandin and insensitive to angiotensin. In this method (Gaddum, 1953), the Tyrode solution superfused the artery first and then the chick rectum preparations mounted in series (Figure 4). First, the sensitivity of the latter preparation to PGE_2 was tested by injecting known concentrations directly above the rectum (bypassing the artery). Superfusion of a dose of angiotensin (0.3 μM) through both the artery and rectum preparations caused maximal contraction of the artery, while no response of the rectum to the perfusate from the arterial strip was detected

Table 3 Effect of reducing the sodium ion concentration in the medium to 80 mM on the responses to vasoconstrictor agents

		*% increase in response**	
Agents	*Concentration* (M)	Coeliac	Mesenteric
Adrenaline	1.5×10^{-7}	29 ± 4	32 ± 5
Angiotensin	5.0×10^{-8}	22 ± 3	15 ± 6
K^+	3.6×10^{-2}	−2 ± 6	−5 ± 5

Average ± s.e.mean from five independent experiments.
*Measured by the response in sodium-deficient medium relative to that in normal medium in the same preparation.

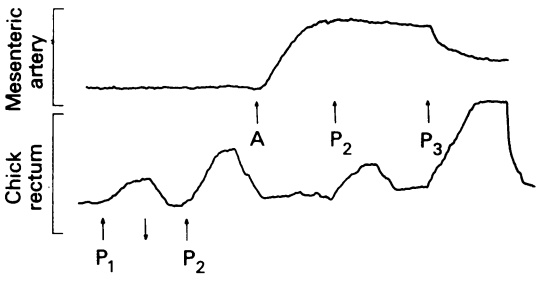

Figure 4 Response of the mesenteric artery and chick rectum preparations mounted in series in the cascade method. Tyrode solution superfused first the artery and then the rectum preparation. Drugs were applied either above the artery (indicated by the arrows below the upper trace) or directly above the rectum, bypassing the artery (indicated by the arrows under the lower trace). A, 0.3 μM angiotensin; P_1, 2.8 nM prostaglandin E_2 (PGE_2); P_2, 5.6 nM PGE_2; P_3. 2.8 μM PGE_2.

($n = 5$). Thus, no prostaglandin release from the arteries treated with angiotensin could be detected. The concentration of exogenous PGE_2 needed to elicit significant relaxation of the angiotensin-contracted artery was found to be three orders of magnitude larger than the limit of detection (1–2 nM) of the chick rectum preparation used in our experiments. Figure 4 shows that a dose of PGE_2 that produced a significant relaxation of the artery preparation during a response to angiotensin, produced a maximum response of the chick rectum.

Discussion

Indomethacin potentiated the responses of the isolated mesenteric and coeliac arteries to the five vasoconstrictor agonists that were studied, but had no effect on the contractile responses to K^+. This indicates that the effect of indomethacin is not a consequence of the smooth muscle's contraction, but appears to be associated with the agonist-receptor-activated chain of events that result in the contractile response.

The first hypothesis to be considered to explain the potentiation by indomethacin of the smooth muscle responses is that agonists, besides triggering the contractile response, also induce the release of prostaglandins of the E type, which in low concentrations, are known to relax arteries that are contracted by agonists or by K^+ (Strong & Bohr, 1967). Indomethacin, by inhibiting prostaglandin synthesis, would decrease the relaxant component of the response resulting in a greater contraction. Some of our findings do not support this hypothesis since the potentiation of the response by indomethacin was not time-dependent, being the same whether the drug was pre-incubated for 1 h or added at the peak of the contraction. Some difference should have been observed since the indomethacin inhibition of prostaglandin synthesis occurs at an early step of the biosynthetic chain from arachidonic acid to prostaglandin, in a time-dependent manner (Smith & Lands, 1971).

Moreover, the contraction of the rabbit coeliac and mesenteric arteries produced by vasoconstrictor substances was not accompanied by detectable prostaglandin release. This is in agreement with previous findings that the release of PGE-like substances by the rabbit mesenteric vascular bed ceased to occur when the small branches of the arterial system were partially removed from the circuit (Grodzinska *et al.*, 1976), indicating that prostaglandin release takes place in the arterioles and capillaries rather than in the arteries.

In addition, in calcium-free medium, in which PGE_2 still has a relaxant effect on the agonist-contracted arteries, indomethacin no longer potentiates the responses to these agonists. Since the extracellular calcium concentration has little effect on prostaglandin biosynthesis. (Oudinet & Hassid, 1983), this finding suggests that the potentiation may be related to a calcium-dependent effect of indomethacin which is not related to its inhibitory action on prostaglandin synthesis.

Indomethacin is known to affect cation transport by the cell, and to inhibit the Ca^+-stimulated (Matsukawa & Takiguchi, 1982) and Na^+-K^+-stimulated (Takahashi, Terao, Hayakawa & Takiguchi, 1982) ATPases of nervous tissue as well as calcium and sodium uptake by smooth muscle cell (Northover, 1971; 1972).

Because of the importance of cation fluxes for smooth muscle contraction, we have investigated whether the effect of indomethacin in the arteries' responses to vasoactive drugs may be due to changes in the translocation of calcium or sodium ions by examining its action in calcium-free medium and in the presence of low sodium concentration.

In calcium-free medium, the contractile response of the arteries depends entirely on the stock of internal calcium, which is sufficient to elicit diminished but reproducible responses to the vasoconstrictor agent for a few hours. Under these conditions, indomethacin did not potentiate the effect of the vasoconstrictor agents, suggesting that the potentiation may involve the component of the response that depends on the influx of external calcium into the cell. Similarly, the agonists that depend more on external Ca^+ for their action, also tend to be more potentiated by indomethacin. However, indomethacin is known to inhibit the net influx of calcium into the cell and it does not seem likely that it may favour calcium entry through receptor-operated calcium channels.

However, receptor-operated calcium channels of

smooth muscles are modulated by sodium ions (van Breemen, Aaronson & Loutzenhiser, 1979), and it is possible that indomethacin may favour calcium influx by acting on sodium translocation. This hypothesis is supported by our results obtained in sodium-deficient medium since lowering the sodium concentration in the medium potentiated the responses of the arteries to vasoconstrictor drugs (Table 3), in agreement with previous findings in the rabbit isolated aorta (Bohr, Brodie & Cheu, 1958). We have also found that, in low sodium medium, indomethacin no longer potentiates those responses. These findings suggest that potentiation by indomethacin of the contractions of coeliac and mesenteric arteries produced by vasoactive substances may be a consequence of its inhibitory effect on sodium influx, resulting in increased calcium entry through receptor-operated channels.

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