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The importance of endogenous prostaglandins other than prostacyclin, for the modulation of contractility of some rabbit blood vessels

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1 Helically cut strips of rabbit aorta, extrapulmonary artery, coeliac artery, and femoral artery were set up in organ baths. Contractions of the strips by noradrenaline and angiotensin II were recorded isotonically. The release of prostaglandins 6-keto-$PGF_{1\alpha}$, E_2, $F_{2\alpha}$, D_2 and thromboxane B_2 from the strips was measured by means of sensitive and specific radioimmunoassays.

2 All blood vessels released a characteristic pattern of cyclo-oxygenase products. Prostacyclin (PGI_2, measured as 6-keto-$PGF_{1\alpha}$) was the major compound formed, followed by smaller amounts of PGE_2 and traces of $PGF_{2\alpha}$, PGD_2 and thromboxane A_2 (measured as thromboxane B_2). The pulmonary and the femoral artery had comparatively high abilities to synthesize PGE_2.

3 Contractions induced by noradrenaline increased prostaglandin release from the pulmonary artery but not from the other blood vessels. Angiotensin II-induced contractions were accompanied by a marked prostaglandin release from the coeliac artery. After angiotensin II, prostaglandin release was also enhanced in the pulmonary artery, but remained essentially unchanged in the aorta and femoral artery.

4 Arachidonic acid markedly increased the levels of all prostaglandin formed.

5 Indomethacin inhibited the formation of all prostaglandins below the detection limits of the respective radioimmunoassays.

6 Indomethacin treatment induced a qualitatively similar shifting of the concentration-response curves of noradrenaline and angiotensin II in some vessels: the concentration-response curves remained unchanged for the aorta, were slightly shifted to the left of the pulmonary artery, were markedly shifted to the left for the coeliac artery, and were shifted to the right for the femoral artery.

7 Exogenous PGI_2 strongly and concentration-dependently inhibited contractions induced by the approximate EC_{50} of noradrenaline in the coeliac artery, but was without effect on the other three preparations. PGE_2 had no effect on noradrenaline-induced contractions of the aorta, inhibited those of the pulmonary and the coeliac artery, but markedly potentiated those of the femoral artery. $PGF_{2\alpha}$ significantly enhanced contractions of the femoral artery, but increased contractions of the other preparations were not significant. PGD_2 was without effect on any preparation.

8 In conclusion, the contractility of the aorta does not seem to be modulated substantially by prostaglandins. The major prostanoid regulating the tone of the coeliac artery was found to be PGI_2. The contractility of the pulmonary and especially the femoral artery is probably not modulated by PGI_2 but rather by PGE_2.

9 These observations suggest that in certain blood vessels, prostaglandins other than PGI_2 are important endogenous modulators of contractility.

Introduction

Prostacyclin (PGI_2) is the major cyclo-oxygenase product generated by blood vessels (Bunting *et al.*, 1976; Moncada *et al.*, 1976). However, other prostaglandins, mainly PGE_2, can also be formed at this site (Wolfe *et al.*, 1979; Sametz & Juan, 1982). Prostaglandins have been implicated as modulators in the action of vasoactive compounds such as noradrenaline and angiotensin II (Messina *et al.*, 1976; Malik, 1978; Moncada & Vane, 1978). Accordingly vasoconstrictor responses to pressor agents are en-

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hanced in several blood vessel preparations, when their endogenous prostaglandin synthesis is blocked by non-steroidal anti-inflammatory drugs like indomethacin. This has been shown e.g. for the mesenteric arteries of lambs and rabbits (Yabek & Avner, 1980; Malik *et al.*, 1976), and for rat aortic strips (Altura & Altura, 1976). PGI_2 could be implicated in these effects since many smooth muscle preparations are relaxed by PGI_2 (Moncada & Vane, 1979). However, some preparations, like rabbit aortic strips, do not respond to PGI_2 (Omini *et al.*, 1977; Furchgott & Zawadzki, 1980) or like the porcine coronary artery (Dusting *et al.*, 1977) and some human and rat veins (Levy, 1978) are even contracted by PGI_2. Similarly the vasoconstrictor responses of some vessels to agonists are not changed after inhibition of cyclo-oxygenase by non-steroidal anti-inflammatory drugs, e.g. the vascular bed of the rabbit kidney (Fink *et al.*, 1977) and the rabbit hindlimb (Gottlieb *et al.*, 1980). In some blood vessels, like the rat portal vein, contractions are even diminished after cyclo-oxygenase inhibition (Altura & Altura, 1976). As non-steroidal anti-inflammatory drug always inhibit the synthesis of all endogenous cyclo-oxygenase products, prostaglandins other than prostacyclin may be involved in this effect.

In the present study we have investigated the effects of indomethacin on contractions induced by two different pressor agents (noradrenaline and angiotensin II) in four different rabbit blood vessels, selected for their different reactivity to cyclo-oxygenase inhibition. Furthermore the cyclo-oxygenase products released by the preparations have been measured and the biological responses to these compounds when given exogenously have been tested on the vessel preparations. An attempt was made to identify the endogenous prostanoid mainly responsible for the modulation of contractility in each individual blood vessel.

A preliminary account of these results has appeared in abstract form (Förstermann & Neufang, 1983).

Methods

Rabbits of either sex (2.5–3.5 kg body weight) were killed by a blow on the head and exsanguinated from the carotic arteries. Then the thoracic aorta, a 1 cm portion of the pulmonary artery taken about 0.5 cm distal to the heart (referred to as the extrapulmonary artery), the coeliac artery, and a femoral artery were dissected out. Care was taken during the following preparation not to disturb the endothelium. The blood vessels were cut into helical strips of about 2 mm × 12 mm (Furchgott, 1960). They were suspended in 3.5 ml organ baths containing Krebs-bicarbonate solution at 37°C as previously described (Förstermann & Hertting, 1979; Simmet *et al.*, 1980). The Krebs-bicarbonate solution had the following composition (mM): NaCl 120.0, KCl 4.75, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, CaCl 1.7, glucose 6.4, and ascorbic acid 0.1. The solution was continuously bubbled with 5% CO_2 in O_2, the pH was 7.4. Contractions of the strips were recorded isotonically with a load of 1.5 g on the aorta and pulmonary artery, 0.5 g on the coeliac artery, and 0.2 g on the femoral artery. The bath fluid was changed every 6 min throughout the experiment. After an equilibration period of about 1 h a stable baseline tone was reached. Then contractions were elicited by noradrenaline (3×10^{-9} M–3×10^{-5} M) or by angiotensin II (10^{-9} M–10^{-5} M). Noradrenaline was dissolved and diluted in 0.001 N HCl and added to the bath in a volume of 50 µl. Angiotensin II was dissolved and diluted in 0.15 M phosphate buffer and added to the bath in the same volume. Contractions were induced at 42 min intervals (7 wash-out periods). When generating concentration-response curves the different concentrations were given at random. Indomethacin was dissolved in 0.15 M phosphate-buffer and added to the Krebs-bicarbonate solution in a concentration of 3×10^{-6} M. When the effect of indomethacin on contractions was tested the medium was changed to the indomethacin-containing solution 24 min (4 wash-out periods) before the next contraction. The inhibitor then remained in the incubation medium until the end of the experiment. Arachidonic acid was dissolved in nitrogen-saturated ethanol (stock solution 10 mg ml^{-1}) and diluted with 20 mM Na_2CO_3 to form the sodium salt. It was added to the bath medium in 50 µl, 1 min after wash-out. The final ethanol concentration resulting in the bath was 0.01%. When exogenous prostaglandins were tested for their modulatory role on contraction, indomethacin (3×10^{-6} M) was present in the bath medium to exclude possible indirect effects, e.g. due to thromboxane formation as recently described (Borda *et al.*, 1983). The prostaglandins were added to the bath in 50 µl immediately after changing the bath fluid, i.e. 1 min prior to the contraction agonist. The prostaglandin vehicles were added to the bath before control contractions. Stock solutions of PGE_2, $PGF_{2\alpha}$, PGD_2, and thromboxane B_2 (1 mg ml^{-1}) were prepared in 70% ethanol and diluted with 2 mM Na_2CO_3. PGI_2 was dissolved and diluted in 10 mM Tris-buffer (pH 10.0). When two control contractions with 3×10^{-7} M noradrenaline had given reproducible responses, prostaglandins to be tested were added to the bath fluid 1 min before the next contraction. Bath fluid samples were collected separately and stored frozen until assayed for prostaglandin and thromboxane content. The details of the five radioimmunoassays developed in our laboratory have been described (Jobke *et al.*, 1973; Peskar & Hertting, 1973; Anhut *et al.*, 1977; 1978; Machleidt *et al.*,

1981). The antibodies used have been previously characterized and found to be highly specific for their respective antigens. Charcoal was used to separate free and antibody-bound fractions. The sensitivities of the assays (detection limits defined as 10% displacement of tracer) were as follows: 11 pg ml^{-1} for 6-keto-$PGF_{1\alpha}$, 4 pg ml^{-1} for PGE_2, 4 pg ml^{-1} $PGF_{2\alpha}$, 7 pg ml^{-1} for PGD_2 and 7 pg ml^{-1} for thromboxane B_2. To be able to determine the whole spectrum of cyclo-oxygenase under basal conditions and after stimulation with contractile agonists or arachidonic acid, the respective bath fluid samples of two consecutive contractions were pooled to obtain enough material (> 5 ml). As both the basal and the stimulated prostaglandin release had a tendency to decrease with time, all determinations were done in pooled samples taken at the same times, i.e. 102 min and 144 min after the beginning of the experiment. In some radioimmunoassays, arachidonic acid caused a small nonspecific crossreaction. Prostanoid levels determined in the presence of arachidonic acid were always corrected for this interference.

Differences in contraction amplitude were tested for statistical significance using Student's *t* test or, when comparing to 100% values without s.e.mean, a parameter test.

Materials

PGD_2, PGE_2 and $PGF_{2\alpha}$ were purchased from Sigma, Munich, F.R. Germany. PGI_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 were generous gifts of Dr J. Pike, Upjohn Co., Kalamazoo, MI, U.S.A. Arachidonic acid (purity > 99%) was from Sigma, Munich, F.R. Germany. (−)-Noradrenaline bitartrate was purchased from Merck-Schuchardt, Munich, F.R. Germany. Angiotensin II-amide 5-valine (Hypertensin) was a generous gift of Ciba, Basle, Switzerland. Indomethacin was donated by MSD Sharp & Dohme, Munich, F.R. Germany. All concentrations given refer to the free bases or acids respectively. Radiolabelled prostaglandins for the radioimmunoassays ([5,6,8,9,12,14,15-^{3}H]-PGD_2, sp. act. 100 Ci $mmol^{-1}$; 5,6,8,11,12,14,15-^{3}H]-PGE_2, sp. act. 160 Ci $mmol^{-1}$; 5,6,8,9,11,12,14,15-^{3}H]-$F_{2\alpha}$, sp. act. 150 Ci $mmol^{-1}$; [5,8,9,11,12,14,15-^{3}H]-6-keto-$PGF_{1\alpha}$, sp. act. 120 Ci $mmol^{-1}$; 5,6,8,9,11,12,14,15-^{3}H]-thromboxane B_2, sp. act. 155 Ci $mmol^{-1}$) were purchased from New England Nuclear, Dreieich, F.R. Germany.

Results

Prostaglandin release from blood vessel strips

When suspended in vessel chambers all four strips continuously released a characteristic pattern of cyclo-oxygenase products into the bath medium. 6-Keto-$PGF_{1\alpha}$ was the major compound synthesized by all four blood vessels followed by smaller amounts of PGE_2 and even lower quantities of the other cyclo-oxygenase products. The crossreactivity of 6-keto-$PGF_{1\alpha}$ in our radioimmunoassay for PGE_2 was about 1%. Therefore the predominant 6-keto-$PGF_{1\alpha}$-like material is unlikely to account for significant amounts of PGE_2-immunoreactivity under our experimental conditions. The basal formation of prostanoids was comparatively high in the pulmonary artery and the coeliac artery but lower in the aorta and the femoral artery (Figure 1a–d).

When the blood vessels were contracted with noradrenaline (3×10^{-7} M) prostaglandin release increased about two fold in the pulmonary artery (Figure 1b) but was not significantly changed in the other preparations (Figure 1a, c, d). When angiotensin II was used as the contractile agonist it produced a strong increase in prostaglandin release from the coeliac artery, with a predominant enhancement of the 6-keto-$PGF_{1\alpha}$-peak (Figure 1c). This release was even greater than that produced by a relatively high concentration of arachidonic acid (see below). Angiotensin II also increased the prostaglandin release from the pulmonary artery (Figure 1b). In the aorta and the femoral artery there was no significant increase in prostaglandin release during angiotensin II-induced contractions (Figure 1a, d). Higher concentrations of both pressor agonists were tested, but did not release larger amounts of prostaglandins from the tissues.

When the precursor of bis-enoic prostaglandins, arachidonic acid (3×10^{-6} M), was added to the bath medium it greatly increased prostaglandin synthesis in all preparations. It enhanced the levels of most prostaglandins determined to a reliable detection level and thus gave more precise information about the relative ability of each blood vessel strip to synthesize prostaglandins. The pulmonary and the femoral artery produced relatively more PGE_2 in comparison to 6-keto-$PGF_{1\alpha}$ than the two other preparations both under basal conditions and after arachidonic acid (Figure 1a–d).

Effects of indomethacin

Indomethacin in a bath concentration of 3×10^{-6} M decreased both the basal and the stimulation-induced release of all prostaglandins measured to below the detection limits of the respective radioimmunoassays. Furthermore, both noradrenaline and angiotensin II-induced contractions were modulated in some preparations in the presence of indomethacin. Concentration-response curves of both noradrenaline and angiotensin II for the aorta remained unchanged after indomethacin treatment (Figure 2a and 3a). Essentially similar results were obtained for the pulmonary artery. However, in some experi-

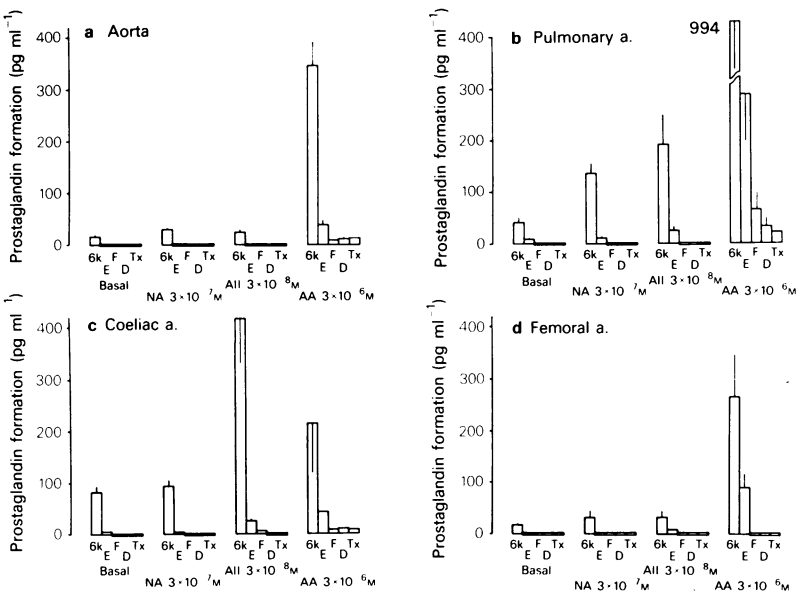

Figure 1 The pattern of cyclo-oxygenase products released by four different rabbit blood vessel strips (a–d) under basal conditions ($n = 14$), during contractions induced by 3×10^{-7} M noradrenaline (NA, $n = 4$), during contractions induced by 3×10^{-8} M angiotensin II (A II, $n = 4$) and after addition of 3×10^{-6} M arachidonic acid (AA, $n = 4$) to the bath medium. Columns represent the mean with s.e.mean (vertical lines) (6k: 6-keto-$PGF_{1\alpha}$-immunoreactivity; E: PGE_2-immunoreactivity; F: $PGF_{2\alpha}$-immunoreactivity; D: PGD_2-immunoreactivity; Tx: thromboxane B_2-immunoreactivity).

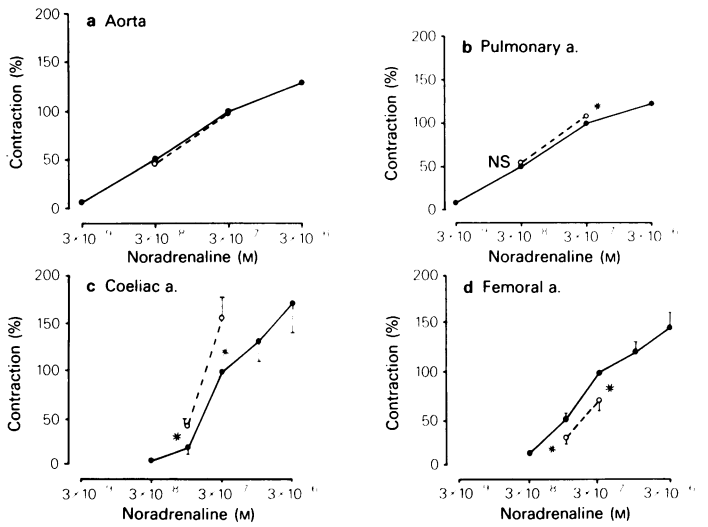

Figure 2 Concentration-response curves for noradrenaline-induced contractions of four different rabbit blood vessel strips (a–d, continuous lines) and the effect of indomethacin (3×10^{-6} M, broken lines). Contractions induced by 3×10^{-7} M noradrenaline were taken as 100%. All other contractions were related to this value. Each value is the mean from 4–6 experiments; vertical lines show s.e.mean. Asterisks indicate significant differences ($P < 0.05$) of contractions in indomethacin containing medium from those in normal medium, NS: not significant.

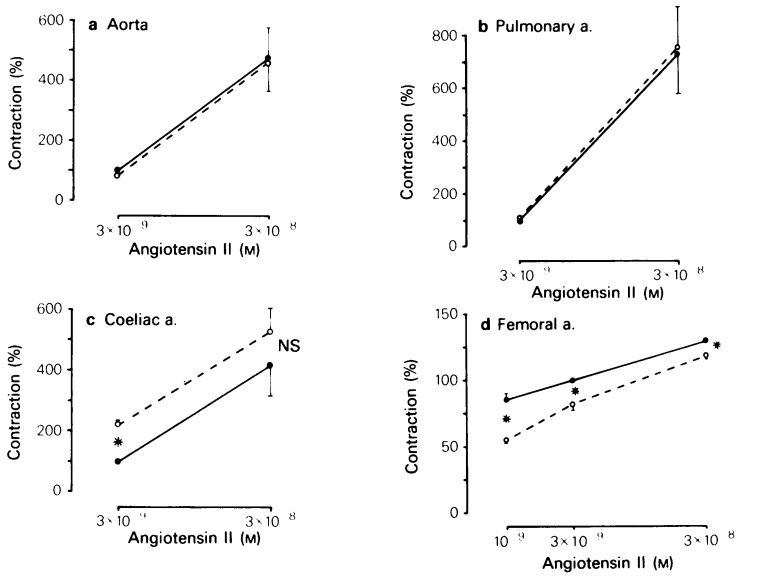

Figure 3 Concentration-response relationship for angiotensin II-induced contractions of four different rabbit blood vessel strips (a–d, continuous lines) and the effect of indomethacin (3×10^{-6} M, broken lines). Contractions induced by 3×10^{-8} M angiotensin II were taken as 100%. The other contraction amplitudes were related to this value. As the magnitude of angiotensin II-induced contractions was very low in the pulmonary artery (b) these data should only be taken as rough estimates. Each value is the mean of 4–6 experiments; vertical lines show s.e.mean. Asterisks indicate significant differences ($P < 0.05$) of contractions in indomethacin containing medium from those in normal medium, NS: not significant.

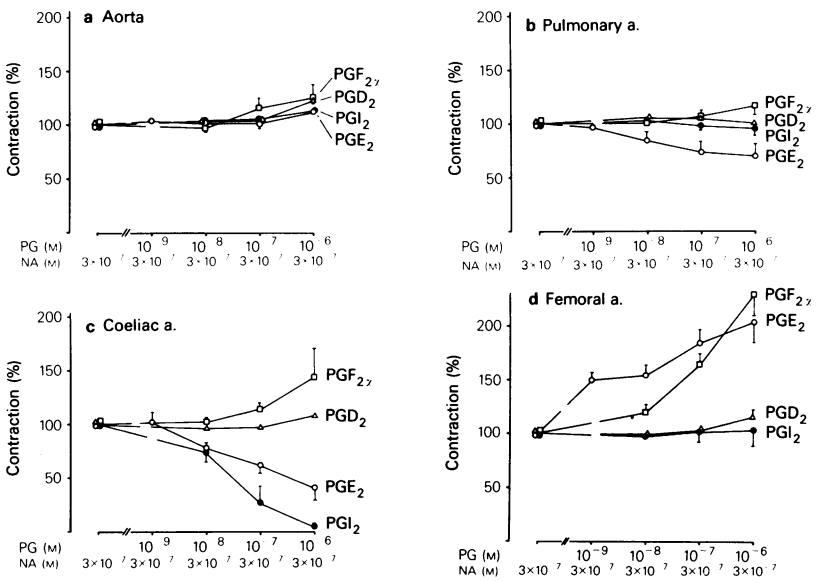

Figure 4 Effect of different concentrations of prostaglandins PGD_2 (△), PGE_2 (○), $PGF_{2\alpha}$ (□), and PGI_2 (●) on noradrenaline (NA, 3×10^{-7} M)-induced contractions in four different rabbit blood vessels (a–d). Indomethacin (3×10^{-6} M) was present in all experiments. Contractions with 3×10^{-7} M noradrenaline alone were taken as 100%. Each value is the mean of 5–6 experiments; vertical lines show s.e.mean.

ments there was a slight shift of the noradrenaline concentration-response curve to the left (Figure 2b). On the other hand the dose-response curves of both pressor agonists were markedly shifted to the left for the coeliac artery (Figures 2c and 3c) whereas the concentration-response curves for both noradrenaline and angiotensin II were significantly shifted to the right for the femoral artery (Figure 2d and 3d).

Effects of exogenous prostaglandins on contractions

In order to investigate which of the endogenous prostaglandins formed might be responsible for the effects observed in the presence of indomethacin, the effects of exogenous prostaglandins were tested on contractions induced by 3×10^{-7} M noradrenaline in the presence of indomethacin (3×10^{-6} M). This concentration of noradrenaline was found to be approximately the EC_{50} for contractions in all four preparations.

Contractions of the aorta were not affected by any of the prostaglandins tested (Figure 4a). At the highest concentration tested (10^{-6} M) all cyclo-oxygenase products induced slight increases in tone which, however, were not statistically significant (Figure 4a). Contractions of the pulmonary artery remained essentially unchanged in the presence of even high concentrations of PGI_2 and PGD_2. $PGF_{2\alpha}$ slightly enhanced the tone at 10^{-6} M, whereas PGE_2 dose-dependently inhibited noradrenaline-induced contractions (Figure 4b). Contractions of the coeliac artery were not affected by PGD_2. They were slightly enhanced by high doses (10^{-6} M) of $PGF_{2\alpha}$; however, this effect was inconsistent and not statistically significant. PGE_2 and PGI_2 dose-dependently relaxed the artery. PGI_2 was by far the most potent agent in this respect, at the highest concentration used (10^{-6} M) it completely supressed the noradrenaline-induced contractions (Figure 4c). Agonist-induced contractions of the femoral artery remained unchanged in the presence of even high concentrations (10^{-6} M) of PGI_2 and PGD_2. However, large, dose-dependent increases in concentration amplitude (up to more than 200% of control) were elicited by both PGE_2 and $PGF_{2\alpha}$ (Figure 4d). At concentrations as low as 10^{-7} M these two prostaglandins produced small contractions by themselves in this tissue.

Discussion

Our results provide evidence that endogenous prostaglandins other than PGI_2 are involved in the modulation of contractility of some blood vessels. Although all four vessels tested made predominantly PGI_2 they all also had the capacity to form PGE_2 and traces of $PGF_{2\alpha}$, PGD_2 and thromboxane A_2. This ability to synthesize prostaglandins was best demonstrated when the strips were incubated with arachidonic acid, the precursor of bis-enoic prostaglandins (Figure 1a–d). The fact that under basal conditions and in the presence of noradrenaline and agiotensin II the levels of PGE_2 and the other non-prostacyclin prostaglandins were near to, or even below the detection limits of our assays in some incubates, does not exclude the possibilty that even these small concentrations could have modulatory effects. When the endogenous prostaglandin synthesis of the strips was blocked by indomethacin, the concentration-response curves for both noradrenaline- and angiotensin II-induced contractions were shifted similarly and independently of the contractile agonist used (Figures 2 and 3). It appears that the basal concentrations of the prostaglandins formed are sufficient to modulate the contractions, since noradrenaline did not stimulate prostaglandin release in the coeliac and femoral artery and angiotensin did not change the release of prostaglandins in the femoral artery.

Contractions of the rabbit aorta were not changed after indomethacin treatment (Figures 2a and 3a). However, this does not seem to be a general phenomenon as non-steroid anti-inflammatory drugs have been reported to inhibit contractions induced by pressor hormones in rat aortic strips (Altura & Altura, 1976). The ineffectiveness of indomethacin in rabbit aortic strips might be explained by their relatively small capacity to synthesize endogenous prostaglandins (Figure 1a). However, this possibility can be excluded since none of the exogenous prostaglandins tested had important effects on contractions of this tissue (Figure 4a), indicating a low sensitivity towards prostaglandins.

Somewhat similar findings were obtained for the pulmonary artery. In the presence of indomethacin, its contractility remained unchanged or was only slightly increased (Figure 2b) although this blood vessel had a relative high capacity to synthesize prostaglandins (Figure 1b). As exogenous PGD_2, $PGF_{2\alpha}$, and PGI_2 were essentially ineffective, the slight increase in contraction after indomethacin treatment can be explained by an inhibition of the synthesis of PGE_2. Indeed this prostaglandin caused a moderate decrease in contraction amplitude when added to the bath medium (Figure 4b). Salzman *et al.* (1980) demonstrated similar effects of prostaglandins on intrapulmonary arteries: PGI_2 had no consistent effect, $PGF_{2\alpha}$ slightly contracted the tissue, but PGE_2 always relaxed the preparation. However, these authors found a marked formation of thromboxane in intrapulmonary arteries after treatment with arachidonic acid, which increased the tone of the preparation. Extrapulmonary arteries were reported to produce lower amounts of thromboxane relative to

PGI_2. In our hands, treatment of the extrapulmonary arteries with arachidonic acid did not produce contractions and only very small amounts of thromboxane B_2 could be detected (Figure 1b). As we used a portion of pulmonary artery very close to the heart, this discrepancy might be explained by an increasing ability of pulmonary artery to synthesize thromboxane with increasing distance from the heart.

The concentration-response curves for contractions of the coeliac artery were markedly shifted to the left by indomethacin (Figures 2c and 3c). Thus the rabbit coeliac artery behaves similarly to rabbit and lamb mesenteric arteries. In these preparations indomethacin has also been reported to enhance vasoconstrictor responses to adrenergic stimuli (Malik *et al.*, 1976; Yabek & Avner, 1980). In another study with rabbit mesenteric artery strips this enhancement could not be demonstrated (Simmet & Hertting, 1980). However, noradrenaline concentrations used in that study were higher (1.5×10^{-6} M–6×10^{-6} M) than the ones used here (3×10^{-8} M–3×10^{-7} M) and thus concentrations might have been less amenable to alteration. The rabbit coeliac artery was found to have a relatively high ability to synthesize prostaglandins (Figure 1d). Exogenous PGI_2 potently relaxed the blood vessel and could completely abolish noradrenaline-induced contractions at 10^{-6} M (Figure 4c). This is in agreement with data originally obtained by Bunting *et al.* (1976). PGE_2 also inhibited contractions but was less potent (Figure 4c). The contractile effect of 10^{-6} M $PGF_{2\alpha}$ was not statistically significant and as the endogenous synthesis of $PGF_{2\alpha}$ is comparatively low (Figure 1c) this is unlikely to be of biological importance.

Contractions of the femoral artery were inhibited by indomethacin (shifting of the concentration-response curve to the right, Figures 2d and 3d). This cannot be explained by an inhibition of the synthesis of PGI_2 or PGD_2 as both compounds proved to be essentially ineffective when given exogenously. On the other hand, PGE_2 and $PGF_{2\alpha}$ potently increased contractions of this preparation (Figure 4d). Although the total capacity of the femoral artery to synthesize prostaglandins was rather low (Figure 1d) it had a relatively high capacity to synthesize PGE_2 as compared to 6-keto-$PGF_{1\alpha}$ (Figure 1d). Furthermore this tissue proved to be highly sensitive to the action of PGE_2. PGE_2 markedly potentiated contractions even at a concentration of 10^{-9} M (Figure 4d). As endogenous prostaglandins can be expected to reach their site of action more easily and thus act at lower bath concentrations than exogenous ones (Simmet *et al.*, 1980), the most likely explanation for the effect of indomethacin in this preparation is the inhibition of the synthesis of endogenous PGE_2. $PGF_{2\alpha}$, which also contracted this preparation when given exogenously, is obviously formed in much lower concentrations (Figure 4d) and thus is probably less important as an endogenous modulator of contractility.

Taken together our findings demonstrate that the tone of the rabbit aorta is not modulated by endogenous prostaglandins. The extrapulmonary artery is rather insensitive to prostaglandin modulation; however, its tone might be slightly decreased by endogenous PGE_2. The coeliac artery is mainly under the relaxing influence of endogenous PGI_2. Finally, the normal tone of the femoral artery seems to be augmented by endogenous PGE_2. It is interesting to note that the two blood vessels which are probably modulated by PGE_2 (the extrapulmonary and femoral artery), also form more PGE_2 relative to PGI_2 than the other two preparations. In conclusion, evidence has been obtained that the tone of some blood vessels can be modulated by prostaglandins other than PGI_2. Care should be taken not to interpret all changes in vascular tone following cyclo-oxygenase inhibition solely to blockade of the synthesis of PGI_2.

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