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Feedback control of arterial smooth muscle tone: the role of prostacyclin

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1 Indomethacin potentiated noradrenaline (NA)-induced contractions of rabbit isolated mesenteric arteries. Mechanical removal of the endothelium did not influence its potentiating effect.

2 Prostacyclin (PGI_2) synthesis was stimulated by NA (0.1–1.0 $\mu mol\,l^{-1}$) and inhibited by indomethacin in a concentration-dependent manner.

3 There was a positive correlation between the indomethacin-induced inhibition of PGI_2 formation and the indomethacin-evoked potentiation of contractile responses to NA.

4 These results suggest that endogenous PGI_2 plays a part in the feedback control of vascular smooth muscle tone.

Introduction

Inhibition of prostacyclin (PGI_2) synthesis has been reported to enhance contractile responses of rabbit mesenteric arteries to noradrenaline (NA) (Malik *et al.*, 1976; Armstrong & Thirsk, 1979; Hadházy *et al.*, 1984). In addition, NA may stimulate the formation of prostacyclin by rabbit mesenteric blood vessels (Pipili & Poyser, 1981). We observed that contractile responses of rabbit mesenteric arterial strips to transmural electrical stimulation increased gradually during the experiment to become maximal after about 3 h (unpublished finding). On the other hand, PGI_2 production by vascular tissue is high at the beginning of the experiment and then declines gradually (Desjardins-Giasson *et al.*, 1982). These results prompted us to study the relationship between prostacyclin formation and contractile responsiveness by measuring these two parameters simultaneously.

Methods

Helical strips of rabbit mesenteric arteries were set up for isometric recording (Hadházy *et al.*, 1984). The endothelial layer was removed mechanically by use of a fine artist's brush within 20–25 s. A group of strips was prepared taking special care not to touch the intimal surface (strips with intact endothelium). These strips were readily relaxed by acetylcholine (Furchgott & Zawadzki, 1980), whereas those without endothelium were not. The presence of endothelial cells was confirmed histologically. Contractile responses to NA (0.03, 0.1 and 0.3 $\mu mol\,l^{-1}$) were determined both in the absence and presence of indomethacin (1 $\mu mol\,l^{-1}$).

The synthesis of prostacyclin was measured by radioimmunoassay (RIA). Samples collected every 10 min were stored at −30°C until their 6-keto-prostaglandin $F_{1\alpha}$ (6-K-PG) content was determined. This hydrolysis product of PGI_2 was measured by 6-K-PG^{125}I RIA kit (Institute of Isotopes, Budapest). Assay detection limit (10% displacement of tracer) was about 5 pg of 6-K-PG ml^{-1}, and 50% displacement of ^{125}I-6-K-PG was obtained with about 25 pg of 6-K-PG ml^{-1}. Authentic 6-K-PG was supplied by the Upjohn Company and the antisera against 6-K-PG was prepared in the Research Institute of Experimental Medicine (Budapest). The cross-reactivity of 6-K-PG antibody was 0.8% for $PGF_{1\alpha}$, 1.4% for PGE_2 and 1.2% for PGE_1. Intra- and inter-assay coefficients of variation were 5–12% and 13–20%, respectively. The amount of 6-K-PG was expressed as pmol g^{-1} min^{-1}.

Results

The contractile responses of the arterial strips to repeated injections of NA (0.1 $\mu mol\,l^{-1}$ evey 20 min) were small at the beginning of the measurements (90 min after placing the tissues in the baths) then increased gradually. By contrast, the formation of PGI_2 by the arteries was high during the initial phase

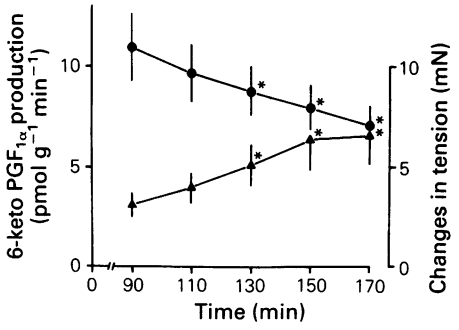

Figure 1 Time-dependent changes in 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) production (●) and in the noradrenaline (0.1 μmol l^{-1}) induced contractions (▲) of rabbit isolated mesenteric arteries ($n = 6$ for both groups). Vertical lines denote s.e.mean. 0 min indicates the time of the suspension of the tissues. *Significantly different from the initial (90 min) value ($P < 0.05$, Student's paired t test).

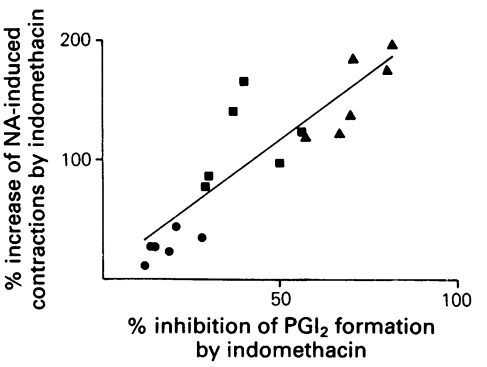

Figure 2 Correlation between the cyclo-oxygenase-inhibitory and the contraction-potentiating effects of indomethacin, 0.07 (●), 0.28 (■) and 1.12 (▲) μmol l^{-1}, in strips of rabbit mesenteric arteries. $y = 0.3487x + 8.6849$; $r = 0.88$. $P < 0.001$.

of the experiments then declined as a function of time. These data are demonstrated in Figure 1.

In 6 strips, NA at concentrations of 0.03, 0.1 and 0.3 μmol l^{-1} produced 2.6 ± 0.4, 9.2 ± 1.7 and 20.1 ± 2.7 mN (mean ± s.e.mean) increase in tension. These contractions were enhanced by indomethacin (1 μmol l^{-1}) by 142, 59 and 20%, respectively. The effect of removing endothelium on indomethacin-induced potentiation was studied in 2 groups of strips ($n = 6$ for both). The tone generated by the vessels with intact endothelium was 9.2 ± 1.5 mN in the presence of NA (0.1 μmol l^{-1}) and the tension produced by NA plus indomethacin (1.0 μmol l^{-1}) was 15.9 ± 2.5 mN (76 ± 8% potentiation). In endothelium-denuded strips, the tone induced by NA and NA plus indomethacin was 8.8 ± 1.4 and 15.3 ± 1.9 mN, respectively (80 ± 10% potentiation). Thus, the contraction-potentiating effect of indomethacin was not altered by the removal of endothelium.

NA, in concentrations of 0.1, 0.3 and 1.0 μmol l^{-1}, increased 6-K-PG release (pmol g^{-1} min^{-1}) from 10.0 ± 1.6 to 15.1 ± 2.9 ($n = 6$, $P < 0.05$), from 9.6 ± 1.5 to 19.1 ± 3.2 ($n = 5$, $P < 0.05$) and from 9.2 ± 1.6 to 25.4 ± 5.1 ($n = 6$, $P < 0.05$), respectively.

Indomethacin, 0.07, 0.28 and 1.12 μmol l^{-1}, potentiated the contractile responses to NA (0.1 μmol l^{-1}), and inhibited PGI_2 production in a concentration-dependent manner. These results are presented in Table 1. When the percentage potentiation was plotted linearly against percentage inhibition of PGI_2 synthesis, a positive correlation was found (Figure 2).

Table 1 Effects of indomethacin on the synthesis of prostacyclin (PGI_2) and on the noradrenaline (NA)-induced contractions of rabbit isolated mesenteric arteries ($n = 6$ for each group)

Effect of indomethacin	*Concentration of indomethacin* (μmol l^{-1}) 0.07	0.28	1.12
Inhibition of PGI_2 formation	18.2 ± 2.4	40.3 ± 4.4	71.0 ± 3.6
Increase in NA induced contraction	28.0 ± 4.7	115.0 ± 14.0	154.0 ± 14.0

Values are percentage changes (± s.e.mean). The amount of PGI_2 formed at the beginning of the measurements was (pmol g^{-1} min^{-1}): 10.3 ± 1.1 ($n = 18$). The tension induced by NA (0.03 μmol l^{-1}) in the absence of indomethacin was (mN ± s.e.mean): 3.0 ± 0.3 ($n = 18$).

Discussion

The results presented in this paper indicate that the smooth muscle of the rabbit mesenteric artery is under feedback control which is mediated, at least in part, by PGI_2. We assume that NA, while contracting arterial smooth muscle cells, stimulates the production of relaxant prostaglandins (mainly PGI_2) which in turn reduce the contractile response to the adrenergic transmitter. This hypothesis is supported by several observations. Firstly, NA is capable of stimulating the synthesis of PGI_2 by rabbit mesenteric blood vessels (Pipili & Poyser, 1981; this paper). Secondly, indomethacin and other cyclo-oxygenase inhibitors enhance adrenergically evoked contractions of rabbit mesenteric arteries (Malik *et al.*, 1976; Armstrong & Thirsk, 1979; Hadházy *et al.*, 1984). Thirdly, vascular smooth muscle cells synthesize substantial amounts of prostacyclin (Baenzinger *et al.*, 1979; Larrue *et al.*, 1980). This also explains the lack of effect of endothelium removal on the potentiation produced by indomethacin in the present study. Fourthly, adrenergically induced contractions of rabbit mesenteric arteries can be readily inhibited by nanomolar concentrations of PGI_2 (Hadházy *et al.*, 1984). Fifthly, similar concentrations of indomethacin will reduce the release of PGI_2 and enhance the contractile responses of vascular tissues to NA (this paper). Finally, there is a close correlation between the cyclo-oxygenase-inhibitory effect of indomethacin and its potentiation of contractions (Figure 2). Nevertheless, there is no direct evidence that the two actions of indomethacin (inhibition of cyclo-oxygenase and potentiation of contractions) are causally related. Inhibition of synthesis should precede the increase in contractility if the former were the cause of the latter. In addition to PGI_2, other prostaglandins, such as PGE_2, may also be involved in the potentiation produced by indomethacin (Armstrong, 1982; Förstermann *et al.*, 1984).

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