Effects of Prostaglandin F\textsubscript{2\alpha} on Vasoconstrictor Responses in the Mesenteric Vascular Bed

PATRICIA M. ARISTEGUI AND MARÍA AMELIA ENERO

SUMMARY Prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}) in a concentration that did not induce vascular contraction (10^{-8} M) potentiated the dose-response curves to norepinephrine and 5-hydroxytryptamine and the contractile response induced by potassium (60 or 100 mM) in isolated mesenteric vascular bed of the rat. After prostaglandin inhibitor treatment with indomethacin (10^{-6} M), the dose-response curve to norepinephrine was reduced, and the dose (10^{-10} M) of PGF\textsubscript{2\alpha}, which was ineffective in control tissues, facilitated the norepinephrine contractile response. In contrast, indomethacin did not change either the contractile response induced by potassium or the PGF\textsubscript{2\alpha} potentiation of this response. Calcium antagonists diltiazem or flunarizine reduced the potassium-induced contractile response. After diltiazem treatment, 10^{-10} M of PGF\textsubscript{2\alpha} was also effective in facilitating this response. The PGF\textsubscript{2\alpha} postjunctional effect was conserved after phosphoinositide hydrolysis inhibition. These results suggest that PGF\textsubscript{2\alpha} potentiation of the contractile response may be independent of PGF\textsubscript{2\alpha} contraction. Low doses of endogenous prostaglandins could be able to facilitate the norepinephrine contractile response in this tissue. This process may be independent of calcium influx and phosphoinositide hydrolysis. (Hypertension 11 [Suppl I]: I-108-I-111, 1988)

KEY WORDS: antagonists • prostaglandins • postjunctional effect • phosphoinositide hydrolysis • Ca\textsuperscript{2+} • norepinephrine • 5-hydroxytryptamine

Materials and Methods

Wistar rats (250 g) were anesthetized by ether and the mesenteric vascular bed was quickly removed. The complete vascular bed was isolated according to the method described by McGregor\textsuperscript{6} and perfused at the rate of 2 ml/min with modified Krebs solution previously gassed with a 95% O\textsubscript{2}, 5% CO\textsubscript{2} mixture. The whole system was maintained at 37°C. Perfusion pressure was monitored by a Statham pressure transducer P23 AC coupled to a Grass pen recorder (Model 7, Quincy, MA, USA). After an equilibration period of 60 minutes, dose-response curves to NE and 5-HT were obtained by injecting the agonist (0.1 ml) into the perfusion stream. The depolarized medium (60 or 100 mM potassium solution) was perfused into the Krebs solution until the steady state of the contractile response was attained. Three consecutive dose-response curves or potassium-stimulated responses were developed at 30-minute intervals in each experiment. Control experiments were performed and showed no significant differences among the three curves or potassium-stimulated responses.

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Results are expressed as the mean ± SEM. Differences between means were evaluated for statistical significance by using paired Student's t test.

The drugs used were norepinephrine (Sigma Chemical), 5-hydroxytryptamine (Sigma Chemical), prostaglandin F<sub>2α</sub> (Syntial Laboratory), diltiazem (Laplex S.A.), flunarizine (Janssen Pharmaceutica), and 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) (Sigma Chemical).

**Results**

The maximum contractile responses to NE and 5-HT were 93.4 ± 5.5 mm Hg (n = 7) and 26.4 ± 8.2 mm Hg (n = 5), respectively. The concentrations of PGF<sub>2α</sub> used, 10<sup>-10</sup> and 10<sup>-8</sup> M, did not induce vascular contractions. When the tissues were perfused with 10<sup>-10</sup> M of PGF<sub>2α</sub> the dose-response curves to NE and 5-HT did not change, whereas 10<sup>-8</sup> M facilitated both contractile responses assayed, increasing the maximum of the dose-response curves by 30 to 40% (Figure 1). The potentiation observed on the contractile response to NE was maintained at low calcium concentration (0.5 mM) in the perfused Krebs solution (data not shown).

The contractile responses induced by potassium, 60 or 100 mM, perfused until its steady state were 46.5 ± 3.6 mm Hg (n = 4) and 125.3 ± 12.6 mm Hg (n = 4), respectively. When the tissues had previously been perfused with 10<sup>-10</sup> M of PGF<sub>2α</sub> the contractile response did not show significant changes, while 10<sup>-8</sup> M of PGF<sub>2α</sub> potentiated the contractile responses to 60 and 100 mM potassium by 40% and 60%, respectively (Table 1).

The inhibition of cyclooxygenase pathways with 10<sup>-6</sup> M of indomethacin reduced the maximum contractile response to NE by about 20% (Figure 2A) without modifying the contractile response induced by a depolarized medium, 60 mM of potassium (control: 46.5 ± 3.6 mm Hg; 10<sup>-6</sup> M indomethacin: 48.0 ± 9.0 mm Hg, n = 4). Inhibition of endogenous cyclooxygenase products with indomethacin facilitated the PGF<sub>2α</sub> potentiation of the NE contractile response. Figure 2B shows that 10<sup>-10</sup> M of PGF<sub>2α</sub>, which was ineffective in control tissues, markedly facilitated the dose-response curve to NE in indomethacin-treated tis-

<table>
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<tr>
<th>Depolarized medium K+ (mM)</th>
<th>Control</th>
<th>PGF&lt;sub&gt;2α&lt;/sub&gt; (10&lt;sup&gt;-10&lt;/sup&gt; M)</th>
<th>PGF&lt;sub&gt;2α&lt;/sub&gt; (10&lt;sup&gt;-8&lt;/sup&gt; M)</th>
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<tr>
<td>60 100</td>
<td>119.9±10.6</td>
<td>245.7±16.5*</td>
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<td>100 100</td>
<td>104.1±10.9</td>
<td>149.9±11.8*</td>
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<td>60 100</td>
<td>129.5±13.3</td>
<td>231.5±34.6*</td>
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* p<0.05 vs control; † p<0.05 vs pretreatment.

**TABLE 1. Effect of PGF<sub>2α</sub> on Potassium-Induced Contractile Response in the Mesenteric Vascular Bed: Influence of Indomethacin and Calcium Antagonists**

**FIGURE 1. Effect of PGF<sub>2α</sub> on NE- or 5-HT-induced contractions in mesenteric vascular bed.** The sequence of experiments was as follows: A. First, control dose-response curve to NE (O); second, dose-response curve after 10<sup>-10</sup> M of perfused indomethacin (△); and last, dose-response curve after 10<sup>-8</sup> M of PGF<sub>2α</sub> (●). Points and bars are the means ± SEM of three to five experiments. * p<0.05 vs control group.

**FIGURE 2. Effect of indomethacin on PGF<sub>2α</sub> potentiation in mesenteric vascular bed.** The sequence of experiments was as follows: A. First, control dose-response curve to NE (O); second, dose-response curve 60 minutes after 10<sup>-8</sup> M of perfused indomethacin (●). B. First, dose-response curve to NE 60 minutes after 10<sup>-8</sup> M perfused indomethacin (△); second, dose-response curve with indomethacin plus 10<sup>-10</sup> M PGF<sub>2α</sub> (●). Points and bars are the means ± SEM of three to five experiments. * p<0.05; ** p<0.025 vs control.
sues. In contrast, this treatment did not modify the potentiation of PGF$_{2\alpha}$ observed in control tissues stimulated with potassium (see Table 1).

The calcium antagonists diltiazem and flunarizine reduced the contractile response to 100 mM of potassium to 40% of maximum. Both concentrations of PGF$_{2\alpha}$, $10^{-8}$ and $10^{-10}$ M, significantly facilitated the contractile response to potassium in tissues treated with $10^{-6}$ M of diltiazem. A similar potentiation was obtained for $10^{-8}$ M of PGF$_{2\alpha}$ in tissues treated with $10^{-7}$ M of flunarizine (see Table 1). The PGF$_{2\alpha}$ potentiation of the NE contractile responses also was not affected by nifedipine, $3 \times 10^{-6}$ M (data not shown).

NCDC ($3 \times 10^{-5}$ M) is an inhibitor of phospholipase C, which inhibits the stimulation of phosphoinositide synthesis by agonists. It reduced the NE-induced contraction. Pretreatment with NCDC failed to change PGF$_{2\alpha}$ potentiation (Figure 3).

Discussion

Prostaglandin F$_{2\alpha}$, in concentrations that did not affect the perfusion pressure of the mesenteric vascular bed, facilitated the dose-response curves to agonists NE and 5-HT, and the contractile response induced by potassium (60 or 100 mM). Endogenous products of cyclooxygenase pathways are involved in the contractile response to NE. After pretreatment with indomethacin, a cyclooxygenase inhibitor, the dose of PGF$_{2\alpha}$ that was ineffective in control tissues facilitated this contractile response. The potentiation of PGF$_{2\alpha}$ may be independent of calcium influx and phosphoinositide hydrolysis.

Cyclooxygenase products mediate prejunctional and postjunctional actions in noradrenergic tissues. PGF$_{2\alpha}$ in concentrations insufficient to alter the vascular tone potentiated contractile response of strips of dog mesenteric arteries to transmural electrical stimulation, but did not affect the postsynaptic response to NE. Nakajima and Toda concluded that PGF$_{2\alpha}$ potentiates the contractile response to nerve stimulation by increasing the release of NE and does not mediate any interaction at the postjunctional level.

In our study in the perfused mesenteric vascular bed of the rat, PGF$_{2\alpha}$ at similar concentrations to those of Nakajima and Toda potentiated the dose-response curves to NE and 5-HT and the contractile response mediated by depolarized medium. The different animal species or the different preparation used could explain the postjunctional effect of PGF$_{2\alpha}$ in our experiments.

Stimulation of $\alpha$-adrenergic receptors modifies prostaglandin synthesis. The $\alpha_1$-adrenergic receptors stimulated with methoxamine increase PGF$_{2\alpha}$ synthesis and decrease PGF$_{2\alpha}$ in mouse vas deferens. In isolated mesenteric vascular bed, $\alpha_1$-adrenergic receptors preferentially mediate vasoconstrictor response. Indomethacin reduced the dose-response curve to NE, suggesting that prostaglandins are involved in this response. Moreover, when the endogenous prostaglandin production was inhibited with indomethacin, a low dose of PGF$_{2\alpha}$ ($10^{-10}$ M) was sufficient to obtain a marked potentiation of the NE contractile response. In contrast, indomethacin did not change either the contractile response induced by potassium or the PGF$_{2\alpha}$ potentiation of this response. These results suggest that low levels of PGF$_{2\alpha}$ produced by the tissues during $\alpha$-adrenergic receptor stimulation could regulate the contractile response in mesenteric vascular bed of the rat.

The calcium antagonists diltiazem and flunarizine reduced the potassium-induced contractile response. After diltiazem treatment, a low dose of PGF$_{2\alpha}$ ($10^{-10}$ M) was sufficient to produce a similar potentiation to those obtained in control tissue. Flunarizine also did not affect the postsynaptic effect of PGF$_{2\alpha}$. Nifedipine, a calcium antagonist, did not affect the PGF$_{2\alpha}$ potentiation of NE responses (data not shown). These results indicate that, unlike PGF$_{2\alpha}$-induced contraction, the potentiation induced by PGF$_{2\alpha}$ on vasoconstrictor responses in mesenteric bed could be independent of the influx of extracellular calcium. From this point of view, the contraction and the potentiation induced by PGF$_{2\alpha}$ on vascular smooth muscle may stimulate different receptors or mechanisms.

Previous studies demonstrated that NE-elicited contraction includes two components. One is calcium channel-mediated and the other is activated by the products of phosphoinositide hydrolysis. An inhibitor of phospholipase C, NCDC, inhibits the activity of phosphoinositide-specific phospholipase C from smooth muscle.

In our experiments, NCDC in concentrations that partially inhibit phosphoinositide hydrolysis reduced the dose-response curve to NE in mesenteric bed. The present data confirm that the $\alpha$-adrenergic receptors stimulated in this tissue are coupled to phosphoinositide hydrolysis. The PGF$_{2\alpha}$ postjunctional action studied was maintained after NCDC treatment. Therefore, phosphoinositide hydrolysis might not be involved in the PGF$_{2\alpha}$ potentiation of NE contractile response in this tissue.

![Figure 3](http://hyper.ahajournals.org/Content/102/9/1140/Figure3.jpg)

**FIGURE 3.** Effect of 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) on PGF$_{2\alpha}$ potentiation in mesenteric vascular bed. The sequence of experiments was first, control dose-response curve to NE (○); second, dose-response curve after $3 \times 10^{-5}$ M of NCDC (▲); third, dose-response curve after NCDC plus $10^{-5}$ M of PGF$_{2\alpha}$ (●). Points and bars are mean ± SEM of three to five experiments. *p < 0.01 vs control; *p < 0.01 vs NCDC.
In conclusion, this study shows that PGF<sub>2α</sub> potentiation of the contractile response in mesenteric bed may be independent of PGF<sub>2α</sub> contraction. This potentiation could be achieved with low concentrations of endogenous prostaglandin produced during the NE response. The process may be independent of calcium influx and phosphoinositide hydrolysis.

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