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

PATRICIA M. ARISTEGUI AND MARÍA AMELIA ENERO

SUMMARY Prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) 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\(_{2\alpha}\), 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\(_{2\alpha}\) potentiation of this response. Calcium antagonists diltiazem or flunarizine reduced the potassium-induced contractile response. After diltiazem treatment, 10\(^{-10}\) M of PGF\(_{2\alpha}\) was also effective in facilitating this response. The PGF\(_{2\alpha}\) postjunctional effect was conserved after phosphoinositide hydrolysis inhibition. These results suggest that PGF\(_{2\alpha}\) potentiation of the contractile response may be independent of PGF\(_{2\alpha}\) 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: prostaglandins • postjunctival effect • phosphoinositide hydrolysis • Ca\(^{2+}\) • norepinephrine • 5-hydroxytryptamine

In all vascular smooth muscle the activation of the contractile process depends, under normal conditions, on an increase in cytosolic calcium concentration, whether derived from the extracellular fluid or from intracellular sources.\(^1,2\) It is generally accepted that norepinephrine (NE) induces release of intracellular calcium (fast component) and influx of calcium (sustained component).\(^3\) In addition, the contractile response induced by serotonin (5-HT) in rat aorta involves, like NE, two components, one calcium channel-mediated and the other through phosphoinositide hydrolysis.\(^6\)

On the other hand, prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) induces contraction by increasing membrane permeability to calcium in rat mesenteric artery.\(^5\) The ability of prostaglandins to mobilize different calcium pools to produce contraction seems to be variable.

The purpose of the present study was to examine the interaction of PGF\(_{2\alpha}\) at concentrations that do not induce vascular contraction, with the contractile response induced by NE or 5-HT and the depolarized medium evoked in perfused mesenteric arteries. We studied the effect on calcium influx of calcium entry blockers diltiazem and flunarizine and the role of phosphoinositide hydrolysis in this interaction. The results showed that PGF\(_{2\alpha}\) facilitates the contractile responses analyzed. This process may be independent of calcium influx and phosphoinositide hydrolysis.

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\(^6\) and perfused at the rate of 2 ml/min with modified Krebs solution previously gassed with a 95% O\(_2\), 5% CO\(_2\) 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.
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\textsubscript{2\alpha} (Syntial Laboratory), diltiazem (Laplex S.A.), flunarizine (Janssen Pharmaceutica), and 2-nitro-4-carboxyphenyl-\textit{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\textsubscript{2\alpha} used, 10\textsuperscript{-10} and 10\textsuperscript{-8} M, did not induce vascular contractions. When the tissues were perfused with 10\textsuperscript{-10} M of PGF\textsubscript{2\alpha} the dose-response curves to NE and 5-HT did not change, whereas 10\textsuperscript{-8} 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\textsuperscript{-10} M of PGF\textsubscript{2\alpha} the contractile response did not show significant changes, while 10\textsuperscript{-8} M of PGF\textsubscript{2\alpha} potentiated the contractile responses to 60 and 100 mM potassium by 40% and 60%, respectively (Table 1).

The inhibition of cyclooxygenase pathways with 10\textsuperscript{-6} 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\textsuperscript{-6} M indomethacin: 48.0 ± 9.0 mm Hg, n = 4). Inhibition of endogenous cyclooxygenase products with indomethacin facilitated the PGF\textsubscript{2\alpha} potentiation of the NE contractile response. Figure 2B shows that 10\textsuperscript{-10} M of PGF\textsubscript{2\alpha}, which was ineffective in control tissues, markedly facilitated the dose-response curve to NE in indomethacin-treated tis-

**Table 1. Effect of PGF\textsubscript{2\alpha} on Potassium-Induced Contractile Response in the Mesenteric Vascular Bed: Influence of Indomethacin and Calcium Antagonists**

<table>
<thead>
<tr>
<th>Depolarized medium</th>
<th>Minimum contractile response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+ (mM)</td>
<td>Control</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Diltiazem (10\textsuperscript{-6} M)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Flunarizine (10\textsuperscript{-7} M)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean ± SEM of three to five experiments is shown. I = indomethacin.

Tissues were stimulated with K+ (60 or 100 mM) three times in each experiment. The first stimulation was the control (100% of the response). PGF\textsubscript{2\alpha} (10\textsuperscript{-10} or 10\textsuperscript{-8} M) was perfused 20 minutes before and during the second or third period of stimulation. Indomethacin 10\textsuperscript{-6} M and Ca\textsuperscript{2+} antagonists were perfused 60 and 20 minutes, respectively, before and during the period of stimulation.

* p<0.05 vs control; † p<0.05 vs pretreatment.

**Figure 1. Effect of PGF\textsubscript{2\alpha} 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 (○); second, dose-response curve 60 minutes after 10\textsuperscript{-8} M of perfused indomethacin (★). B, First, dose-response curve to NE 60 minutes after 10\textsuperscript{-8} M of perfused indomethacin (★); second, dose-response curve with indomethacin plus 10\textsuperscript{-10} M PGF\textsubscript{2\alpha} (○). Points and bars are the means ± SEM of three to five experiments. * p<0.05 vs control.**

**Figure 2. Effect of indomethacin on PGF\textsubscript{2\alpha} potentiation in mesenteric vascular bed. The sequence of experiments was as follows. A, First, control dose-response curve to NE (○); second, dose-response curve 60 minutes after 10\textsuperscript{-8} M of perfused indomethacin (★). B, First, dose-response curve to NE 60 minutes after 10\textsuperscript{-8} M of perfused indomethacin (★); second, dose-response curve with indomethacin plus 10\textsuperscript{-10} M PGF\textsubscript{2\alpha} (○). 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 PGF2α 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 PGF2α, 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 PGF2α in tissues treated with 10^{-7} M of flunarizine (see Table 1). The PGF2α potentiation of the NE contractile responses also was not affected by nifedipine, 3 × 10^{-6} M (data not shown).

NCDC (3 × 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 PGF2α potentiation (Figure 3).

**Discussion**

Prostaglandin F2α, 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 PGF2α that was ineffective in control tissues facilitated this contractile response. The potentiation of PGF2α may be independent of calcium influx and phosphoinositide hydrolysis.

Cyclooxygenase products mediate prejunctional and postjunctional actions in noradrenergic tissues. PGF2α 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 Toda7 concluded that PGF2α 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, PGF2α at similar concentrations to those of Nakajima and Toda7 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 PGF2α in our experiments.

Stimulation of α-adrenergic receptors modifies prostaglandin synthesis. The α1-adrenergic receptors stimulated with methoxamine increase PGF2 synthesis and decrease PGE2 in mouse vas deferens.8 In isolated mesenteric vascular bed, α1-adrenergic receptors preferentially mediate vasoconstrictor response.9 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 PGF2α (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 PGF2α potentiation of this response. These results suggest that low levels of PGF2α produced by the tissues during α-adrenergic receptor stimulation could regulate the contractile response in mesenteric vascular bed of the rat.

The calcium antagonists diltiazem and flunarizine10 reduced the potassium-induced contractile response. After diltiazem treatment, a low dose of PGF2α (10^{-10} M) was sufficient to produce a similar potentiation to those obtained in control tissue. Flunarizine also did not affect the postjunctional effect of PGF2α. Nifedipine, a calcium antagonist, did not affect the PGF2α potentiation of NE responses (data not shown). These results indicate that, unlike PGF2α-induced contraction, the potentiation induced by PGF2α 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 PGF2α 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.11 An inhibitor of phospholipase C, NCDC, inhibits the activity of phosphoinositide-specific phospholipase C from smooth muscle.4 In our experiments, NCDC in concentrations that partially inhibit phosphoinositide hydrolysis4 reduced the dose-response curve to NE in mesenteric bed. The present data confirm that the α1-adrenergic receptors stimulated in this tissue are coupled to phosphoinositide hydrolysis. The PGF2α postjunctional action studied was maintained after NCDC treatment. Therefore, phosphoinositide hydrolysis might not be involved in the PGF2α potentiation of NE contractile response in this tissue.
In conclusion, this study shows that PGF$_{2\alpha}$ potentiation of the contractile response in mesenteric bed may be independent of PGF$_{2\alpha}$ 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.

References
4. Nakaki T, Roth BL, Chuang D-M, Costa E. Phasic and tonic components in 5-HT$_2$ receptor-mediated rat aorta contraction: participation of Ca$^{2+}$ channels and phospholipase C. J Pharmacol Exp Ther 1985;234:442-446
5. Godfraind T, Miller RC. Actions of prostaglandin F$_{2\alpha}$ and noradrenaline on calcium exchange and contraction in rat mesenteric arteries and their sensitivity to calcium entry blockers. Br J Pharmacol 1982;75:229-236
7. Nakajima M, Toda N. Prejunctional and postjunctional actions of prostaglandins F$_{2\alpha}$ and I$_2$ and carbocyclic thromboxane A$_2$ in isolated dog mesenteric arteries. Eur J Pharmacol 1986;120:309-318
Effects of prostaglandin F2 alpha on vasoconstrictor responses in the mesenteric vascular bed.

P M Aristegui and M A Enero

_Hypertension_. 1988;11:I108
doi: 10.1161/01.HYP.11.2_Pt_2.I108

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/11/2_Pt_2/I108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/