Prostaglandin-Mediated Inhibition of the Vasoconstrictor Responses of the Isolated Perfused Rat Splenic Vasculature to Adrenergic Stimuli

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SUMMARY In isolated rat spleen perfused with Tyrode’s solution, prostaglandin (PG) E₁ and E₂, 0.2–2.5 ng/ml, reduced the vasoconstrictor responses to sympathetic nerve stimulation by 38–89% and 35–73% and to injected norepinephrine by 18–53% and 18–34%, respectively. In contrast, PGF₂α and thromboxane B₂ (TxB₂) produced vasoconstriction and potentiated the vascular responses to both adrenergic stimuli, whereas PGD₂ had variable effects. Stimulation of adrenergic nerves or an injection of norepinephrine enhanced the efflux of a PGE-like substance from the rat spleen. Administration of a PG synthetase inhibitor, indomethacin, 100 ng/ml, abolished the efflux of PGE evoked by adrenergic stimuli and potentiated the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. The facilitatory effect of indomethacin on the vasoconstrictor responses to both adrenergic stimuli was abolished by the infusion of PGE₂. A PG precursor, arachidonic acid, inhibited the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. Because the inhibitory effect of arachidonic acid was abolished by the administration of indomethacin, it appears to be mediated, at least in part, through the conversion of the acid to a PG(s). These observations and the effect of exogenous PGE compounds suggest that PGs of the E series participate in the modulation of the vascular responses of the rat spleen to adrenergic stimuli. However, the modulatory effect of PGE compounds varies in different vascular beds of rat, viz, facilitatory in renal and mesenteric vasculature and inhibitory in the splenic vasculature. This study also indicates that other products of arachidonic acid metabolism, including PGF₂α and TxB₂, also may contribute to the modulation of vascular responses to adrenergic stimuli and affect the actions of PGE₂ at the adrenergic neuroeffector junction in the rat spleen.

STIMULATION of sympathetic nerves, as well as administration of norepinephrine, increases the output of prostaglandins (PGs) and particularly of a PGE-like substance from various tissues of different species. Since (1) the administration of PGE compounds diminishes the output of norepinephrine evoked by sympathetic nerve stimulation and reduces the response of several tissues to adrenergic stimuli, and (2) blockade of PG synthesis facilitates norepinephrine release and augments the vascular responses to adrenergic stimuli, it was proposed that PGE compounds function as inhibitory modulators of the vasoconstrictor responses to adrenergic stimuli. However, the demonstration that PGE compounds, which inhibit the vasoconstrictor responses of the rabbit renal and mesenteric vasculature, produce an opposite effect in the rat, viz, potentiate the vascular responses to adrenergic stimuli, suggested that the modulatory effect of PGE compounds on the adrenergic neuroeffector junction is species dependent.

The present study was undertaken to examine the effects of PGE₁, PGE₂, and some other products of arachidonic acid metabolism, PGF₂α, PGD₂, and thromboxane (Tx) B₂, on the vasoconstrictor responses of the isolated rat splenic vasculature, perfused with Tyrode’s solution, to periarterial nerve stimulation and to injected norepinephrine. To determine the contribution of endogenous PGs in the modulation of vascular reactivity to adrenergic stimuli, the output of PGs evoked by sympathetic nerve stimulation and injected norepinephrine and the consequence of blockade of PG synthesis with indomethacin on the vascular responses to adrenergic stimuli were examined. The results of this study indicate an important difference in the effect of PGs on vascular tone and vascular reactivity to adrenergic stimuli in various organs of the rat.

Methods

Experiments were performed on albino rats (Wistar and Sprague-Dawley) of both sexes weighing 300–350 g. Rats were anesthetized with ether and the abdomen opened by a midline incision. The vascular connections of the spleen with stomach, pancreas, and intestine were ligated and cut free from these organs. The coeliac artery with

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its nerve plexus and the portal vein were dissected free from the surrounding tissue. A stainless steel cannula was advanced through the coeliac artery and the tip of the cannula positioned close to the splenic artery. The spleen was then flushed with heparinized saline (100 U/ml). The rat was killed by an incision in the heart. The portal vein was cut, and the spleen was immediately isolated by dissecting the surrounding tissue and was transferred to a thermostatically controlled Plexiglas box, where it was covered with cotton gauze moistened with Tyrode’s solution. The spleen was perfused at a constant rate of 5 ml/min, with a Harvard peristaltic pump (model 1203). The millimolar composition of the Tyrode’s solution was: NaCl, 137; KCl, 2.7; CaCl2, 1.8; MgCl2, 1.1; NaHCO3, 12; NaH2PO4, 0.42; and d-glucose, 5.6. The fluid perfusing the spleen flowed from the cut end of the portal vein. The perfusion fluid was maintained at a temperature of 37°C and aerated with a mixture of 95% O2-5% CO2. Changes in perfusion pressure were measured with a mercury manometer and recorded on a kymograph equipped with an isotonic frontal writing lever. Before cannulation of the coeliac artery, the pressure in the cannula was 15 mm Hg at a flow rate of 5 ml/min. During perfusion of the spleen, the pressure in the cannula increased by an average of 75 mm Hg (range, 60–85 mm Hg).

A bipolar platinum electrode was placed on the periarternal nerve plexus about 2 mm distal to the cannula. The nerves were stimulated at 0.3 Hz, using supramaximal biphasic rectangular pulses, 1 msec in duration, for 22 seconds at 4-minute intervals with a Grass stimulator (S44). At 4-minute intervals, either the periarternal nerves were stimulated or norepinephrine was infused directly into the arterial cannula in a volume of 0.05 ml for 10–20 seconds with an infusion pump (Braun-Melsungen), using an automatic timer. The dose of norepinephrine was varied (range, 50–70 ng) to produce control vasoconstrictor responses, the magnitude of which was not significantly different (P > 0.5) from the control responses produced by stimulation of periarterial nerves for 22 seconds. All other drugs were infused into the spleen randomly. Changes in the vasoconstrictor responses to nerve stimulation and to injected norepinephrine produced by various agents were calculated, without taking into account alterations in the basal perfusion pressure, by comparing the mean height of the vasoconstrictor responses during the infusion of an agent for 16–24 minutes with the mean height of control responses in the absence of the drug.

Determination of Prostaglandins

Release of prostaglandin-like substance from the rat spleen perfused with Tyrode’s solution in response to nerve stimulation (0.3 Hz, 1 msec duration, supramaximal voltage) or to norepinephrine (30–50 ng), injected directly into the cannula leading to the coeliac artery, was detected in the perfusate from rat spleen by continuously superfusing a series of assay tissues, according to the procedure of Vane.15,16 Assay tissues included the rat stomach strip, chick rectum and rat colon. The initial resting load on the tissues was 2–3 g, and their contractions were measured by Harvard smooth muscle transducers and recorded on a physiograph (Rikadenki). The tissues were made insensitive to acetylcholine, histamine, 5-hydroxytryptamine, and catecholamines by continuously infusing a mixture of antagonists2 into the superfusing fluid to give the following final concentration of the active bases: hyoscine, 10−7 g/ml; methysergide, 2 × 10−7 g/ml; phenoxbenzamine, 10−6 g/ml; pyrilamine, 10−6 g/ml; and propranolol, 2 × 10−8 g/ml. Indomethacin, an inhibitor of PG synthesis,14 also was included in the fluid superfusing the assay organs distal to spleen to give a final concentration of 5 μg/ml. Prostaglandins E2, F2α, D2, and TxB2 standards were administered directly into the fluid superfusing the assay organs.

The nature of PG-like material released from rat spleen in response to adrenergic stimuli was further investigated by collecting the splenic perfusate for 10-minute periods before and during periarterial nerve stimulation (0.3 Hz, 1 msec) in the absence and presence of indomethacin. The splenic effluent was adjusted to pH 3 with formic acid, and the acidic lipids were separated from the neutral lipids by extraction with ethyl acetate followed by extraction with potassium phosphate buffer and, finally, with chloroform. The acidic lipids so obtained were separated by thin layer chromatography on 0.5-mm thick silica gel plates (Brinkman Instruments) using the solvent system chloroform-methanol-acetic acid (18:2:1, by volume). Marker plates prepared by spotting 10 μg of authentic PGE2, PGF2α, PPD, and TxB2 were run concurrently with preparative plates. Spots of authentic PGs on the marker plates were localized by spraying with 10% phosphomolybdic acid in ethanol, followed by heating at 100°C for 15 minutes. The zones on the preparative plates corresponding to the position of authentic PGE2, PGF2α, PPD, and TxB2 on the marker plates were scraped, as were the areas between them, and the remainder of each plate divided into five 2-cm zones and scraped. Each zone was eluted separately with chloroform-methanol (4:1 vol/vol). Eluates were dried in a nitrogen atmosphere, reconstituted in 1 ml of saline, and 0.1-ml samples of this solution were tested for PG-like activity, using PGE2, PGF2α, PPD, and TxB2 as reference standards, respectively, on rat stomach strip and rat colon. The isolated organs were suspended in glass chambers and superfused in series with Krebs solution (3 ml/min, 37°C), and their contractions were measured with Harvard muscle transducers and recorded on a physiograph.

Drugs

The concentration of norepinephrine bitartrate (Winthrop) is expressed as the free base. The following drugs were used: hexamethonium bromide (K & K Labs), 6-hydroxydopamine hydrobromide (Aldrich), guanethidine (Ciba), cocaine hydrochloride and hyoscine hydrobromide (British Drug Houses), phenoxbenzamine hydrochloride (Smith, Kline & French), pyrilamine maleate (Pfaltz & Bauer), methysergide maleate (Sandoz), propranolol hydrochloride (Ayerst Labs), prostaglandins E1, E2, D2, and prostaglandin F3, tromethamine and thromboxane B2 (Upjohn Co.), indomethacin (Merck, Sharp &
Dohme), and arachidonic acid (Applied Science Labs).

Prostaglandins E1, E2, D2, and F2 are dissolved in ethanol (1 mg/ml); arachidonic acid (10 mg) was dissolved in 1 ml of hexane and mixed with 2 ml of sodium carbonate (0.1%) and then exposed to nitrogen to evaporate the hexane. Further dilutions of PGs and arachidonic acid solutions were made with Tyrode's solution. These diluted solutions were then added to the perfusion fluid to obtain the final concentration. Control Tyrode's solution perfusing the splenic blood vessels contained the same amount of ethanol (0.2-2.5 nl/ml) or sodium carbonate (20 ng/ml) as did the drug-containing Tyrode's solution. The amount of ethanol or sodium carbonate present in the control Tyrode's solution did not affect the vascular tone or the vasoconstrictor responses to nerve stimulation or injected norepinephrine. 6-Hydroxydopamine hydrobromide, which depletes sympathetic nerves of their catecholamines, was dissolved in 1 ml of saline and immediately injected intraperitoneally (100 mg/kg) twice at 24-hour intervals. All other drugs were dissolved in Tyrode's solution and added to the perfusion fluid to obtain the final concentration, whereas norepinephrine was injected directly into the arterial cannula.

Statistical Analysis
Paired and unpaired t-tests were performed according to the methods described by Steel and Torrie.

Results
Effect of Periarterial Nerve Stimulation and Injected Norepinephrine on Perfusion Pressure in Isolated Perfused Rat Splenic Vasculature and Modification by Ganglionic and Adrenergic Neuron Blocking Agents and by Chemical Sympathectomy

Stimulation of periarterial nerves or injections of norepinephrine directly into the arterial cannula constricted splenic vessels and increased the perfusion pressure. The rise in perfusion pressure was related to the frequency of nerve stimulation, as well as the amount of norepinephrine injected into the arterial cannula (Fig. 1). Repeated stimulation of periarterial nerves (0.1-0.5 Hz) or injections of norepinephrine (30-80 ng) at 4-minute intervals during a 3-hour period did not cause diminution of the vasoconstrictor responses. The vasoconstrictor responses of rat spleen to both adrenergic stimuli remained unaltered during the infusion of a ganglionic blocking agent, hexamethonium, 10-100 µg/ml, (five preparations), whereas administration of guanethidine, 0.5 µg/ml (six preparations), which blocks sympathetic transmission, inhibited the vasoconstrictor responses to nerve stimulation but increased the responses to injected norepinephrine by 14 ± 3% (± SEM); the change was statistically insignificant. Moreover, in four preparations isolated from rats pretreated with 6-hydroxydopamine, stimulation of periarterial nerves did not alter the perfusion pressure, whereas injections of norepinephrine (50-80 ng) produced a 30 ± 4% greater degree of constriction of splenic vessels than was obtained from untreated rats. Therefore, it appears that the periarterial nerves to rat splenic vasculature are postganglionic adrenergic.

Effects of Prostaglandin E1, E2, F2, D2, and Thromboxane B2 on Vasoconstrictor Responses of Rat Splenic Vasculature to Periarterial Nerve Stimulation and to Injected Norepinephrine

Prostaglandins E1 and E2
Infusion of either PGE1 or PGE2, 0.2-1 ng/ml, did not alter the basal perfusion pressure, but invariably reduced the vasoconstrictor responses of rat splenic vasculature to periarterial nerve stimulation (0.3 Hz) and, to a lesser degree, responses to injected norepinephrine (50-70 ng). Higher concentrations of PGE1 and PGE2, 2.5 ng/ml,
produced a fall in basal perfusion pressure and decreased the vasconstrictor responses to both adrenergic stimuli (Fig. 2 and Table 1).

Prostaglandin \( \text{PGE}_2 \)

\( \text{PGE}_2 \), 0.2-1 ng/ml, consistently increased the basal perfusion pressure and potentiated the vasconstrictor responses of splenic vasculature to both adrenergic stimuli (Fig. 3 and Table 1). In five preparations, infusion of \( \text{PGE}_2 \), 0.5-1 ng/ml, abolished the fall in basal perfusion pressure and the inhibitory effect on the vasconstrictor responses to nerve stimulation and to injected norepinephrine produced by \( \text{PGE}_2 \), 2.5 ng/ml (Fig. 4).

Prostaglandin \( \text{D}_2 \)

The infusion of \( \text{PGD}_2 \), 0.2 ng/ml, altered neither the basal perfusion pressure nor the vasconstrictor responses to nerve stimulation or to injected norepinephrine. \( \text{PGD}_2 \), 1, 2.5, 5, and 25 ng/ml, in 24 of 32 rat spleens, decreased the basal perfusion pressure (3-5 mm Hg) and reduced the vasconstrictor responses to nerve stimulation by 12-37\%, whereas in eight preparations, the basal perfusion pressure was increased by 4-7 mm Hg, and the responses to nerve stimulation were potentiated by 20-49\%. The vasconstrictor responses to injected norepinephrine also were affected variably by \( \text{PGD}_2 \), viz, inhibited in 14 of 18 preparations by 9-40\% and increased in four preparations by 12-50\%.

Thromboxane \( \text{B}_2 \)

Thromboxane \( \text{B}_2 \) (\( \text{TXB}_2 \)) is derived by the breakdown of a biologically active and an unstable product of arachidonic acid metabolism, \( \text{TXA}_2 \), in platelets and some other tissues including spleen. \( \text{TXB}_2 \) has been synthesized by Nelson and Jackson and shown to be similar to the naturally occurring compound. Infusion of \( \text{TXB}_2 \), 0.2-2.5 ng/ml, affected neither the basal perfusion pressure nor the vasconstrictor responses to nerve stimulation or injected norepinephrine. \( \text{TXB}_2 \), in concentrations of 5-100 ng/ml, invariably increased the basal perfusion pressure (3-6 mm Hg) and potentiated the vasconstrictor responses to nerve stimulation and to injected norepinephrine produced by \( \text{PGE}_2 \), 2.5 ng/ml.

In four preparations, the infusions of \( \text{TXB}_2 \), 100-150 ng/ml, abolished the fall in basal perfusion pressure and the inhibition of the vasconstrictor responses to nerve stimulation and to injected norepinephrine produced by \( \text{PGE}_2 \), 2.5 ng/ml.

Release of Prostaglandins from the Isolated Perfused Rat Spleen in Response to Periarterial Nerve Stimulation and to Injected Norepinephrine

In the rat spleen, stimulation of periarterial nerves for 1 minute (0.3-0.5 Hz) or an injection of norepinephrine (20-50 ng) directly into the arterial cannula raised the perfusion pressure and released a substance having a smooth muscle-stimulating activity like \( \text{PGE}_2 \) (five preparations). It caused contraction of rat stomach strip and chick rectum but had no effect on rat colon (Fig. 5). Direct administration of \( \text{PGE}_2 \), 1-4 ng, into the fluid superfusing the assay organs caused contraction of rat stomach strip and chick rectum, but had little or no effect on the rat colon, whereas \( \text{PGF}_2 \) contracted the rat colon but usually had only 1/3-1/5th the potency of \( \text{PGE}_2 \) in contracting rat stomach strip and chick rectum. Prostaglandin \( \text{D}_2 \) and \( \text{TXB}_2 \) had 1/200th and 1/1000th the musculotropic activity of \( \text{PGE}_2 \) on the assay organs (not shown in figures). Since the assay organs were constantly exposed to an inhibitor of PG synthesis, release of a PG-like substance could only arise from the perfused rat spleen. In addition, the fluid superfusing the assay organs contained receptor antagonists of acetylcholine, histamine, serotonin, and catecholamines; therefore, norepinephrine or any of the other substances released in re-
TABLE 1  Effect of Prostaglandins E, E, and F,, TxB, Indomethacin and Arachidonic Acid on Equiconstrictor Responses of Rat Splenic Vasculature to Periarterial Nerve Stimulation and to Injected Norepinephrine

<table>
<thead>
<tr>
<th>Substance (ng/ml)</th>
<th>Responses to nerve stimulation (NS)</th>
<th>Responses to injected norepinephrine (NE)</th>
<th>Difference between NS and NE, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE, 0.2</td>
<td>-38 ± 4*</td>
<td>-18 ± 3†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PGE, 1</td>
<td>-61 ± 6‡</td>
<td>-33 ± 5*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PGE, 2.5</td>
<td>-89 ± 2‡</td>
<td>-53 ± 4†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PGF, 0.2</td>
<td>-35 ± 4*</td>
<td>-18 ± 1*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PGF, 1</td>
<td>-56 ± 4‡</td>
<td>-33 ± 3†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PGF, 2.5</td>
<td>-73 ± 3†</td>
<td>-34 ± 3†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TxB, 5</td>
<td>+9 ± 2</td>
<td>+9 ± 2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TxB, 25</td>
<td>+21 ± 3*</td>
<td>+21 ± 2*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TxB, 50</td>
<td>+31 ± 5*</td>
<td>+33 ± 5*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TxB, 100</td>
<td>+45 ± 6*</td>
<td>+49 ± 6*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>+34 ± 3†</td>
<td>+21 ± 2†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1000</td>
<td>-28 ± 3‡</td>
<td>-26 ± 3*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>5000</td>
<td>-53 ± 3‡</td>
<td>-40 ± 6†</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid 100</td>
<td>-33 ± 2*</td>
<td>-25 ± 2*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Response values are means ± SE.
* P < 0.01 compared to control.
† P < 0.05 compared to control.
‡ P < 0.001 compared to control.

Table 1 shows the effects of various substances on the responses of rat splenic vasculature to periarterial nerve stimulation and to injected norepinephrine. The table includes substances such as PGE, PGF, TxB, Indomethacin, and Arachidonic Acid, along with their effects on nerve stimulation and injected norepinephrine, as well as the differences between the responses. The table provides a clear overview of the modulatory effects of these substances on vascular reactivity.
Effect of prostaglandin F2α (PGF2α) on the vasoconstrictor responses of isolated perfused rat spleen to periarterial nerve stimulation (NS) (0.3 Hz) and to injected norepinephrine (NE) (55 ng). Each tracing illustrates vasoconstrictor responses of a different rat spleen.

Effect of Prostaglandin E2 (PGE2) on the Inhibitory Effect of PGF2α on the Vascular Tone and on the Vasoconstrictor Responses of the Rat Splenic Vasculature to Nerve Stimulation (NS) (0.3 Hz) and to Injected Norepinephrine (NE) (50 ng).

Effect of Arachidonic Acid on the Vasoconstrictor Responses of Perfused Rat Spleen to Periarterial Nerve Stimulation and Injected Norepinephrine

In six splenic preparations, infusion of a precursor of PGs, arachidonic acid, invariably reduced the vasoconstrictor responses to nerve stimulation to a greater degree than responses to injected norepinephrine (Table 1 and Fig. 7). Simultaneous infusion of indomethacin, 100 ng/ml, abolished the inhibitory effect of arachidonic acid on the vasoconstrictor responses to both adrenergic stimuli. However, addition of PGE2, 1 ng/ml, to the perfusion fluid restored the inhibitory effect of arachidonic acid on the vasoconstrictor responses to adrenergic stimuli (Fig. 7).

Discussion

The present findings indicate that PGs of the E series affect vascular responses of the rat spleen to sympathetic nerve stimulation and to injected norepinephrine in a direction opposite to that reported for the renal and mesenteric vasculature of this species. Thus, PGE1 and PGE2, which potentiated the vascular responses of the rat renal and mesenteric vasculature to adrenergic stimuli, produced vasodilation and reduced the vasoconstrictor responses of the splenic vasculature to sympa-
FIGURE 5 Efflux of prostaglandins (PGs) from the isolated rat spleen perfused with Tyrode's solution in response to periarterial nerve stimulation (NS) and to injected norepinephrine (NE). Prostaglandins $E_2$ and $F_2$ were given as bolus injections directly (DIR) into the fluid superfusing the rat stomach strip (RSS), chick rectum (CR), and rat colon (RC). The periarterial nerves were stimulated for 1 minute at 0.3 Hz, supramaximal voltage, and 1-msec duration. Norepinephrine solution in a volume of 0.05 ml containing 50 ng was injected directly into the cannula leading to the coeliac artery. The splenic vessels constricted and caused a rise in perfusion pressure and an output of PGs into the effluent from the spleen as indicated by contraction of RSS and CR. Infusion of indomethacin, 100 ng/ml, into the spleen abolished the output of PGs and potentiated the vasoconstrictor responses to adrenergic stimuli. Prostaglandins $F_2$ or $E_2$ applied directly into the fluid superfusing the assay organs during infusion of indomethacin caused contraction of the tissues.

The inhibitory effect of PGE compounds on the adrenergically induced vasoconstrictor responses of rat splenic vasculature is in accordance with the observations reported for the pancreatic vessels, 23 cat spleen, 5, 6 and other organs of different species. 3, 5, 8 Reduction by PGE1 and PGE2 of the vascular responses in the rat spleen to adrenergic stimuli could be attributed to decreased vascular reactivity to norepinephrine. Since PGE compounds reduced the vasoconstrictor responses to nerve stimulation to a greater degree than responses to injected norepinephrine, diminished release of norepinephrine from nerve fibers appears to contribute to the inhibitory effect of these agents on the vasoconstrictor responses to nerve stimulation. Supporting this view is my recent observation that PGE2 diminished the efflux of $^3$H-norepinephrine elicited by nerve stimulation from the rat spleen (unpublished observation). The ability of PGE compounds to inhibit the release of norepinephrine evoked by the stimulation of postganglionic sympathetic nerves has been demonstrated in several tissues of different species. 1, 5, 6, 7

These observations and the demonstration that stimulation of periarterial nerves or administration of norepinephrine released a PGE-like substance from the rat spleen suggest that PG release could in turn attenuate the vascular responses to adrenergic stimuli. If this is true, then alterations of PG synthesis should affect the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. Thus, blockade of PG synthesis with indomethacin potentiated the vasoconstrictor responses to adrenergic stimuli. The demonstration that administration of PGE2 nullified the potentiating effect of indomethacin on the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. The demonstration that administration of PGE2 nullified the potentiating effect of indomethacin on the vasoconstrictor responses to adrenergic stimuli indicates that PGs of the $E$ series participate in the modulation of vascular responses to adrenergic stimuli. 11, 12, 24 Since indomethacin produced a greater degree of augmentation of the vasoconstrictor responses to nerve stimulation than those to injected norepinephrine, an enhanced release of the adrenergic transmitter as a result of diminished PG synthesis presumably contributes to the facilitatory effect of indomethacin on responses to nerve stimulation. The ability of indomethacin to augment the output of norepinephrine evoked by sympathetic nerve stimulation has been demonstrated in the heart 20 and kidney 26 of rabbits. Reduction of the vasoconstrictor responses to both adrenergic stimuli and vasodilation produced by higher con-
FIGURE 6 Effect of indomethacin (Ind) on the vasoconstrictor responses of the isolated perfused rat spleen to periarterial nerve stimulation (NS) (0.3 Hz) and to injected norepinephrine (NE) (65 ng). Each tracing represents vasoconstrictor responses of a different rat spleen.

FIGURE 7 Effect of arachidonic acid (AA) on the vasoconstrictor responses of the isolated perfused rat spleen to periarterial nerve stimulation (NS) (0.3 Hz) and to injected norepinephrine (NE) (55 ng) and its modification by indomethacin (Ind) and prostaglandin E\(_1\) (PGE\(_1\)). Each tracing represents vasoconstrictor responses of a different rat spleen.

Concentrations of indomethacin in the rat spleen is most likely due to a direct effect of this agent on the vascular smooth muscle, since termination of the infusion of indomethacin resulted in restoration of vascular tone and vasoconstrictor responses to control levels despite persistent inhibition of PG synthesis.

The role of endogenous PGs as modulators of the vasoconstrictor responses to adrenergic stimuli also is supported by the demonstration that arachidonic acid, a precursor of PGs, inhibited the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. Since the inhibitory effect of arachidonic acid was abolished during the infusion of the PG synthesis inhibitor, indomethacin, it appears that a product or products of arachidonic acid metabolism participate in its action on the vascular responses to adrenergic stimuli. These observations and the present demonstration that PGE\(_1\) and PGE\(_2\) inhibited the vasoconstrictor responses to adrenergic stimuli are consistent with the hypothesis\(^{10-12}\) that PGE compounds participate in the modulation of vascular responses to adrenergic stimuli. These data, however, do not permit the exclusion of the contribution of other...
products of arachidonic acid metabolism in the rat spleen, i.e., cyclic endoperoxides PGF2a and PGH2, TxA2, PGI2, PGF2aa, and PGD2,20,27 in the modulation of vascular responses to adrenergic stimuli. This is so because indomethacin inhibits primarily the conversion of arachidonic acid to cyclic endoperoxides PGG2 and PGH2, which give rise to TxA2 and various prostaglandins. Although the major product of arachidonic acid metabolism in blood vessels appears to be PGI2,20 rat spleen has been shown to synthesize larger amounts of TxB2, a stable derivative of TxA2, than PGH2, PGE2, PGF2aa, or PGD2,20. These observations and the present demonstration that TxB2 caused vasoconstriction and potentiated the vascular responses to nerve stimulation and to injected norepinephrine suggest that TxB2 and probably its biologically active precursor TxA2 also could participate in the modulation of vascular responses to adrenergic stimuli. Moreover, in view of the presence in several tissues of an enzyme, PGE2-9-keto-reductase,20,30 which converts PGE to PGF compounds, and the demonstration that PGF2a, like TxB2, caused vasoconstriction and augmented the vascular responses to adrenergic stimuli, it is possible that PGF2a and TxB2—depending upon the species and vascular bed—could either oppose (rat, dog, and cat spleen, rabbit renal and mesenteric vasculature) or contribute to (rat renal and mesenteric vasculature) the modulatory effect of PGE2 on vascular reactivity to adrenergic stimuli. Supporting this view was the demonstration that the vasodilator and the inhibitory effects of PGE2 on the vasoconstrictor responses of the rat spleen to nerve stimulation and to injected norepinephrine were abolished by the administration of either PGF2a or TxB2.

The present study indicates that PGs may participate in the modulation of the vasoconstrictor responses of the isolated rat spleen to sympathetic nerve stimulation and to injected norepinephrine. However, the direction of modulation may vary with the type of PG and vascular bed within the same species. Thus, PGF2a and TxB2 increased vascular tone and potentiated the vasoconstrictor responses to adrenergic stimuli, whereas PGE2 and PGD2, which potentiated the vascular responses to adrenergic stimuli in the rat renal and mesenteric vessels, produced an inhibitory effect in the spleen, viz, reduced the vasoconstrictor responses to adrenergic stimuli. The opposite direction of modulation by PGE compounds and PGF2a and TxB2 in the rat splenic vasculature and of PG compounds in the renal and mesenteric and splenic vessels of this species, which could be attributed to differences in PG receptors or the events resulting from PG receptor interaction, remains to be determined.

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References


Prostaglandin-mediated inhibition of the vasoconstrictor responses of the isolated perfused art splenic vasculature to adrenergic stimuli.

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