Prostaglandin B₂-Induced Cutaneous Vasoconstriction of the Canine Hind Paw

By Stanley Greenberg, James A. Engelbrecht, and William R. Wilson

ABSTRACT

The cutaneous vascular effects of prostaglandin B₂ (PGB₂) were studied in dogs during constant-flow perfusion of the hind paw. The effects of PGB₂ (50–800 ng/kg min⁻¹, ia) on systemic pressure, hind-paw perfusion pressure, and responses to local heating at 45°C (30 seconds) and cooling at 4°C (90 seconds) were measured in 44 dogs. PGB₂ increased perfusion pressure by 50 ± 19 mm Hg to 218 ± 21 mm Hg without any effect on systemic arterial blood pressure. The pressor response to cooling increased from 34 mm Hg to 53 mm Hg (50 ng/kg min⁻¹PGB₂), but the dilator response to heating was reduced significantly during infusions of PGB₂ (50 and 100 ng/kg). Acute denervation and reserpine treatment (0.5 mg/kg dog⁻¹ for 2 days) reduced the constrictor responses to PGB₂. The abilities of PGB₂ to produce intense vasoconstriction, which is blocked by acute denervation and reserpine, to enhance responses to cooling, and to antagonize responses to heating make this preparation a useful model for the study of Raynaud's phenomenon and suggest that a prostaglandin, perhaps PGB₂, may participate in cutaneous vasospastic disorders.

KEY WORDS Raynaud's phenomenon skin blood flow reserpine cold-induced vasoconstriction enhanced by PGB₂ heat-induced vasodilation antagonized by PGB₂ denervation pressor response

Methods

Mongrel dogs of either sex (14–18 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and, after endotracheal intubation, they were artificially ventilated with room air with a Harvard ventilator (15 strokes/min, 250 ml air/stroke). Arterial blood pressure was measured through a cannula inserted into the left femoral artery with a Statham P23AA arterial blood pressure transducer. A cannula inserted into the left femoral vein was used for intravenous administration of drugs. The right hind paw was perfused according to the method of Zimmerman and Gomez (7). Heparin (5 mg/kg, iv) was administered to each dog. After 10 minutes the right tibial artery was cannulated, and the hind paw was perfused with autologous blood obtained from the right iliac artery. Flow was maintained constant with a Harvard peristaltic pump (model 1215). A T-tube inserted into the perfusion circuit between the pump and the tibial artery was used for monitoring perfusion pressure. Flow, which averaged 24.4 ± 1.4 ml/min in 25 experiments, was initially set at a level which resulted in a perfusion pressure equal to systemic pressure; it was not reset during the experiment. Isolation of the hind paw was confirmed by turning off the perfusion pump and measuring the residual pressure which approached small vein pressure (22 ± 1.6 mm Hg). Since blood flow was maintained constant, changes in perfusion pressure reflected changes in vascular resistance. All pressures were measured with Statham pressure transducers and recorded on a Beckman type RM dynograph.

Responses to intra-arterial injections of tyramine...
monohydrochloride (50 and 200 µg), norepinephrine (0.1–1.0 µg), and nitroglycerin (10–100 µg) were obtained prior to, during, and after constant intra-arterial infusions of PGB2.1 Injections (0.1–0.10 ml) and infusions (1.0 ml/min) were made directly into the perfusion circuit prior to its entrance into the hind paw. Responses to the agonists during infusions of the prostaglandins were measured after the elevation in perfusion pressure (when it occurred) was well maintained, usually 15 minutes after initiation of the PGB2 infusion. Responses to cold were obtained by applying ice water (4°C) in a polyethylene bag directly to the hind paw for 90 seconds. Responses to heat were obtained by applying a polyethylene bag containing water at 45°C directly to the hind paw for 30 seconds. These procedures were repeated during infusions of PGB2.

The effect of PGB2 on cutaneous vascular resistance was evaluated by intra-arterial infusion of graded concentrations (50–3200 ng/kg min−1) into the hind paw (1 ml/min). Experiments were repeated in acutely denervated hind paws after sectioning of the sciatic nerve (8) and in dogs treated with reserpine (0.5 mg/kg. 48 and 24 hours prior to the experiment), phenolamine (2 mg/kg. iv), or bretylium (20 mg/kg, iv).2

Nerve stimulation to the paw was performed in a separate group of dogs. The sciatic nerve was sectioned and Harvard bipolar shielded electrodes were placed around the distal portion of the cut sciatic nerve. The nerves were stimulated with an American Electronics Laboratory stimulator at variable frequencies with 2-msec, 20–30-v pulses for 15 seconds. Nerve stimulation was repeated 15 minutes after starting a constant infusion of PGB2.

**Statistical Analyses**

Data were analyzed with analysis of variance using orthogonal comparisons. Means were compared with Student’s or group t-test and either Tukey’s procedure or Dunnett’s test (9); P ≤ 0.05 was statistically significant.

**Results**

Table 1 summarizes the effects of intra-arterial infusions of PGB2 on perfusion pressure of the innervated canine hind paw. PGB2 produced concentration-dependent increases in cutaneous vascular resistance but did not significantly affect systemic arterial blood pressure. Mean arterial blood pressure immediately before infusion of PGB2 was 122 ± 12 mm Hg; 10 minutes after each infusion rate of PGB2 mean arterial blood pressure was 125 ± 14 mm Hg (50 ng/kg min−1), 119 ± 12 mm Hg (200 ng/kg min−1), and 121 ± 10 mm Hg (800 ng/kg min−1).

**Effect of Reserpine, Acute Denervation, and Adrenergic Blockade on Vascular Reactivity and PGB2-Induced Vasodilatation**

The effects of acute denervation, reserpine (0.5 mg/kg day−1) for 2 consecutive days prior to the experiment, bretylium (20 mg/kg, iv), and phenolamine (2 mg/kg, iv) on systemic arterial blood pressure and hind-paw perfusion pressure are summarized in Table 1. Acute denervation achieved by sectioning the sciatic nerve reduced hind-paw perfusion pressure but did not affect systemic pressure. Systemic and perfusion pressures increased after intravenous administration of bretylium. However, both parameters were below control values prior to the evaluation of the responses to the vasoactive stimuli. Both reserpine and phenolamine reduced systemic arterial blood pressure and hind-paw perfusion pressure significantly (Table 1). The concentrations of the antagonists used are sufficient to deplete the catecholamine stores or block the pressor responses to the appropriate stimuli (10–13). Table 1 summarizes the effects of each of the pharmacologic antagonists on the responses of the hind paw to PGB2. Acute denervation, bretylium, phenolamine, and reserpine significantly reduced the constrictor responses and unmasked a vasodilator response to PGB2. Reserpine produced a greater reduction (P < 0.05) in the response to PGB2 than did either acute denervation, bretylium, or phenolamine. These results agree with previous findings (1–3).

**Nerve Stimulation**

The effects of PGB2 on the vasconstrictor responses to nerve stimulation are summarized in Table 2. PGB2 produced a shift to the left in the frequency-response curve to nerve stimulation. The F-ratio of the PGB2-nerve stimulation interaction term in the analysis of variance was not statistically significant (P > 0.2). This finding indicates that the slopes of nerve stimulation frequency-response curves before and during PGB2 infusions did not deviate significantly from parallelism (9). Acute denervation of the hind paw did not affect the magnitude of PGB2-induced enhancement of the responses to nerve stimulation (Table 3) despite the fact that PGB2-induced increases in perfusion pressure were reduced in the denervated hind paw (Table 1).

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1The generous supply of PGB2 used in this study was donated by Dr. J. R. Weeks and Mr. J. E. Pike of the Upjohn Company.

2Bretylium tosylate was kindly supplied by Dr. R. A. Maxwell of the Burroughs Wellcome Company.
TABLE 1
Effect of Acute Denervation, Reserpine, Phentolamine, and Bretylium on PGB₂-Induced Cutaneous Vasoconstriction

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean arterial blood pressure (mm Hg)</th>
<th>Initial perfusion pressure (mm Hg)</th>
<th>Δ Perfusion pressure after PGB₂ infusion (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 ng/kg min⁻¹</td>
</tr>
<tr>
<td>Innervated</td>
<td>10</td>
<td>122 ± 12</td>
<td>143 ± 12</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Acute denervation</td>
<td>14</td>
<td>121 ± 8</td>
<td>108 ± 5</td>
<td>16 ± 8*</td>
</tr>
<tr>
<td>Reserpine (0.5 mg/kg, im)</td>
<td>10</td>
<td>86 ± 4</td>
<td>115 ± 7</td>
<td>-30 ± 4*</td>
</tr>
<tr>
<td>Phentolamine (2 mg/kg, iv)</td>
<td>5</td>
<td>82 ± 10</td>
<td>112 ± 20</td>
<td>-11 ± 2.4*</td>
</tr>
<tr>
<td>Bretylium (20 mg/kg, iv)</td>
<td>5</td>
<td>124 ± 12</td>
<td>127 ± 5</td>
<td>10 ± 11*</td>
</tr>
</tbody>
</table>

All values are means ± SE. Acute denervation was achieved by sectioning the sciatic nerves; reserpine was administered 48 and 24 hours before the experiment; phentolamine and bretylium were administered 20 minutes before infusions of PGB₂. Intra-arterial infusions of PGB₂ were maintained for 15 minutes and perfusion pressure was returned to base-line values before infusion of the next highest concentration.

*Differs significantly from responses in the innervated hind paw (P < 0.05).

NOREPINEPHRINE AND TYRAMINE

Figure 1 illustrates the effects of intra-arterial infusions of PGB₂ on vascular resistance and the pressor responses to bolus injections of norepinephrine and tyramine. Despite the intensive vasoconstriction produced by the high dose of PGB₂, the increases in norepinephrine-induced perfusion pressures were essentially similar to control values. During infusions of the low dose of PGB₂ (50 ng/kg min⁻¹) the pressor response to the high dose of norepinephrine was slightly enhanced. However, as the infusion rate of PGB₂ was increased, no significant differences appeared in the pressor responses to norepinephrine before and during infusions of PGB₂ (Fig. 1 middle). In contrast to these findings, PGB₂ enhanced the pressor responses to the low dose of tyramine. This effect did not depend on the dose of PGB₂ used (Fig. 1 middle and Fig. 2 center). In the acutely denervated hind paw, PGB₂ did not affect the pressor responses to either norepinephrine or tyramine (Table 3).

LOCAL COOLING

The initial constrictor responses to local cooling at 4°C for 90 seconds varied from experiment to experiment but were consistent within the hind paw of an individual dog. A typical record of PGB₂-induced enhancement of cutaneous vasoconstrictor responses to local cooling is shown in Figure 3. During intra-arterial infusions of PGB₂ the constrictor responses to local cooling were enhanced (Fig. 2).

TABLE 2
Effect of Intra-Arterial Infusions of PGB₂ on Vasoconstrictor Responses of the Innervated and Denervated Hind Paw to Nerve Stimulation

<table>
<thead>
<tr>
<th>PGB₂ (ng/kg min⁻¹)</th>
<th>Δ Perfusion pressure after nerve stimulation (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hz</td>
</tr>
<tr>
<td>Innervated</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12.8 ± 3</td>
</tr>
<tr>
<td>200</td>
<td>41.9 ± 1*</td>
</tr>
<tr>
<td>Denervated</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>50</td>
<td>29 ± 4*</td>
</tr>
<tr>
<td>200</td>
<td>49 ± 20*</td>
</tr>
</tbody>
</table>

Each value represents the mean response ± SE to stimulation (2 msec, 20-30 V) of the sciatic nerve at 1, 4, and 16 Hz for 15 seconds. Six dogs were studied in each group.

*Differs significantly from control responses (P < 0.05).
TABLE 3
Effect of PGB₂ on the Pressor Responses of the Acutely Denervated Hind Paw to Intra-Arterial Bolus Injections of Norepinephrine and Tyramine

<table>
<thead>
<tr>
<th>PGB₂ (ng/kg min⁻¹)</th>
<th>Norepinephrine</th>
<th>Tyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 μg</td>
<td>0.3 μg</td>
</tr>
<tr>
<td>0</td>
<td>21 ± 0.1</td>
<td>55 ± 4.9</td>
</tr>
<tr>
<td>50</td>
<td>24 ± 2.2</td>
<td>51 ± 5.1</td>
</tr>
<tr>
<td>200</td>
<td>21 ± 1.2</td>
<td>50 ± 4.9</td>
</tr>
</tbody>
</table>

Responses to each dose of agonist were obtained before and during continuous infusions of PGB₂. Each value represents the mean response from eight dogs. Responses do not differ significantly (P > 0.05) from control values.

The effect of these infusions of PGB₂ on vasoconstrictor responses to local cooling was not studied in the acutely denervated perfused hind paw, because the responses were extremely small and were not reproducible within an individual dog.

EFFECT OF PGB₂ ON VASODILATION PRODUCED BY LOCAL HEATING AND NITROGLYCERIN

A polyethylene bag of water at 45°C applied directly to the perfused hind paw for 30 seconds caused cutaneous vasodilation. The magnitude of heat-induced dilation was significantly reduced during infusions of PGB₂ (Figs. 3 and 4). In contrast to these findings, PGB₂ did not affect the magnitude of nitroglycerin-induced decreases in cutaneous vascular resistance (Figs. 1 and 4).

Discussion

The results of this study show that changes in resistance produced by PGB₂ do not influence significantly the magnitude of norepinephrine-induced increases in vascular resistance. Pressor responses to vasoactive stimuli at a perfusion pressure of 150 mm Hg may not be comparable with a change in pressure at 500 mm Hg despite constant-flow perfusion since smaller changes in smooth muscle wall tension are required to produce equivalent degrees of vascular smooth muscle shortening. Similarly, as the ratio of wall to lumen thickness is increased as a consequence of PGB₂-induced vasoconstriction, equivalent decreases in the radius of the vascular bed produce disproportionately higher increases in perfusion pressure. Therefore, whether PGB₂-induced enhancement of the cold-induced vasoconstriction was related to a direct effect of PGB₂ or to a nonspecific effect of the pressure elevation produced by the drug does not appear to be established by this study.

Other investigations in our laboratory (14), however, which are supported by the findings of Kadowitz et al. (15, 16), demonstrate that the pressor responses of the perfused hind paw to exogenously administered norepinephrine and nerve stimulation are independent of the initial vascular resistance encountered in this study. Furthermore, as perfusion pressure increases to very high levels, the pressor responses to norepinephrine and nerve stimulation should decrease (17). Finally, studies performed with PGB₁ demonstrate that it produces effects on cold-induced vasoconstriction similar to those produced by PGB₂, although the magnitude of vasoconstriction is less (18). Therefore, although...
PGB$_2$-INDUCED VASOCONSTRICTION

It cannot be discounted entirely, it is unlikely that PGB$_2$-induced enhancement of vasoconstrictor responses to cold represents a nonspecific effect resulting from increased pressure elevation.

The results of the present experiments indicate that the response of the normally innervated hind paw to intra-arterial infusions of PGB$_2$ is vasoconstriction. PGB$_2$-induced vasoconstriction is neurogenic in origin and is mediated by release or enhancement of release of catecholamines from the adrenergic nerve endings innervating the cutaneous vasculature (1–3). PGB$_2$ also possesses intrinsic direct smooth muscle vasoconstrictor activity (19). Depletion of catecholamines by reserpine and blockade of both smooth muscle adrenergic receptors (phenotamine) and neurotransmitter release (bretlyium) shifted the dose-response curve for PGB$_2$ to the right but did not abolish PGB$_2$-induced vasoconstriction. However, these interventions abolished the vasoconstrictor responses to nerve stimulation and tyramine, interventions which release norepinephrine from adrenergic nerves (3, 10–13). Therefore, the ability of acute denervation to reduce PGB$_2$-induced vasoconstriction also sug-

FIGURE 2
Effect of intra-arterial infusions of prostaglandin B$_2$ (PGB$_2$) on the pressor responses of the perfused hind paw to norepinephrine (left), tyramine (center), and local cooling (4°C, 90 seconds) (right). Ordinate: Change in perfusion pressure (Δ mm Hg from base-line pressure) after administration of the agonist. Abscissa: Concentration of agonist. Responses to agonists were obtained before and during continuous infusions of PGB$_2$. Values listed on the bottom of the bars are percent increases in perfusion pressure. Asterisks indicate responses to the agonists during infusions of PGB$_2$ which differed (P < 0.05) from control. CV = coefficient of variation and IA = intra-arterial.

FIGURE 3
Typical record of the potentiating effect of prostaglandin B$_2$ (PGB$_2$) on cold-induced vasoconstriction and the inhibitory effect on heat-induced vasodilation. Responses to the agonists were obtained 15 minutes after initiation of PGB$_2$ infusions. IA = intra-arterial.

FIGURE 4
Effect of prostaglandin B$_2$ (PGB$_2$) on the vasodilator responses of the perfused innervated canine hind paw to local heating (45°C for 30 seconds) and intra-arterial (IA) infusion of nitroglycerin. Ordinate: Decrease in perfusion pressure expressed as a percent of control pressure which averaged 152 ± 8 (SE) mm Hg, in response to heat (left) or nitroglycerin (right). Numbers in the bars represent the mean decrease in perfusion pressure (mm Hg) resulting from local heating. Asterisks indicate the responses to the interventions which differed significantly (P < 0.05) from control.
gests that this prostaglandin not only releases catecholamines but also enhances resting or tonic release of neurotransmitter. In addition, PGB₂ has intrinsic vasodilator activity which is masked by its ability to release catecholamines. Previous studies (1–3) have demonstrated that PGB₂-induced vasodilation reflects a direct smooth muscle site of action that is independent of activation of cholinergic, histaminergic, or beta receptors.

Enhancement of reflexly mediated (cold-induced) vasoconstriction and neurally mediated vasoconstriction by PGB₂ is evident from this study. Although PGB₂ enhances the vasoconstrictor responses to norepinephrine and tyramine in the innervated preparation, these effects are minimal compared with the enhancement of the responses to nerve stimulation and cold-induced vasoconstriction. In addition, acute denervation abolishes PGB₂-induced enhancement of norepinephrine and tyramine-mediated vasoconstriction without any significant effect on the PGB₂-induced enhancement of the responses to nerve stimulation. These data are consistent with the conclusion that PGB₂ enhances the release of neurotransmitter that is susceptible to activation by the nerve action potential (1–3). However, the data do not allow the same conclusion concerning PGB₂-induced enhancement of the vasoconstrictor responses to cold. Since this intervention has both an afferent and an efferent component (20, 21), we cannot conclude with any certainty that PGB₂ preferentially affects either component. However, Figures 2 and 3 demonstrate that the vasoconstriction in response to local cooling is enhanced during intra-arterial infusions of PGB₂.

Intra-arterial infusions of PGB₂ were effective in blocking the cutaneous dilator responses to local warming but did not inhibit the vasodilator responses to intra-arterially administered nitroglycerin, suggesting that PGB₂ modified the cutaneous responses to local heating by an effect on the afferent or efferent limb of the reflex response to heat rather than by an effect on the vascular smooth muscle cells themselves. PGB₂ enhances the release of and releases the adrenergic neurotransmitter, norepinephrine; therefore, assuming that heat-induced vasodilation is mediated in part by the inhibition of sympathetic activity to the cutaneous vasculature (20, 21), PGB₂ might prevent or delay the inhibition of adrenergic transmitter release.

Our data suggest that cutaneous vasoconstriction produced by PGB₂ is partly mediated by increased release of norepinephrine from the adrenergic nerves innervating the canine hind paw. Also PGB₂-induced enhancement of the cutaneous vasoconstrictor responses to nerve stimulation and cooling and inhibition of the vasodilator responses to local warming may be mediated by an enhanced release of neurotransmitter and a decreased ability of the nerves to terminate the release process, respectively, rather than by a direct effect of PGB₂ on the vasculature. The ability of PGB₂ to (1) produce intense cutaneous vasoconstriction which is reduced by acute denervation, reserpine treatment, and alpha-receptor and adrenergic neuronal blockade, (2) enhance vasoconstrictor responses to cold and nerve stimulation, and (3) antagonize the responses to heating makes this preparation a potential model for the study of Raynaud’s phenomenon.

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References


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