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Enhancement of Neural and Thermal Vasoconstriction by Prostaglandin B_1[1,2] (38619)

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Recent reports from our laboratory demonstrated that prostaglandin B_2 (PGB_2) enhanced the constrictor response of the perfused canine paw to both sympathetic nerve stimulation and local cooling while antagonizing the vasodilator response to local heating (1, 2). These effects were manifested at concentrations of PGB_2 that profoundly constricted the canine paw. It was suggested that the ability of PGB_2 to enhance thermal and neurogenic vasoconstriction resulted from a selective ability of PGB compounds to facilitate catecholamine release. However, the possibility that these effects of PGB_2 were secondary to the increased wall stress resulting from the higher distending pressure could not be entirely eliminated (1–3).

Prostaglandin B_1 (PGB_1) is less potent a constrictor of the canine paw than is PGB_2 and possesses intrinsic vasodilator properties at low concentrations (1–3). This study examines the effects of PGB_1 on the responses of the perfused canine paw to local cooling and heating to evaluate whether the effects of PGB compounds on these interventions are intrinsic to the prostaglandin B molecule or reflect the increased wall stress and transmural pressure associated with the constricted paw.

Materials and Methods. Fifteen mongrel dogs of either sex (14–18 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv) and, after endotracheal intubation, artificially ventilated with room air with a Harvard ventilator (15 strokes/min; 250 ml of air/stroke). Arterial pressure was measured with a Statham P23AA arterial-pressure transducer through a cannula inserted into the left femoral artery. A cannula inserted in the left femoral vein was utilized for iv administration of drugs. The right hindpaw was perfused according to the method of Zimmerman and Gomez (4). This technique has been previously described in detail (4–5). Flow [24.9 ± 2.3 (SE) ml/min in 15 experiments], was set at a level which resulted in a perfusion pressure equal to systemic pressure and was not reset during the experiment. Isolation of the hindpaw was confirmed by turning off the perfusion pump and measuring the residual pressure which approached small vein pressure (23 ± 2.1 mmHg). 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), (Nutritional Biochemicals, Cleveland, OH), norepinephrine (0.1–1.0 μg), (Levophed bitartrate, Winthrop Laboratories, New York, NY), and nitroglycerin (10–100 μg, Parke Davis, Inc., Detroit, MI) were obtained prior to and during constant intra-arterial infusions of PGB_1. Injections (0.01–0.10 ml) and infusions (1.0 ml/min) were made directly into the perfusion circuit prior to its entrance into the paw. Responses to the agonists during infusions of PGB_1 were measured 15 min after initiation of the PGB infusion. Responses to cold were

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obtained by applying ice water (at 4°C) contained in a polyethylene bag directly to the paw for 90 sec. Responses to heat were obtained by application of a polyethylene bag containing water (45°C) applied directly to the paw for 60 sec. These procedures were repeated during infusions of PGB_1.

Nerve stimulation to the paw was performed in a separate group of animals. 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, 2-msec duration, 20–30 V, for 15-sec duration. Nerve stimulation was repeated 15 min after starting a constant infusion of PGB_1.

Data were analyzed with analyses of variance utilizing orthogonal comparisons. Means were compared with Student's t test for paired data (6). A P value of 0.05 or less was chosen for statistical significance.

Results. Table I summarizes the effects of intra-arterial infusions of prostaglandin B_1 (PGB_1) on perfusion pressure of the innervated canine paw. PGB_1 produced biphasic changes in cutaneous vascular resistance but did not significantly affect systemic mean arterial pressure.

Nerve stimulation. The effect of PGB_1 on the vasoconstrictor responses to sympathetic nerve stimulation are summarized in Table II. PGB_1 (200 ng/kg/mm ia) produced a shift to the left in the frequency-response curve to nerve stimulation. The F ratio of the PGB-nerve stimulation interaction term in the analysis of variance was not statistically significant ($P > 0.3$). This finding means that the slope of the nerve stimulation-frequency response curve before and during PGB_1 infusions did not deviate significantly from parallelism. However, significant enhancement of the response to nerve stimulation required extremely high concentrations of PGB_1 (Table II).

TABLE I. EFFECT OF INTRA-ARTERIAL INFUSIONS OF PROSTAGLANDIN B_1 (PGB_1) ON SYSTEMIC AND PERFUSION PRESSURES OF THE PERFUSED, INNERVATED CANINE PAW.[a, b]

Pressures	PGB_1 infusion rate (ng/kg/min) ia			
	Control	50	100	200
	(mmHg ± SEM)			
MAP	121±12	121±12	116±10	114±11
P.P.	154±10	113±11*	102±9*	161±18

[a] Intra-arterial infusions of PGB_1 into the perfused, innervated canine paw were continued for 15 min before measurement of mean arterial pressure (MAP) and perfusion pressure (P.P.).

[b] Each value represents the responses from 15 animals. An asterisk denotes that the pressures differ ($P < 0.05$) from control values.

TABLE II. EFFECT OF PGB_1 ON RESPONSES OF THE PERFUSED DENERVATED PAW TO SYMPATHETIC NERVE STIMULATION.[a]

PGB_1 (ng/kg/min ia)	Stimulus frequency (Hz)				
	1	2	4	8	16
	Δ Perfusion pressure (mmHg ± SEM)				
0	17 ±6[b]	35 ±11	64 ±11	112 ±17	160 ±28
50	21 ±9	42 ±13	64 ±14	96 ±16	162 ±26
200	28 ±9	42 ±10	81 ±18	142 ±21*	197 ±30*
800	48 ±14*	81 ±20*	102 ±25*	156 ±22*	194 ±28*

[a] Responses to stimulation of the sciatic nerve obtained prior to and during infusion with PGB_1 or saline.

[b] $N = 5$.

* Differ from responses obtained during infusion of PGB_1 (0 ng/kg/min) – $P < 0.05$.

Norepinephrine and tyramine. Figure 1 (top and middle panels) illustrates the effects of intra-arterial infusions of PGB_1 on vascular resistance and the pressor responses to bolus injections of norepinephrine and tyramine. Despite the decrease in perfusion pressure by PGB_1, the increases in norepinephrine-induced perfusion pressures were essentially similar to control values. PGB_1 did not affect the pressor responses to tyramine (Figs. 1 and 2, middle panels).

Local cooling. The initial constrictor responses to local cooling at 4°C for 90 sec were variable from experiment to experiment but were consistent within the paw of an individual dog. During ia infusions of PGB_1

the constrictor responses to local cooling were enhanced (Fig. 2). This occurred with concentrations of PGB_1 significantly lower than those required to enhance the response to nerve stimulation and was unrelated to the level of perfusion pressure of the paw (Table I).

Effect of PGB_1 on vasodilation produced by local heating and nitroglycerin. Application of a polyethylene bag of water (45°C) directly to the perfused canine paw for 60 sec resulted in cutaneous vasodilation. The magnitude of heat-induced dilation was significantly reduced during infusions of PGB_1 (Fig. 3). PGB_1 decreased the magnitude of nitroglycerin-induced decrease in cutaneous vascular resistance (Figs. 1 and 3).

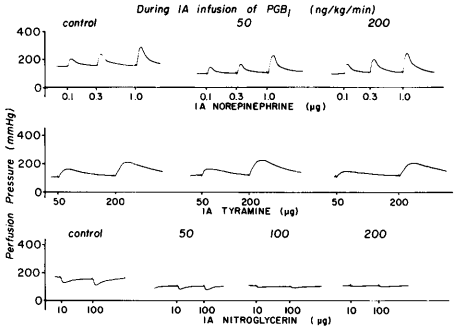

FIG. 1. A typical record illustrating the effects of PGB_1 on cutaneous vascular resistance of the perfused innervated canine paw and on vascular responses to intra-arterial norepinephrine (top panel), tyramine (middle panel), and nitroglycerin (bottom panel). Responses to the agonists were obtained 15 min after the initiation of PGB_1 infusions.

Discussion. The results of this study show that prostaglandin B_1 selectively enhances the pressor response to local cooling and nerve stimulation without any significant effect on the pressor responses to norepinephrine or tyramine. Furthermore, the dilator responses to local heating and nitroglycerin are significantly depressed. These findings suggest that PGB_1 does not act by inhibition of alpha receptors; it may act to depress the vasodilatory mechanisms of nitroglycerin, and also to facilitate neurotransmitter release at the adrenergic nerve terminal. Moreover, the results clearly demonstrate that PGB-induced enhancement of the vasoconstrictor responses to cold (1–3) cannot result from an increase in vessel wall tension since PGB_1 initially decreases rather than increases, intravascular pressure.

Pressor responses to vasoactive stimuli at a perfusion pressure of 150 mmHg are not comparable with changes in pressure at 100 mmHg despite constant flow perfusion since greater changes in smooth-muscle wall tension would be required to produce equivalent degrees of vascular smooth-

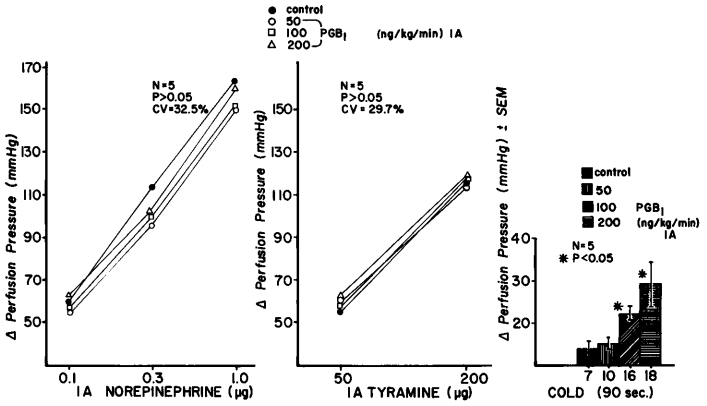

FIG. 2. Effect of intra-arterial infusions of PGB_1 on the pressor responses of the perfused paw to norepinephrine, tyramine, and local cooling. Ordinate: change in perfusion pressure (Δ mmHg from baseline pressure) after administration of the agonist. Abscissa: concentration of agonist. Responses to agonists were obtained before and during continuous infusions of PGB_1. C. V.: coefficient of variation. Left panel: norepinephrine; middle panel: tyramine; right panel: local cooling, 90 sec 4°C. The values listed on the bottom of the bars of the right panel are the percentage increases in perfusion pressure. An asterisk denotes that responses to the agonists during infusions of PGB_1 differ ($P < 0.05$) from control.

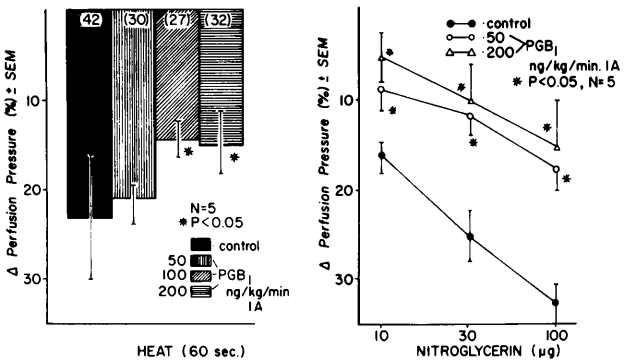

FIG. 3. Effect of PGB_1 on the vasodilator responses of the perfused innervated canine hindpaw to local heating (45°C for 60 sec) and intra-arterial nitroglycerin. Ordinate: decrease in perfusion pressure [expressed as a percentage of control pressure which averaged 162 ± 11 (SE) mmHg] in response to heating (left panel) or nitroglycerin (right panel). The numbers in the bars in the left panel represent the mean mmHg decrease in perfusion pressure to local heating. An asterisk denotes that the responses to the interventions differ ($P < 0.05$) from control.

muscle shortening. However, investigations from our laboratory (7) and by Kadowitz *et al.* (8, 9) demonstrate that the pressor responses of the perfused paw to exogenously administered norepinephrine and nerve stimulation are independent of the level of initial vascular resistance encountered in this study. If the potentiating effects of PGB_1 on vascular responses to neural and thermal vasoconstriction were secondary only to a decrease in perfusion pressure, then as perfusion pressure decreased to low levels, the equivalent pressor responses to norepinephrine, tyramine, local cooling, and nerve stimulation should all have increased similarly. Our studies show that the effects of PGB_1 on cold vasoconstriction are concentration related (Fig. 2), whereas, its effects on perfusion pressure are biphasic (Table I; see also refs. 2 and 13). These findings clearly suggest that the effects of PGB_1 on vascular responses to cold, heat, and nitroglycerin are not directly related to changes in perfusion pressure.

Enhancement of reflexly mediated (cold) vasoconstriction and neuronally mediated vasoconstriction by PGB_1 is evident from this study. These data are consistent with the conclusion that PGB_1 enhances the release of neurotransmitter susceptible to activation by the nerve action potential (1–3). However, the data do not allow the same conclusion concerning PGB_1-induced enhancement of the vasoconstrictor responses to cold. Since this intervention has both an afferent and an efferent component (10), we cannot conclude with any certainty that PGB_1 preferentially affects either the former or latter component. In addition, the vasoconstriction in response to local cooling is enhanced during ia infusions of concentrations of PGB_1 that do not affect significantly the responses to nerve stimulation. Whether this phenomenon reflects different sensitivities of the thermal and neural responses to PGB_1, or two different actions of PGB_1, remains to be elucidated.

Intra-arterial infusions of PGB_1 were effective both in blocking the cutaneous dilator responses to local warming and the vasodilator responses to intra-arterial nitroglycerin, suggesting that PGB_1 modified the cutaneous responses to local heating through an effect on the afferent or efferent limb of the reflex response to warming and/or the vascular smooth muscle cells themselves. Since PGB_1 enhances the release of the adrenergic neurotransmitter, norepinephrine, it is possible [assuming heat vasodilation is mediated in part by the inhibition of sympathetic activity to the cutaneous vasculature (10)] that PGB_1 may prevent or delay the inhibition of adrenergic transmitter release by local heating. In addition, PGB_1 may inhibit sulfhydryl groups essential for nitroglycerin-induced vasodilation (11) and perhaps intraneuronal neurotransmitter retention (12). We have recently demon-

strated that PGB_1 decreases vascular smooth-muscle content of sulfhydryl groups (13). Whether PGB_1-induced inhibition of vascular and neural sulfhydryl groups is related to its facilitation of transmitter release is currently under investigation.

Summary. The vascular effects of prostaglandin B_1 (PGB_1) were studied during constant-flow perfusion of the canine hindpaw. The effects of PGB_1 (50–200 ng/kg/min ia) on systemic and hindpaw perfusion pressures and on responses to local cooling (4°C for 90 sec) and local heating (45°C for 60 sec) were measured in 15 dogs. PGB_1 (50–100 ng/kg/min) decreased perfusion pressure without any significant effect on systemic arterial pressure. Higher concentrations of PGB_1 (200 ng/kg/min) elevated perfusion pressure to control values. The pressor responses to local cooling were increased from 11 to 32 mmHg while the dilator responses to local heating and nitroglycerin were reduced during infusions of PGB_1. PGB_1 also enhanced the pressor responses to norepinephrine or tyramine. These findings support the conclusions that (1) low concentrations of prostaglandin B_1 enhance neurotransmitter release with minimal effects on vascular smooth muscle cells and (2) these effects are not secondary to increased perfusion pressures or vascular wall stresses since infusions of PGB_1 resulted in vasodilation.

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