Central Effects of Prostaglandin E2 on Blood Pressure and Plasma Renin Activity in Rats
Role of the Sympathoadrenal System and Vasopressin

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SUMMARY This study was designed to determine the roles of the sympathetic nervous system, adrenal medulla, and arginine vasopressin (AVP) in mediating pressor and plasma activity (PRA) responses to intraventricularly (ICV) administered prostaglandin E2 (PGE2) in conscious rats. The ICV PGE2 elevated blood pressure and caused increases in PRA, plasma AVP, and plasma norepinephrine and epinephrine. The pressor effect of ICV PGE2 was not influenced by pretreatment with captopril, but was attenuated by the AVP antagonist, d(CH2)5Tyr(Me)AVP, and by phenoxybenzamine, and was completely abolished by the combination of the AVP antagonist and phenoxybenzamine. The PRA response to ICV PGE2 was not affected by bilateral renal denervation or by phenoxybenzamine alone, but was attenuated by propranolol alone and was completely abolished by the combination of propranolol and phenoxybenzamine. Bilateral adrenomedullectomy did not affect the pressor response to ICV PGE2, whereas it attenuated the increase in PRA and completely abolished the increase in plasma epinephrine. These results suggest that the pressor effect of ICV PGE2 is the result of increased sympathetic nervous system activity and is dependent on the stimulation of alpha-adrenergic receptors and on AVP release. The pressor response to ICV PGE2 is accompanied by but not dependent on an increase in PRA. The renin-stimulating effect of centrally administered PGE2 is, at least in part, dependent on beta-adrenergic receptor stimulation by increased circulating catecholamines. (Hypertension 4: 809-816, 1982)

KEY WORDS • Prostaglandin E2 • central injection • blood pressure • plasma renin activity • vasopressin • the sympathoadrenal system

BLOOD pressure rises dramatically when prostaglandin E2 (PGE2) is injected into the lateral cerebral ventricle of the rat.1-3 Observations by others that pretreatment with phenoxybenzamine may abolish2 and that hypophysectomy attenuates3 this pressor response, as well as the fact that ventriculocisternal PGE2 infusions elevate plasma arginine vasopressin (AVP) levels in another species,4 suggest roles for the sympathetic nervous system and AVP secretion in mediating intraventricularly (ICV) administered PGE2-induced hypertension. Other mechanisms that also may be involved but have not been investigated include the renin-angiotensin system. It is known that central nervous system stimulation increases renin release,5,6 and thus it is possible that ICV PGE2 stimulates the peripheral renin-angiotensin system.

We designed the present studies to characterize the actions of ICV PGE2, focusing on the sympathetic nervous system, adrenal medulla, renin-angiotensin system, and AVP. By utilizing a potent antagonist of the vascular actions of AVP and a variety of drugs which oppose the actions of catecholamines and the renin-angiotensin system, we were able to demonstrate the relative contributions of the sympathetic nervous system and AVP to the rise in blood pressure evoked by ICV PGE2 in conscious rats and to show that the renin-angiotensin system is not involved in this pressor phenomenon.
Methods

Male Wistar rats (Charles River Breeding Laboratory, Wilmington, Massachusetts) weighing 500 to 600 g, eating normal rat chow, were anesthetized with ether. PE 50 cannulas (Clay Adams, Parsippany, New Jersey) were placed in the femoral artery and a femoral and/or jugular vein. The cannulas were filled with heparin-saline solution (20 U/ml), tunneled subcutaneously to the back and fixed in position. At the same time a PE 10 cannula was placed stereotaxically into the lateral ventricle through a hole drilled in the skull (approximately 1 mm posterior to the bregma, 1.5 mm lateral to the midline, and 4.5 mm ventral to the skull surface) and fixed to the skull with screws and dental cement.

The rat was allowed to waken and, after an interval of at least 24 hours, experiments were started in which PGE₂ (Sigma Chemical Company, St. Louis, Missouri). 1 μg/kg dissolved in 10 μl of a solution containing 0.9% NaCl and 0.5% ethanol, was injected into the lateral ventricle of anesthetized animals. The PGE₂ was dissolved in 95% ethanol at 10 μg/ml as a stock solution and then diluted with 0.9% NaCl just before injection. After completion of the experiments, dye was injected into the ventricle, the animal sacrificed, and the position of the cannula verified. The following other interventions were performed.

Angiotensin-Converting Enzyme Inhibition

One mg/kg of SQ 14,225 (captopril, Squibb Institute for Medical Research, Princeton, New Jersey) was injected intravenously 5 minutes prior to the ICV injection of PGE₂. Control animals received an injection of 0.3 ml of 0.9% NaCl.

Bilateral Renal Denervation

In another series of experiments, bilateral renal denervation was performed 1 week before the ICV PGE₂ study. The rat was anesthetized with ether. Then through a flank incision the renal adventitia was stripped and the renal artery painted with 20% phenol (weight/volume) in absolute alcohol. Control animals were also anesthetized and subjected to a sham operation that consisted of flank incisions only. After the ICV PGE₂ study, the rat was sacrificed, and the kidneys were quickly removed, frozen in liquid nitrogen, and stored at −80°C for subsequent determination of renal norepinephrine content.

Infusion of an AVP Antagonist

In these experiments 30 μg of d(CH₂)₅Tyr(Me)AVP (kindly supplied by Dr. M. Manning, Toledo, Ohio), an AVP analog that antagonizes the vascular effects of AVP, was injected rapidly into a femoral vein after which 1 μg/min was infused for 30 minutes with the aid of a Harvard constant infusion pump (Harvard Apparatus Company, Millis, Massachusetts). PGE₂ was injected intracerebrally at the midpoint of this study. In some experiments the AVP analog was infused in animals pretreated with the alpha-adrenergic antagonist phenoxybenzamine, as described below.

Adrenergic Antagonists

In these studies 1 mg/kg of phenoxybenzamine (Dibenzyline, Smith Kline and French, Philadelphia, Pennsylvania) was administered rapidly intravenously 10 minutes before the ICV PGE₂ injection. In other experiments 10 mg/kg of propranolol (Sigma Chemical Company, St. Louis, Missouri) was administered intraperitoneally 30 minutes prior to the ICV PGE₂ injection.

Bilateral Adrenomedullectomy

Bilateral adrenomedullectomy was performed through flank incisions one week prior to the experiment. A small slit was made in the adrenal capsule and the medulla removed by gently squeezing the gland with a forceps. Control animals underwent sham surgery which consisted of flank incisions only.

In all of the protocols above, arterial blood (1 ml) was drawn before and 15 minutes after ICV PGE₂ injection. Samples for plasma renin activity (PRA) were collected in iced tubes containing EDTA (1 mg/ml); samples for norepinephrine and epinephrine were collected in iced tubes containing EGTA (90 mg/ml) and glutathione (60 mg/ml). Blood removed for sampling was immediately replaced with an equal volume of 0.9% saline. In a separate series of experiments, awake animals were killed by decapitation 15 minutes after ICV injection of PGE₂ or vehicle alone. Blood was collected in chilled heparinized tubes for determination of plasma osmolality and AVP.

Blood pressure and heart rate were continuously recorded via a Statham P23Db pressure transducer (Statham Laboratories, Gould-Statham, Oxnard, California) coupled to a Hewlett-Packard 7758B recorder. PRA was measured by radioimmunoassay according to the method of Haber et al. and renal norepinephrine content and plasma norepinephrine and epinephrine by a modification of the radioenzymatic assay of Peuler et al. Plasma for AVP determination was extracted with acetone and assayed with a highly specific antiserum (10169) which does not cross react with oxytocin, vasotocin, or angiotensin II. Antiserum 10169, utilized at a final dilution of 1:3,000,000 in a nonequilibrium assay in which bound and free vasopressin were separated by charcoal, has a sensitivity of 0.1 pg/assay tube. Plasma osmolality was measured by freezing point depression using an Advanced Osmometer (Advanced Instruments, Inc., Needham Heights, Massachusetts).

Results were expressed as mean ± SEM. Statistical analysis was performed by both paired and unpaired Student’s t tests; results were considered significant when p < 0.05.

Results

Injection of PGE₂ into the lateral ventricle resulted in an immediate increase in blood pressure, which was maximal at approximately 15 minutes, and returned to basal levels within 1 hour (fig. 1 A). Mean blood
Effects of intraventricular (ICV) prostaglandin E₂ (PGE₂) injection on mean blood pressure (A) in intact rats, (B) in rats pretreated with the arginine vasopressin (AVP) antagonist d(CH₂)₅Tyr(Me)AVP (MEAVP 60 μg IV), (C) in rats pretreated with phenoxybenzamine (1 mg/kg IV) and (D) in rats pretreated with phenoxybenzamine (1 mg/kg IV) and the AVP antagonist (60 μg IV).

Pressure was 122 ± 3 mm Hg before injection and 153 ± 3 mm Hg after injection (p < 0.001). Heart rate increased simultaneously from 416 ± 11 to 499 ± 14 beats/min (p < 0.001) and PRA from 3.0 ± 0.5 to 5.8 ± 0.8 ng/ml/hr (p < 0.01). Injection of vehicle alone produced no changes (fig. 2). Plasma AVP levels, measured in separate studies, were 2.5 ± 0.5 pg/ml in vehicle injected rats (n = 7) compared to 29 ± 8 pg/ml in PGE₂-treated animals (n = 7) (p < 0.01). Plasma osmolality was similar in both groups.
In parallel experiments, 1 μg/kg of PGE₂ was injected into a jugular vein instead of the cerebral ventricle (n = 6). Blood pressure decreased transiently by 5 ± 1 mm Hg (p < 0.001), but there were no significant changes in PRA (3.7 ± 0.2 ng/ml/hr preinjection and 3.4 ± 0.5 ng/ml/hr at 15 minutes postinjection). These results demonstrate that the effects of ICV PGE₂ described above are not dependent on leakage of PGE₂ into the periphery.

Angiotensin-Converting Enzyme Inhibition

The dose of captopril injected has been shown to completely block the pressor effect of 310 ng/kg of angiotensin I. In the current study the converting enzyme inhibitor produced a small but significant (p < 0.01) reduction in basal blood pressure (—6 ± 1 mm Hg) but failed to alter the pressor response to ICV PGE₂ (25 ± 3 mm Hg in treated (n = 8) and 28 ± 3 mm Hg in controls (n = 7)).

Bilateral Renal Denervation

Kidney norepinephrine content in renal-denervated animals averaged 10% of the controls, demonstrating the adequacy of the procedure. There were no significant differences in the responses to ICV PGE₂ when results in renal-denervated animals (n = 8) were compared to those of sham-operated rats (n = 8) (fig. 3), even though mean basal PRA was lower in the denervated group (1.6 ± 0.2 vs 3.3 ± 0.5 ng/ml/hr in controls; p < 0.01). The absolute increase in PRA induced by ICV PGE₂ was slightly but not significantly lower in renal-denervated rats. The percent change, however, was similar in both groups, averaging 109% ± 14% in renal-denervated and 90% ± 29% in sham-operated rats. These data suggest that the pressor and renin responses to ICV PGE₂ are not mediated by the renal nerves.

Alpha-Adrenergic Blockade

In preliminary experiments, 1 mg/kg of phenoxybenzamine caused a decrease in the pressor effects of 50 μg/kg of methoxamine HCl (Burroughs Wellcome Co., Research Triangle Park, North Carolina) from 32 ± 2 to 3 ± 1 mm Hg (n = 5), demonstrating effective blockade of alpha-adrenergic receptors. Basal blood pressure was significantly lower in rats pretreated with phenoxybenzamine (119 ± 3 mm Hg in drug-treated (n = 7) vs 139 ± 2 mm Hg in controls (n = 7); p < 0.001), but PRA was similar in both groups (3.3 ± 0.4 ng/ml/hr in treated and 3.0 ± 0.4 ng/ml/hr in controls). Phenoxybenzamine attenuated the maximum pressor response to ICV PGE₂ (+28 ± 4 mm Hg in controls (n = 7) vs +15 ± 2 mm Hg in treated; p < 0.01), but did not significantly reduce the renin response (fig. 1 C and 4).

Infusion of an AVP Antagonist Alone or During Alpha-Adrenergic Blockade

Efficacy of the AVP antagonist d(CH₂)₅Tyr(Me)AVP was evaluated in preliminary studies in which 10 mU of AVP (Sigma Chemical Co., St. Louis, Missouri) was injected intravenously into five rats, followed by a 30-minute infusion of the antagonist. Initially, blood pressure increased by 35 ± 2 mm Hg, but this pressor effect was almost completely abolished during the 30-minute infusion of the antagonist (3 ± 2 mm Hg during and 2 ± 2 mm Hg 30 minutes following discontinuance of the infusion).

The AVP antagonist did not alter basal blood pressure, but attenuated the maximum pressor response to ICV PGE₂ (+28 ± 4 mm Hg in controls (n = 7) vs +14 ± 2 mm Hg with the antagonist (n = 7); p < 0.01) (figs. 1 B and 4). In contrast, basal and PGE₂-stimulated PRA were similar in both groups.

Basal blood pressure was significantly lower in rats pretreated with the AVP antagonist as well as phe-
CENTRAL EFFECTS OF PGE₂ ON BLOOD PRESSURE AND PRA/Okuno et al.

**Figure 4.** Effects of ICV PGE₂ (1 μg/kg) on mean blood pressure and PRA in saline-infused controls, in phenoxybenzamine-treated (1 mg/kg IV) rats, in rats pretreated with the AVP antagonist d(CH₂)₅Tyr(ME)AVP (MEAVP 60 μg IV), or in rats pretreated with the AVP antagonist (60 ng IV) and phenoxybenzamine (1 mg/kg IV). Bars indicate SEM; NS = not significant.

**Beta-Adrenergic Blockade**

Propranolol pretreatment, in a dose that completely abolishes the effects of isoproterenol (100 μg/kg) on blood pressure and PRA release in the rat,10 failed to influence the pressor effect of ICV PGE₂ (fig. 5). This dose, however, attenuated the increase in heart rate from 56 ± 10 to 17 ± 9 beats/min (p < 0.02). Basal PRA was significantly lower in treated animals (n = 7) (1.2 ± 0.2 ng/ml/hr) compared to controls (n = 7) (2.5 ± 0.3 ng/ml/hr; p < 0.01), and propranolol attenuated the increase in PRA evoked by ICV PGE₂ from 2.9 ± 0.6 to 0.8 ± 0.2 ng/ml/hr (p < 0.01).

**Effects of Combined Alpha- and Beta-Adrenergic Blockade**

Pretreatment with both propranolol and phenoxybenzamine lowered both blood pressure (treated (n = 8), 112 ± 4 mm Hg; control (n = 7), 132 ± 2 mm Hg; p < 0.001) and PRA (treated, 1.3 ± 0.2 ng/ml/hr; control, 2.5 ± 0.3 ng/ml/hr, p < 0.01) in the basal state and significantly attenuated the maximal pressor response to ICV PGE₂ (+ 15 ± 1 mm Hg in treated vs + 26 ± 2 mm Hg in control). Combined treatment with propranolol and phenoxybenzamine almost completely blocked the expected increment in PRA levels (fig. 5).

**Adrenomedullectomy**

Basal values for plasma norepinephrine and epinephrine were 356 ± 40 pg/ml and 413 ± 90 pg/ml...
respectively in sham-operated control animals (n = 7). ICV PGE₂ increased plasma norepinephrine to 729 ± 87 pg/ml (p < 0.02) and epinephrine levels to 1074 ± 134 pg/ml (p < 0.01), while injection of vehicle alone had no effect on catecholamine levels. With adrenomedullectomy (n = 7), basal values for norepinephrine (318 ± 42 pg/ml) were unchanged, and the increase evoked by ICV PGE₂ was similar to that of the controls. As expected, plasma epinephrine was markedly reduced by adrenomedullectomy, averaging 19 ± 6.6 pg/ml in the basal state (p < 0.001), and did not increase with ICV PGE₂.

Basal PRA (1.9 ± 0.5 ng/ml/hr) in the adrenomedullectomized rats was similar to that in the sham-operated controls (2.9 ± 0.6 ng/ml/hr). However, the increment in PRA with ICV PGE₂ was markedly attenuated (+ 0.9 ± 0.2 ng/ml/hr) compared to sham-operated controls (+ 3.0 ± 0.6 ng/ml/hr, p < 0.01). Despite these differences in circulating epinephrine and renin, there were no significant differences in pressor effects of ICV PGE₂ in adrenomedullectomized animals and sham-operated control rats (fig. 6).

Discussion

These data confirm that ICV PGE₂ administration increases systemic blood pressure in the conscious rat. The results extend those of others by demonstrating that intracerebral injection of PGE₂ also elevated PRA, AVP levels, and catecholamine concentrations. Furthermore, we demonstrate that the pressor action of ICV PGE₂ is dependent, in part, on the sympathetic nervous system and on AVP release, but not on increments in PRA. Our results also show partial dependence of the renin response to ICV PGE₂ on beta-adrenergic receptor stimulation by increased circulating catecholamines, but no dependency on functioning renal nerves.

Hoffman and Schmid² have reported that pretreatment with phenoxybenzamine virtually eliminated the blood pressure rise evoked by intracerebral PGE₂ and suggested that the pressor effect of ICV PGE₂ was primarily mediated via the sympathetic outflow. However, when Takahashi and Bühag⁴ studied rats after section of the spinal cord, they noted that only the early (2-minute) phase of the ICV PGE₂ pressor response was attenuated, while the late (15-minute) phase of the pressure rise still occurred. These authors suggested, therefore, that the pressor effect was mediated in part by mechanisms other than the sympathetic nervous system. In the present study, the ICV PGE₂-induced increase in blood pressure was accompanied by increments in circulating norepinephrine, and the pressor response to intracerebral PGE₂ was attenuated by only about 50% following combined alpha and beta blockade. Reasons for the discrepancy in the amount of suppression produced by alpha blockade in the study of Hoffman and Schmid⁵ compared to our results are not obvious, since both groups used identical doses of phenoxybenzamine. One possibility is the difference in the timing of blood pressure recording in the two protocols. Of importance, however, is that the results of both studies support an important role of centrally mediated sympathetic discharge in the pressor response to ICV PGE₂.

Having demonstrated that ICV PGE₂ increases plasma epinephrine as well as norepinephrine, we performed further studies aimed at identifying the source of these catecholamines. Aware that electrical stimulation of the diencephalon and mesencephalon increases both blood pressure and total plasma catecholamines and that this effect is greatly attenuated by removal of the adrenal medulla,¹¹,¹² we performed bilateral adrenomedullectomies in rats subsequently subjected to ICV PGE₂. This procedure did not alter the pressor or norepinephrine response, although plasma epinephrine, which was reduced to 5% of normal before prostaglandin injection, failed to increase when the blood pressure rose. Thus, it appears that the adrenal medulla and circulating epinephrine are not involved in mediating the pressor response to ICV PGE₂.

Our results demonstrate that ICV PGE₂ increases plasma AVP levels and suggest that circulating AVP may be involved in mediating the PGE₂-evoked response. Noting that median eminence lesions will abolish the antidiuretic action of ICV PGE₂, but leave the pressor response intact, Hoffman and Schmid¹ concluded that AVP was not a major factor in the PGE₂-evoked rise in pressure. In contrast, Takahashi and
Buñaño reported that the blood pressure rise induced by ICV PGE$_2$ in hypophysectomized rats was half that in intact animals, suggesting a pressor role for AVP. Such evidence, however, is indirect since it is possible that destruction of the median eminence or hypophysectomy could inhibit release of other pressor pituitary hormones such as ACTH or somatostatin. In this study, we approached the problem by using a highly specific antagonist of the pressor effects of AVP. This antagonist significantly attenuated the blood pressure rise due to ICV PGE$_2$, and the combination of AVP antagonist and phenoxybenzamine completely abolished the PGE$_2$-evoked pressor response. These results suggest that the pressor effect of ICV PGE$_2$ is dependent on activation of the central sympathetic nervous system with a resultant increase in alpha-adrenergic stimulation of resistance vessels and an enhanced release of AVP.

The pressor effect of ICV PGE$_2$ was accompanied by a significant rise in PRA. We therefore examined the contribution of angiotensin II to the pressor response by injecting PGE$_2$ intracerebrally in captopril-treated animals. Captopril had no effect on the pressor response, demonstrating that the PGE$_2$-evoked pressure rise is not angiotensin II-dependent.

Bilateral renal denervation did not reduce the renin or pressor response to ICV PGE$_2$. This is surprising since the renin release that occurs in response to electrical stimulation of the brainstem is abolished by bilateral renal denervation. It has been shown that clonidine, a centrally acting alpha-adrenergic agonist that produces a decrease in sympathetic nervous system activity, reduces PRA, and that pentolinium, a ganglion blocker, and bilateral renal denervation abolish the clonidine-induced suppression of PRA. Such data suggest that central nervous stimulation increases PRA via a mechanism that involves the renal nerves. The discrepancy between these data and the result of our study (failure of renal denervation to suppress the renin response) is hard to explain, but could be due to differences in the brain region stimulated. The increase in PRA was accompanied by increments in plasma norepinephrine and epinephrine. Since circulating catecholamines stimulate renin release, it is possible that the elevated levels of these sympathetic hormones are responsible for the increased renin. It is generally accepted that renin release provoked by catecholamine infusion or sympathetic nerve stimulation is mediated by beta-adrenergic receptors. Therefore, we examined the renin response to ICV PGE$_2$ in rats pretreated with propranolol. Propranolol markedly attenuated the renin response to ICV PGE$_2$. Since bilateral renal denervation failed, while propranolol succeeded in reducing the renin response, it appears that the increase in PRA induced by ICV PGE$_2$ is in part dependent on stimulation of beta-adrenergic receptors by increased circulating catecholamines.

We also demonstrated that phenoxybenzamine pretreatment did not alter the renin response to ICV PGE$_2$, suggesting that alpha-adrenergic receptor mechanisms are not involved in that response. The role of alpha-adrenergic receptors in the control of renin release is still controversial. Intravenous and intrarenal infusions of alpha-agonists are variously reported to increase and to decrease PRA. The alpha-adrenergic blocker phenoxybenzamine is reported to increase the renin release evoked by hypoglycemia, to completely block the increase in PRA following renal nerve stimulation and to have no effect on renin release induced by renal nerve stimulation. The results of the current study are compatible with the notion that alpha-adrenergic receptors do not play a major role in mediating renin release. However, combined administration of propranolol and phenoxybenzamine almost completely abolished the renin response to ICV PGE$_2$. In addition, bilateral adrenomedullectomy, which completely abolished the increase in plasma epinephrine but did not alter the norepinephrine response, significantly attenuated the renin response to ICV PGE$_2$. These observations are consistent with the previous demonstration that the increase in PRA evoked by electrical stimulation of the medulla oblongata of the dog was attenuated by propranolol alone and was completely abolished by the combination of propranolol and phenoxybenzamine. Blair recently examined, in anesthetized dogs, the role of alpha-adrenergic receptors in mediating tonic neural stimulation of renin secretion. She observed that intravenous phenoxybenzamine alone did not change PRA, but that when beta-adrenergic receptors were blocked by intravenous propranolol, subsequent administration of phenoxybenzamine decreased PRA by about 50%. From these findings, Blair suggested that, when beta-adrenergic receptors are blocked by propranolol, tonic neural stimulation of renin secretion is mediated by alpha-adrenergic receptors. These results and those of the current study suggest that alpha-adrenergic receptors may play a role in the renin response to ICV PGE$_2$ after the blockade of beta-adrenergic receptors.

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