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Influence of Adrenergic Drugs on Prostaglandin E Release from the Dog Kidney[1] (38361)

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(Introduced by G. E. Cartwright)

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Of the prostaglandins extractable from renomedullary tissue in various species, prostaglandin E (PGE) is considered to be the most abundant and the most potent renal vasodilator (1–3). Significant increases in the levels of PGE or PGE-like substances in renal venous blood of dogs following sympathetic renal nerve stimulation or infusion of norepinephrine into the renal arterial circulation have been reported (4–6). Since PGE is rapidly metabolized in the lungs, and since the levels found in renal venous blood were considered too low to exert a systemic effect, it was suggested that if renomedullary PGE has a physiologic role, it is primarily intrarenal in nature (4, 5). Subsequently, Lonigro and his associates reported that infusion of PGE_2 into the renal artery of dogs inhibited the renal vasoconstriction and antidiuresis produced by renal nerve stimulation or norepinephrine infusion (3).

We have employed radioimmunoassay to estimate PGE levels in the renal venous blood of dogs following injections of norepinephrine, epinephrine and isoproterenol into the renal artery. Effects of pretreatment with phenoxybenzamine and the prostaglandin-synthetase inhibitors acetylsalicylic acid and indomethacin were also investigated. The purpose of this paper is to present the results of our studies and to discuss possible implications as to the mechanism of renal PGE release.

Methods. Twenty-two mongrel dogs of both sexes, with an average weight of 27 kg were anesthetized with 30 mg/kg sodium pentobarbital injected intravenously. Mechanical ventilation was maintained throughout the experiments using a gas mixture containing 5% CO_2 and 20% O_2. The tip of a number 8 Cournand catheter was positioned in the left renal vein via the external jugular vein with the aid of fluoroscopy. Proper catheter positioning was verified in each experiment by direct palpation. A second number 8 Cournand catheter was inserted into the ipsilateral internal carotid artery to measure central aortic pressure. The left kidney and abdominal aorta were exposed through a flank incision. A perfusion cannula connected in series to a roller pump and constant temperature reservoir was introduced into the distal aorta and advanced retrograde to the bifurcation of the left renal artery. The abdominal aorta was then ligated both proximal and distal to the tip of the cannula to isolate the flow of blood from the pump to the left renal artery. Since this vessel usually originated from the aorta about 3 cm distal to the right renal artery, perfusion of the contralateral kidney was avoided in most experiments. It was often difficult to determine whether one or two renal arteries were being perfused until the area was exposed at autopsy. In nine of the experiments both kidneys were perfused. Reservoir volume was initially established with 500 ml donor blood and subsequently maintained through a cannula from the left carotid artery. Renal artery perfusion pressure was measured through a Y tube extension of the perfusion cannula. Catheters and the cannula were connected to Statham P23Db pressure transducers. Pressures and the ECG were recorded on an oscillographic recorder (Minneapolis Honeywell,

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Denver, CO). The pump rate was adjusted to provide a perfusion pressure of 100–120 mm Hg. Flow rate was maintained constant and was measured at the conclusion of each experiment.

Drugs tested included norepinephrine (4–10 μg/kg), epinephrine (2–9 μg/kg), and isoproterenol (2–8 μg/kg). The sequence of injections varied and not all drugs were given in each experiment. All drugs were injected directly into the perfusion cannula. In five experiments norepinephrine and epinephrine were given before and after phenoxybenzamine (Smith, Kline and French) administration (2 mg/kg). In nine experiments norepinephrine was given before and after either acetylsalicylic acid (3 mg/kg in four experiments) or indomethacin (3 mg/kg in six experiments). Acetylsalicylic acid (Merck and Co.) was dissolved in 3 ml ethanol and diluted to 10 ml with normal saline. Indomethacin (Merck, Sharp and Dohme) was dissolved in 10 ml 0.1 *M* phosphate buffer, pH 7.4, and diluted to 500 ml with normal saline. Both drugs were placed in the reservoir rather than in the perfusion cannula. The effects of phosphate alone or ethanol alone were not tested.

Blood samples (5 ml) for PG analysis were obtained from the renal vein before the test injection (control level), at 1 min after injection (*i.e.*, at the peak of the pressure change), and 15 min after injection. The latter sample served as the control for the subsequent drug when drugs were tested in sequence. Samples were centrifuged immediately at approximately 4000*g*. The plasma was separated and frozen at −20°. Prostaglandin E levels were later determined by radioimmunoassay using the method of Jubiz and his associates (7).

Results. The results obtained with norepinephrine are presented in Fig. 1. Mean renal artery perfusion pressure rose from a baseline of 116 ± 6 mm Hg to 235 ± 11 mm Hg one minute after injection. Concomitantly, renal venous plasma PGE levels rose from 1.62 ± 0.25 ng/ml to 5.07 ± 1.23 ng/ml (mean ± SEM). Changes in perfusion pressure and PGE levels were statistically significant ($P < 0.001$ and < 0.003, respectively). By 15 min, perfusion pressure and PGE levels had returned to preinjection values. Changes in PGE levels and perfusion pressures were no different in the nine experiments in which both kidneys were perfused than in the others in which only one kidney was perfused. Phenoxybenzamine inhibited the norepinephrine

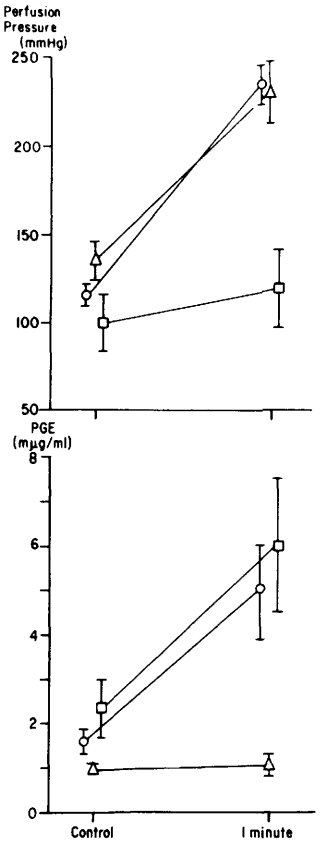

FIG. 1. Changes in perfusion pressure and PGE levels in renal venous blood following injection of 4–10 μg/kg of norepinephrine (mean ± SEM). o = norepinephrine alone ($N=22$), □ = pretreatment with phenoxybenzamine ($N = 5$), Δ = pretreatment with acetylsalicylic acid ($N = 4$) or indomethacin ($N = 6$).

induced pressor response but not the rise in PGE levels. Conversely, acetylsalicylic acid and indomethacin inhibited the rise in PGE levels following norepinephrine but did not alter the increase in perfusion pressure.

The results obtained with epinephrine are presented in Fig. 2. Mean renal artery perfusion pressure rose from a baseline of 119 ± 8 mm Hg to 229 ± 18 mm Hg. Concomitantly, renal venous plasma PGE levels rose from 2.09 ± 0.52 ng/ml to 6.21 ng/ml. Changes in perfusion pressure and PGE levels were statistically significant ($P < 0.001$ and < 0.01, respectively). Phenoxybenzamine inhibited the epinephrine-induced pressor response but not the rise in PGE levels. Acetylsalicylic acid and indomethacin were not tested with epinephrine.

With isoproterenol, perfusion pressure fell

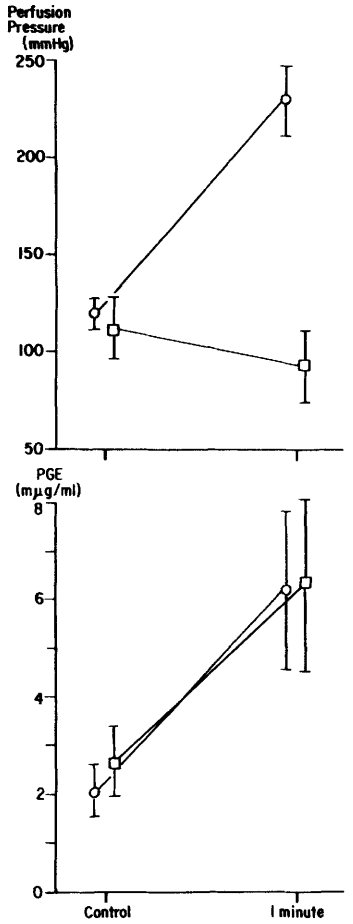

FIG. 2. Changes in perfusion pressure and PGE levels in renal venous blood following injection of 2–9 μg/kg of epinephrine (mean ± SEM). o = epinephrine alone (N = 12), □ = pretreatment with phenoxybenzamine (N = 5).

from 128 ± 9 mm Hg to 95 ± 9 mm Hg ($P < 0.001$) and PGE levels increased slightly from 2.31 ± 0.77 ng/ml to 2.65 ± 0.80 ng/ml ($P < 0.05$).

Renal blood flow averaged 13.3 ± 1.5 ml/min/kg in the dogs in which both kidneys were perfused, and 9.4 ± 1.0 ml/min/kg in the dogs in which only one kidney was perfused.

Discussion. We have found a significant increase in PGE levels in the renal venous blood of dogs following norepinephrine or epinephrine administration. The basal PGE levels of 2.0 ng/ml of plasma are higher than those reported by Terragno and his associates of 0.37 ng/ml of blood and by Dunham and Zimmerman of 0.21 ng/ml of blood, not corrected for a recovery of 30% (4, 6). Several factors may explain this discrepancy: In our study, plasma rather than blood was assayed and radioimmunoassay rather than bioassay was employed; also, values obtained with the assay in unextracted plasma (as presented here) are generally higher than those obtained after extraction in our laboratory. It is also conceivable that rather than measurement differences there was an absolute increase in PGE response to the nonspecific stress of the procedure that we used. While intravascular volume was maintained constant there was interruption of the vascular supply of several beds. In support of this possibility is the fact that renal vein PGE levels that we have obtained subsequently in basically intact animals are considerably lower (0.4–0.8 ng/ml). As yet we are not aware of any other study available for comparison in which renal venous prostaglandin levels in dogs have been determined by radioimmunoassay.

Our finding of increased PGE levels in response to norepinephrine is in agreement with the findings of Terragno and his associates and Dunham and Zimmerman (4, 6). Quantitative comparison of results cannot be made, however, since we used bolus injections of drugs rather than infusions, and the amount of norepinephrine that we administered was considerably greater than that used in their experiments. The effects of epinephrine and isoproterenol were not studied by either of the other groups.

In our preparation, and in that used by Dunham and Zimmerman, renal blood flow rate was kept constant (4). This suggests that the rise in PGE levels associated with the administration of the α-adrenergic drugs is not necessarily dependent on changes in total renal blood flow. However, nothing can be said about the influence of blood flow redistribution taking place within the kidney. Relevant to this point is the report by Pomeranz and his associates that sympathetically-induced vasoconstriction is associated with an increase in blood flow to the outer medulla while decreasing outer cortical flow (8).

Alpha-adrenergic blockade of renal vasoconstriction with phenoxybenzamine did not prevent the rise in PGE levels associated with administration of α-adrenergic drugs indicating that PGE release is not necessarily dependent on an increased total renal perfusion pressure, though the possibility that intra-renal changes in blood flow occur cannot be excluded.

Finally, while our data are not conclusive we

feel that norepinephrine and epinephrine are involved in the release of PGE by a mechanism other than the renal vascular constriction they elicit. Takeguchi and his associates have reported that L-norepinephrine and L-epinephrine, among other substances, increased the rate of conversion of arachidonic acid to PGE_2 *in vitro* when incubated with the enzyme prostaglandin synthetase (9). This provided the basis for the hypothesis that the α-adrenergic drugs act as co-factors in the reaction. Moreover, our finding that acetylsalicylic acid and indomethacin, both potent inhibitors of prostaglandin synthetase, appear to block the adrenergically-induced rise in PGE levels in renal venous blood is further support of the postulate that norepinephrine and epinephrine stimulate the synthesis of prostaglandins rather than the release of stored prostaglandins from the kidney. This tends to support the suggestion made by Crowshaw and others that there are no sites for prostaglandin storage within the cellular elements of renomedullary tissues (10).

Summary. PGE levels in renal venous blood of *in situ* perfused dog kidneys were measured by radioimmunoassay. A basal efflux of PGE was found and a significant increase in the levels was elicited by injection of norepinephrine or epinephrine into the renal arterial circulation. Isoproterenol had a small but possibly unimportant effect. α-adrenergic blockade with phenoxybenzamine did not inhibit the PGE increase but did block the pressor response to norepinephrine and epinephrine. Thus, PGE release in these experiments was apparently not dependent on total renal blood flow or perfusion pressure. Acetylsalicylic acid and indomethacin blocked the PGE response to norepinephrine and epinephrine but did not affect the pressor response suggesting that PGE synthesis rather than the release of stored PGE occurs when α-adrenergic drugs are administered. Our findings lend support to the suggestion that PGE may play a physiologic role in the intrarenal vascular adjustments.

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