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Hemodynamic Effects of Furosemide in Isolated Perfused Rat Kidneys[1] (40203)

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In anesthetized dogs, the diuretic furosemide has been shown to increase renal blood flow, an effect which can be blocked by the prostaglandin synthetase inhibitor, indomethacin (1, 2). Furosemide also caused an increase in PGE_2 release into renal venous blood and this increase was inhibited by indomethacin (2). These data suggest a relationship between the hemodynamic effect of furosemide and the renal prostaglandins.

PGE_2 is a vasodilator in the dog kidney; however, Malik and MgGiff (3) demonstrated that this prostaglandin increases renal vascular resistance in the rat. This apparent species difference between the rat and other mammals (4) offers a potential tool for elucidating the relationship between furosemide and PGE_2 on renal hemodynamics. If the action of furosemide to dilate the renal vasculature in dogs is dependent on release of PGE_2, and if PGE_2 is a vasoconstrictor in rats, then furosemide would be expected to decrease renal blood flow in the rat. Alternatively, if the primary effect of furosemide is to produce hemodynamic changes which secondarily influence prostaglandin synthesis, furosemide might increase renal blood flow in rats as it does in dogs.

The purpose of this investigation was to determine the effect of furosemide on renal vascular resistance and to elucidate the interactions between this diuretic and PGE_2 in isolated perfused rat kidneys. Since the renin-angiotensin system may also influence the renal vasculature, experiments were performed to evaluate interactions between this system and PGE_2 and furosemide.

Methods. All experiments were performed on kidneys obtained from male, Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 300–375 g. Rats were anesthetized with 50 mg/kg sodium pentobarbital, intraperitoneally. The abdomen was opened by a midline incision and the right kidney, the right renal artery, and the abdominal aorta were exposed. All rats received 3 mg of heparin via the femoral vein five minutes prior to cannulation of the inferior vena cava. The right ureter and the inferior vena cava were cannulated for collection of urine and venous effluent, respectively. Following cannulation of the right renal artery, perfusion with modified Tyrode's solution containing 1% inulin and saturated with a 95% O_2–5% CO_2 mixture was immediately initiated using a Harvard Apparatus Peristaltic Pump, Model 1203. Tyrode's solution was modified as follows: 124 m*M* NaCl, 2.7 m*M* KCl, 1.8 m*M* $CaCl_2$, 1.1 m*M* $MgCl_2$, 25 m*M* $NaHCO_3$, 0.42 m*M* $NaHPO_4$, and 5.6 m*M* D-glucose. The kidney was excised from the animal, placed on a nylon covered ring and covered with cotton gauze moistened with modified Tyrode's solution. Temperature of the perfusate and kidney was maintained at 35–39° with a heat lamp. The perfusate did not recirculate.

An equilibrium period followed attachment of the isolated kidney to the perfusion apparatus. During this time the flow rate was adjusted with the pump to maintain an effective perfusion pressure of 100 mmHg. Average arterial flow rate was 14 ml/min. Perfusion pressure was monitored on a Beckman type RS dynograph. A 20-min control urine collection period followed the equilibration period. Arterial and venous samples were obtained at 5 and 15 min of the urine collection period for determination of glomerular filtration rate. In some experiments, venous effluent was also collected at 5 and 15 min and assayed for PGE_2 and renin concentration. Urine collections during drug perfusion periods were also 20 minutes.

Experiment A—effects of PGE_2 on perfusion pressure. After the control period, PGE_2 [5 ng/ml (2 experiments) and 10 ng/ml (3 ex-

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periments)] was added to the perfusate. PGE_2 was initially dissolved in ethanol (1 mg/ml) but further dilutions were made with modified Tyrode's solution. Venous effluent from kidneys perfused with 5 ng/ml PGE_2 were assayed for renin.

Experiment B—effects of norepinephrine depletion on PGE_2 vasoactivity. Five rats were anesthetized with 50 mg/kg sodium pentobarbital and 100 mg/kg 6-hydroxydopamine in saline, containing 1 mg/ml ascorbic acid, was administered via a tail vein in a volume of 0.1 ml/100 g. The animals were allowed to recover. After 48 hours, the left kidney was removed, homogenized, and assayed for norepinephrine (6). The right kidney was isolated and perfused with modified Tyrode's solution without PGE_2 (control) and then with PGE_2 (10 ng/ml).

Experiment C—effects of furosemide on renal perfusion pressure and PGE_2 release. Following control periods, furosemide in concentrations of 10^{-4} *M* (three rats), 5×10^{-4} *M* (four rats) and 10^{-3} *M* (four rats) was infused into isolated perfused rat kidneys. Venous effluent samples were assayed for PGE_2.

Experiment D—effects of furosemide on release of PGE_2 and renin in constant pressure perfused isolated rat kidneys. Furosemide (5×10^{-4} *M*) was infused into the isolated kidney (5 rats) and perfusion pressure was maintained constant at 100 mmHg by adjusting the flow rate. Venous effluent was assayed for PGE_2 and renin concentrations.

Experiment E—effects of indomethacin perfusion on furosemide vasoactivity. Isolated kidneys (eight rats) were perfused sequentially with modified Tyrode's solution, modified Tyrode's solution containing indomethacin (5 μg/ml), and modified Tyrode's solution containing furosemide (5×10^{-4} *M*). Renal venous PGE_2 and renin concentration were determined.

Prostaglandins in perfusate were extracted into ethyl acetate. The ethyl acetate fraction was dried and then taken up into Tris buffer in a small volume representing a 20–40× concentration. This solution was assayed for PGE_2 by radioimmunoassay as described by Stygles *et al.* (5). This method is sensitive to 1 ng/ml, has approximately 10% cross reactivity with PGE_1, 3–4% with $F_{2\alpha}$ and less than 1% with PGE and PGB. Reproducibility is within ± 4%. Addition of 1 ng/ml PGE_2 to buffer resulted in measured concentrations of 1.4 ± 0.1 ng/ml; addition of 10 ng/ml measured as 9.2 ± 1.2 ng/ml in six replicate experiments.

Norepinephrine in kidney homogenate was assayed by the method of Chang (6). Venous effluent renin concentration was determined by radioimmunoassay for the generated angiotensin I after addition of exogenous homologous renin substrate (7).

Data were analyzed statistically by randomized complete block analysis of variance. Treatment differences were detected by the least significant difference test (8). The 0.05 level of probability was used as the criterion of significance.

Results. PGE_2 increased renal vascular resistance in isolated perfused rat kidneys (Experiment A) (Fig. 1). In two experiments addition of PGE_2 (5 ng/ml) to the perfusion solution increased the perfusion pressure to a maximum of 20 and 25 mmHg above control. Perfusion with 10 ng/ml PGE_2 increased perfusion pressure to a maximum of 34 ± 6 mmHg over control. Maximum perfusion pressure occurred approximately 2 min after initiation of PGE_2 perfusion and usually remained constant during the drug treatment period. PGE_2 had no effect on glomerular filtration rate. In two experiments, perfusion with 5 ng/ml PGE_2 had no effect on venous effluent renin concentration (4.95 and 7.25 ng/ml during control; 6.61 and 7.16 ng/ml

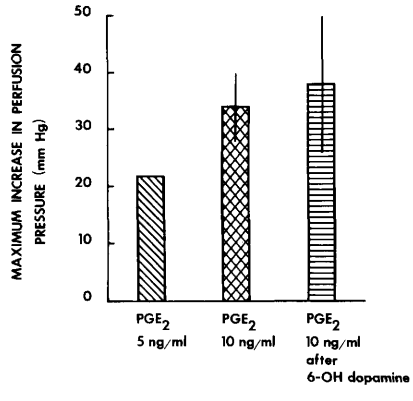

FIG. 1. Effect of addition of PGE_2 to fluid perfusing isolated rat kidneys. Values represent means of 2, 3 and 5 experiments. Vertical bars represent ± 1 SE.

during treatment). Pretreatment of rats with 6-hydroxydopamine reduced mean norepinephrine concentration in the left kidney from 0.06 ± 0.01 μg/g to less than 0.01 μg/g (n = 5) (Experiment B). This treatment did not influence the ability of PGE_2 (10 ng/ml) to increase perfusion pressure (38 ± 12 mmHg) (Fig. 1).

Furosemide infusion (Experiment C) significantly reduced renal perfusion pressure (Table I). Although not measured in all experiments, PGE_2 concentration in venous effluent was clearly reduced by furosemide (Table I).

In order to determine the effects of furosemide on the release of PGE_2 independent of changes in perfusion pressure, furosemide was infused and flow rate was increased to maintain the perfusion pressure constant (Experiment D). Furosemide significantly decreased the release of PGE_2 and increased the release of renin into the venous effluent (Fig. 2).

Indomethacin (5 μg/ml) blocked the furosemide-induced decrease in perfusion pressure and reduced PGE_2 release from the kidney (Experiment F) (Table II). Venous effluent PGE_2 concentration during the subsequent furosemide perfusion also remained lower than during the control period. Indomethacin perfusion did not prevent the furosemide-induced increase in renin release (Table II).

Discussion. While the isolated perfused rat kidney is useful for physiological studies of renal function, interpretation of results must be tempered due to the limits of the preparation. Perfusion with a noncolloidal fluid could alter organ responses to stimuli as could the absence of normal blood borne constituents (nutrients, hormones, etc.). The relatively high concentrations of the vasoconstrictor, PGE_2, and vasodilator, furosemide, required in this study suggests that vascular reactivity is reduced. Nevertheless, these experiments provide some new insight into the renal hemodynamic effects of furosemide.

Although PGE_2 is a vasodilator in most species, high concentrations of PGE_2 have been reported to produce vasoconstriction in isolated rat kidneys (3). The present study confirmed these findings. In addition, Malik and McGiff (3) demonstrated that PGE_2 potentiated vasoconstrictor responses to both sympathetic nerve stimulation and injected norepinephrine. In our experiments the isolated perfused rat kidney was completely denervated and treatment with 6-hydroxydopamine resulted in norepinephrine depletion of the kidney. However, these kidneys still responded to PGE_2 with an increase in resistance (Fig. 1). Although the vasoconstrictor effect of PGE_2 may be, in part, correlated with the release of catecholamines from sym-

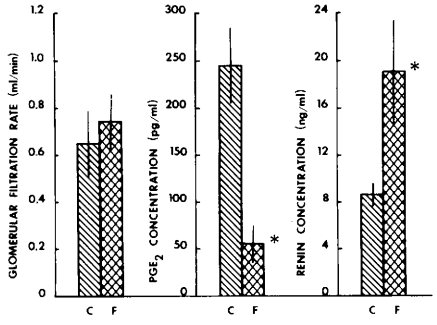

FIG. 2. Effects of furosemide (5×10^{-4} M) on glomerular filtration rate and venous effluent concentrations of PGE_2 and renin in constant pressure perfused isolated rat kidney. C = control, F = furosemide. Mean ± 1 SE are shown. N = 5. * Values significantly different from control ($P < 0.05$).

TABLE I. EFFECT OF FUROSEMIDE ON PERFUSION PRESSURE AND VENOUS EFFLUENT CONCENTRATION OF PGE_2.

Drug		Perfusion pressure (mmHg)	PGE_2 concentration (pg/ml)
10^{-4} M Furosemide	Control period	100 ± 1[a]	246, 152[c]
(N = 3)	treatment	88 ± 2[b]	78, 82
5×10^{-4} M Furosemide	Control period	100 ± 1	154
(N = 4)	treatment	91 ± 2[b]	24
10^{-3} M Furosemide	Control period	100 ± 1	130
(N = 4)	treatment	80 ± 2[b]	20

[a] Values represent mean ± SE.
[b] Significantly different from control ($P < 0.05$).
[c] Concentrations of PGE_2 represent individual values.

TABLE II. EFFECTS OF INDOMETHACIN ON FUROSEMIDE-INDUCED CHANGES IN PERFUSION PRESSURE AND VENOUS EFFLUENT CONCENTRATIONS OF PGE_2 AND RENIN.[a]

Treatment	Perfusion pressure (mmHg)	PGE_2 (pg/ml)	Renin (ng/ml)
Control	103 ± 1	214 ± 27[b]	10.27 ± 2.27
5 μg/ml Indomethacin	97 ± 2	42 ± 24[b,c]	14.31 ± 2.08
5×10^{-4} M Furosemide	103 ± 3	Unmeasurable[b,c]	28.86 ± 5.05[c]

[a] Values represent mean ± SE of eight experiments.
[b] $N = 4$.
[c] Significantly different from control ($P < 0.05$).

pathetic nerve terminals, these observations indicate a direct effect of PGE_2 on the renal vasculature.

In dogs, furosemide inhibits electrolyte transport and dilates the renal vasculature leading to an increase in renal blood flow (9, 10). Williamson *et al.* (2) showed that the release of PGE_2 into renal venous blood increased following infusion of furosemide into dogs. PGE_2 produced vasodilation in isolated rabbit kidneys (3) and increased renal blood flow in dogs (11). Furthermore, furosemide-induced increases in renal blood flow were blocked by the prostaglandin synthetase inhibitor, indomethacin (2). These data suggest that furosemide-induced renal vasodilation is mediated by prostaglandins of the E series.

In the present group of experiments, furosemide decreased renal resistance and PGE_2 release in isolated perfused rat kidneys (Table I). Since PGE_2 is a renal vasoconstrictor in rats (3) (Fig. 1), the removal of this vasoconstrictor could be the mechanism whereby furosemide reduced renal vascular resistance. On the other hand, since in this preparation renal perfusion pressure fell after furosemide the decrease in renal venous PGE_2 might be related to the change in perfusion pressure rather than to an effect of the drug. To assess this possibility, arterial flow rate was increased during furosemide infusion to maintain perfusion pressure at 100 mmHg. Data from these experiments indicate that the decreased PGE_2 release produced by furosemide is independent of changes in perfusion pressure (Fig. 2).

Pretreatment of dogs with indomethacin prevented the renal vasodilation observed following furosemide (1, 2). Furthermore, Williamson *et al.* (2) showed that indomethacin pretreatment eliminated the increase in PGE_2 release produced by furosemide. When indomethacin was infused directly into the isolated rat kidney, PGE_2 release was decreased and the vasodilating effect of furosemide was blocked (Table II). However, PGE_2 was reduced even further by furosemide.

These data do not eliminate the possibility that the hemodynamic changes were produced by chemicals other than PGE_2. Although indomethacin infusion markedly reduced release of PGE_2, it had no significant effect on perfusion pressure as might be expected if PGE_2 was a major tonic constrictor in the isolated rat kidney (Table 2). The radioimmunoassay utilized in this study measured PGE_2 specifically. Perfusion with indomethacin could have inhibited synthesis of other (nonmeasured) prostaglandins which influence the hemodynamic state of the kidney and which mediate the vasoactivity of furosemide. In addition, indomethacin may have a direct effect on renal hemodynamics independent of prostaglandins.

PGE_2 has been shown to stimulate renin release in a rabbit renal cortical cell suspension (12) and increase plasma renin activity in anesthetized dogs (13). Furosemide also stimulates renin secretion in dog (14) and man (15). Bailie *et al.* (14) reported that indomethacin inhibited furosemide induced renin secretion in the dog. In the present experiments, exogenous PGE_2 had no effect on renin secretion. The inconsistency of this data with previous findings in rabbit and dog may be due in part to the fact that PGE_2 is a vasoconstrictor in isolated rat kidneys and a vasodilator in most other mammals. Furosemide stimulated renin secretion in the constant pressure perfused isolated kidney (Fig. 2). Indomethacin had no effect upon increased renin release produced by the diuretic, although indomethacin did block furosemide-induced vasodilation (Table II).

Thus, enhanced renin release after furosemide can be separated from prostaglandin and hemodynamic changes.

In summary, these experiments confirm that exogenous PGE_2 is a vasoconstrictor in isolated non-protein perfused rat kidneys. Furosemide decreased renal vascular resistance and PGE_2 release from the preparation. Superficially, these events appear related as to cause and effect; removal of the vasoconstrictor, PGE_2, by furosemide could cause the decrease in renal vascular resistance. Although indomethacin reduced PGE_2 release and blocked the furosemide induced decrease in renal vascular resistance, the inhibitor alone did not change renal resistance. Furosemide decreases renal vascular resistance in both dog and rat kidney, whereas PGE_2 release is increased in the dog and decreased in the rat. While consistent with the physiological effects, it is difficult to envision the premier obligatory biochemical action of furosemide to be diametrically opposed in the two species. Rather, it is tempting to speculate that another, (unmeasured) vasodilator PG is increased after furosemide in both species and changes in PGE_2 are only secondary.

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