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Effects of Dimaprit, Prostacyclin, and Acetylcholine on Renal Blood Flow and Function (40775)[1]

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Many previous reports have shown that direct intraarterial administration of potent vasodilators into the renal artery of dogs causes a significant increase in the excretion of water and electrolytes. As recently reviewed by Baylis and Brenner (1) it has also been reported that many drug-induced increases in renal blood flow are not accompanied by significant increases in glomerular filtration rate (GFR). The finding that dilator agents evoke insignificant changes in GFR, despite an increase in total renal blood flow, has been related to compensatory effects of the drugs on factors which determine the filtration rate. Recently, Baylis *et al.* (2) and Deen *et al.* (3) reported that prostaglandin E_1, acetylcholine, bradykinin, and papaverine increase renal blood flow due to a marked decrease in both afferent and efferent arteriolar resistance, but that an offsetting decrease in the ultrafiltration coefficient (K_f) resulted in the maintenance of a constant GFR.

Vasodilator-induced increases in sodium chloride excretion could depend on either changes in passive reabsorptive forces at the peritubular level, a direct inhibition by the drug of sodium chloride reabsorption at the tubular level, or on a combination of both factors. No general agreement has been reached about the mechanism primarily responsible for the changes in renal function during administration of vasodilator agents.

The present study was undertaken to explore some of the possible mechanisms mediating the renal excretory response to vasodilator agents. The agents we investigated included dimaprit, acetylcholine, and prostacyclin. Histamine itself has no apparent tubular effects (4) and its enhancement of renal excretion appears to depend upon stimulation of vascular H_2-receptors (5). Consequently, we studied effects of the histamine H_2 receptor agonist dimaprit. Acetylcholine has been reported to have direct inhibitory effects on sodium chloride reabsorption (6–8) in addition to its vasodilatory actions (6–11). Prostacyclin is a potent vasodilator agent whose actions on the tubule have remained in doubt (12–14). Based upon our results we concluded that prostacyclin and dimaprit exert their diuretic effects through a hemodynamic mechanism.

Methods. A total of 18 dogs of either sex weighing 15–25 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv) and supplemental doses were given as needed throughout the experiment for maintenance. An endotracheal tube was inserted and the animals were allowed to breathe spontaneously. Body temperature was sustained with a heating pad connected to a temperature-controlled system (Yellow Springs, Model 63RC). Mean arterial blood pressure was measured through a right femoral arterial catheter (PE-100) connected to a pressure transducer (Physiograph, Model P-1000A). Another catheter was placed in the left femoral artery for withdrawal of blood samples.

After infusing a moderate volume of saline (1 ml/kg-min for 15 min), saline was administered at a rate of 1.0 ml/minute throughout each experiment via a catheter placed in the right femoral vein. The left femoral vein was also catheterized for infusion of saline containing creatinine (3g%) at a rate of 0.2 ml/minute.

Through a small abdominal incision, both

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ureters were exposed and catheterized. The left renal artery was exposed and isolated through a retroperitoneal incision. Renal blood flow was measured with an electromagnetic blood flow meter (Narco Bio-Systems Model RT 400 with digital display meter Model DD 350). An electromagnetic blood-flow transducer (i.d. 3–3.5 mm) was positioned on the renal artery. At the beginning and end of each experiment, zero blood flow was obtained by occlusion of the renal artery distal to the transducer. A curved 23-gauge needle connected to PE-50 tubing and a syringe infusion pump system (Harvard Apparatus Model 975) was inserted into the renal artery proximal to the transducer for intraarterial infusion of the vasoactive agents. During the control periods, saline was infused into the renal artery at a rate of 0.2 ml/minute. Mean arterial pressure and renal blood flow were recorded on a polygraph (Physiograph Model 4).

Following surgery, the dogs were allowed adequate time for stabilization (2–4 hr) prior to commencing the experiment. The criterion used to determine when an animal was in a steady state was a constant osmolar excretion rate over a 30-min time interval. When urinary flow rate and osmolality were stabilized, a control renal clearance (C) was begun. Immediately following this control period, infusion of an agent was started. To insure a steady state situation during infusion of the vasodilator agents three 2-min clearance periods were conducted during administration of each agent, namely at t + 21, t + 33, and t + 45 min. Results of these three clearance values were averaged. Individual drugs were dissolved in isotonic saline and infused into the renal artery for 50 min each. The dosages of each dilator drug were: acetylcholine chloride (Sigma) 0.5–1.0 μg/kg-min; prostacyclin (Upjohn) 0.01 μg/kg-min, and dimaprit (Smith Kline & French Laboratories) 2.5–5 μg/kg-min. In some experiments with dimaprit we also simultaneously infused the H_2 receptor antagonist cimetidine (Smith, Kline & French) at 10^{-6} mol/minute. Comparisons were made between the responses with dimaprit alone and with the same dose of dimaprit after blockade of H_2 receptors with cimetidine. As in control periods, the volume flow entering the renal artery during vasodilator infusion was 0.2 ml/minute.

Plasma and urinary concentrations of creatinine (cr) and chloride (cl) were measured by the method of Folin and Wu (15) and ampherametric titration with silver (Bulcher–Cotlove chloridometer), respectively. The clearance of creatinine (C_{cr}) was equated with the GFR. Urinary osmolality was measured by freezing-point depression (Precision Systems). At the end of each experiment the left kidney was excised and weighed. Data are presented as mean ± standard error of the mean. Statistical significance was determined by using Student's t test for paired or pooled data.

Results. The effect of the vasodilators of renal blood flow and other parameters of kidney function is summarized in Table 1.

Intraarterial infusion of dimaprit resulted in a significant increase in total renal blood flow and produced no change in systemic arterial pressure. Dimaprit significantly elevated absolute as well as fractional chloride excretion ($FE_{cl} = C_{cl}$/GFR). Total solute excretion ($U_{os}V$ where U_{os} = osmolar concentration of urine and V = urine flow rate) was also markedly increased. Of particular interest is the fact that GFR significantly increased by 24.0%. The above observed response in renal blood flow and excretory function was slow in onset and reached a peak value 30–50 min after the onset of infusion. In four dogs, dimaprit + cimetidine was administerred intraarterially. Cimetidine completely prevented all the dimaprit-induced changes in renal blood flow and function. Cimetidine when infused into the renal artery by itself did not produce any significant changes in either renal blood flow or kidney function.

Infusion of acetylcholine into the left renal artery significantly increased both renal blood flow and excretion of osmolar constituents. Total and fractional excretions of chloride were also significantly augmented in the infused kidney, as was GFR which increased by 36.3% during infusion of acetylcholine. Blood pressure was not alttered during infusion of acetylcholine.

TABLE I. EFFECT OF VASODILATORS ON RENAL FUNCTION [a]

RBF (ml/min-g kidney wt)		GFR (ml/min-g kidney wt)		$U_{cl}V$ (μeq/min-g kidney wt)		$U_{os}V$ (μOsm/min-g kidney wt)		FE_{cl} (%)	
Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Dimaprit (n = 9)									
3.99 ± 0.31	5.96 ± 0.55	0.70 ± 0.04	0.80 ± 0.06	3.19 ± 0.42	4.76 ± 0.52	11.04 ± 0.98	14.22 ± 1.20	3.87 ± 0.42	5.73 ± 0.65
P [b]	< 0.001	< 0.001		< 0.001		< 0.001		< 0.005	
Dimaprit + Cimetidine (n = 4)									
3.82 ± 0.66	4.25 ± 0.80	0.80 ± 0.06	0.82 ± 0.07	3.55 ± 0.95	4.04 ± 1.28	11.98 ± 2.56	13.55 ± 2.99	3.74 ± 0.98	4.34 ± 1.11
P	N.S.	N.S.		N.S.		N.S.		N.S.	
Acetylcholine (n = 6)									
4.47 ± 0.41	7.34 ± 0.96	0.65 ± 0.08	0.90 ± 0.13	2.03 ± 0.51	6.03 ± 0.89	8.23 ± 1.05	17.93 ± 1.80	2.81 ± 0.58	6.42 ± 1.13
P	< 0.005	< 0.005		< 0.005		< 0.001		< 0.001	
Prostacyclin (n = 10)									
4.43 ± 0.29	7.16 ± 0.43	0.60 ± 0.04	0.74 ± 0.05	2.13 ± 0.44	3.11 ± 0.68	7.67 ± 1.13	10.62 ± 1.52	2.54 ± 0.46	3.30 ± 0.59
P	< 0.001	< 0.001		< 0.025		< 0.02		N.S.	

[a] Values expressed as means ± SE.
[b] P values based on Student's t test for paired data.

Infusion of prostacyclin into the renal artery caused significant increments in total blood flow and *GFR* but did not affect systemic arterial pressure. Prostacyclin also evoked significant increases in excretion of chloride and in total solute excretion, whereas the observed rise in fractional chloride excretion was not significantly different from the control valves.

By comparing the effects of different vasodilators on the percentage increase in total solute excretion ($\Delta\%U_{os}V$) with the increase in renal blood flow ($\Delta\%RBF$) direct effects of a drug on tubular solute reabsorption might be more apparent (Fig. 1). Indeed, in spite of the fact that the percentage increases in renal blood flow observed with acetylcholine, prostacyclin, and dimaprit were not significantly different from each other, infusion of acetylcholine resulted in more than a threefold greater increase in solute excretion than that obtained with the other vasodilators. Similarly, analysis of percentage changes in solute excretion versus percentage changes in glomerular filtration rate (Δ%GFR) also revealed larger increases in solute excretion with acetylcholine per unit change in GFR (Fig. 2). Despite the fact that the percentage increase in GFR observed with acetylcholine and dimaprit were not significantly different, the percentage increase in solute excretion was greater with acetylcholine. By contrast, dimaprit and prostacyclin produced similar percentage increases in both GFR and total solute excretion.

Discussion. The increase in chloride and total solute excretion observed in the current study during renal vasodilation with all three agents is in general agreement with published reports (5, 9–14, 16, 17). Our laboratory has previously reported that histamine H_1 and H_2 receptors are present in the vasculature of the kidney and that stimulation of these receptors significantly affects either renal blood flow or function or both (5). We had also observed that histamine and dimaprit increased urine flow and chloride excretion (5). Similar findings were noted with histamine by Selkurt (16) and by O'Brien and Williamson (4). Furthermore, the latter authors concluded that the natriuretic effect of histamine is purely vascular. Our previous and current obser-

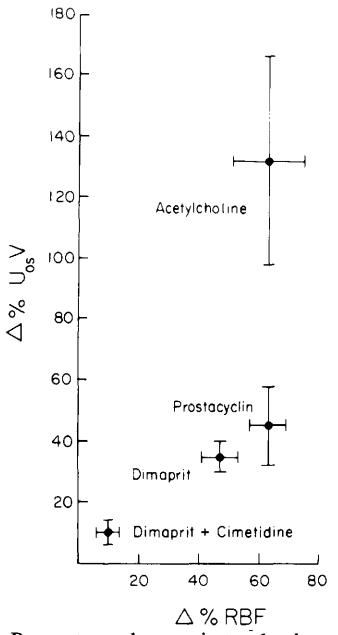

FIG. 1. Percentage changes in total solute excretion ($\Delta\%U_{os}V$) vs percentage increases in renal blood flow (Δ%RBF) during infusion of acetycholine, prostacyclin dimaprit, and dimaprit + cimetidine. For unfusion details see text.

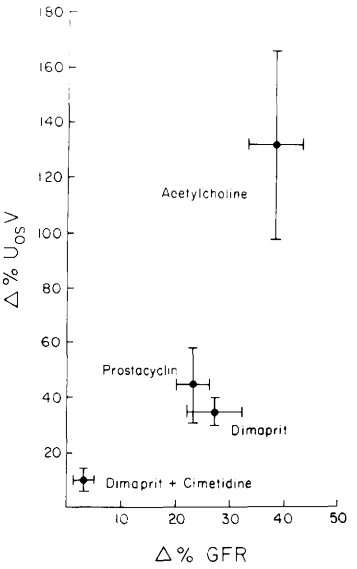

FIG. 2. Same as Fig. 1 except that $\Delta\%U_{os}V$ is shown as a function of percentage increases in glomerular filtration rate (Δ%GFR) during infusion of vasodilators.

vations with dimaprit suggest that these changes in renal function depend upon the hyperemia evoked by the drug. The effect of H_2 receptor inhibition on renal vascular and functional responses to dimaprit was evaluated in the present study. We had previously observed that cimetidine (H_2 receptor antagonist) inhibited responses to histamine in the kidney (5). In the present study we found that cimetidine abolished renal vascular and excretory responses to dimaprit. These experiments with cimetidine provide additional evidence that dimaprit induced changes in renal function are mediated by histamine H_2 receptors.

Several reports have suggested that increases in urine flow rate, chloride, and other ion excretion accompanying acetylcholine-induced renal vasodilation are due to blood flow-related decreases in tubular reabsorption as well as to direct inhibition of solute reabsorption by the drug (6–8). The current study is consistent with a combined vascular and tubular effect of acetylcholine since, for a given change in renal blood flow, acetylcholine caused a threefold greater increase in solute excretion compared with dimaprit.

The present investigation demonstrated that prostacyclin prompts renal excretion of chloride and total solute as well as augmenting renal blood flow. Similar findings with prostacyclin have been reported by others (12–14). Data from the current study also suggest that the diuresis which occurs during prostacyclin administration is related to indirect rather than to direct effects of the drug on tubular functions. This conclusion is based on the similarity of changes in renal parameters produced by prostacyclin and dimaprit. Prostacyclin-induced increases in solute excretion appear to be solely a function of increases in GFR and/or vascular-related changes in reabsorptive forces.

Presumably, the vasodilators used in the current study exert their action upon renal blood flow by diminishing the vasoconstrictor tone of the afferent and/or efferent renal arterioles. Other workers have shown that despite an increase in renal blood flow, GFR failed to increase in the dog during intraarterial vasodilator administration (1), although variable changes in GFR have been noted with acetylcholine (9, 10).

In the current study we have consistently observed increases in GFR with the three vasodilator agents. A possible explanation for the discrepancy between our results and those of others may be related to differences in experimental conditions. In particular, we allowed a lengthy control period before initiating infusion of each dilator agent, since we found that a 2- to 4-hr period of time is necessary to achieve steady state conditions. We did not commence any experiment until urinary flow rate and osmolality had stabilized. We also infused dilator drugs at rates which did not alter systemic arterial blood pressure over a relatively long period of time (45 min). In our previous study (5), dimaprit was only infused for 10 to 15 min and no increase in GFR was observed.

Summary. Renal excretion of water and solutes is enhanced during intraarterial infusion of various vasodilator drugs into the canine kidney, although the mechanism mediating increased excretion is uncertain. We studied effects of infusing dimaprit, acetylcholine, and prostacyclin into the left renal artery of anesthetized dogs. Blood flow in the vessel was estimated with an electromagnetic blood flowmeter. GFR was determined from the clearance of exogenously administered creatinine. Other estimated renal functions included excretion of chloride and total osmolytes. Infusion of each of the vasodilator drugs in doses which increased renal blood flow by approximately 50% caused significant increases in GFR, fractional and total chloride excretion, and total solute excretion. Both circulatory and excretory responses to dimaprit were virtually abolished by cimetidine. Dimaprit and prostacyclin appear to increase renal excretion of water and solutes mainly through their vasodilator actions, whereas acetylcholine also acts directly upon the renal tubule to enhance renal excretion. All three dilator agents increased glomerular filtration rate in our experiments.

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1. Baylis, C., and Brenner, B. M., Rev. Physiol. Biochem. Pharmacol. **80**, 1 (1978).
2. Baylis, C., Deen, W. M., Myers, B. D., and Brenner, B. M., Amer. J. Physiol. **230**, 1148 (1976).
3. Deen, W. M., Robertson, C. R., and Brenner, B. M., Fed. Proc. **33**, 14 (1974).
4. O'Brien, I. P., and Williamson, H. E., Eur. J. Pharmacol. **16**, 385 (1971).
5. Banks, R. O., Fondacaro, J. D., Schwaiger, M. M., and Jacobson, E. D., Amer. J. Physiol. **235**, F570 (1978).
6. Parmelee, M. L., and Carter, M. K., Arch. Int. Pharmacodyn. Ther. **1**, 174 (1968).
7. May, D. G., and Carter, M. K., Amer. J. Physiol. **218**, 417 (1970).
8. Stein, J. H., Congbalay, R. C., Karsh, D. L., Osgood, R. W., and Ferris, T. F., J. Clin. Invest. **51**, 1709 (1972).
9. Vander, A. J., Amer. J. Physiol. **206**, 492 (1964).
10. Pinter, G. G., O'Morchoe, C. C. C., and Sikand, R. S., Amer. J. Physiol. **207**, 979 (1964).
11. Harvey, R. B., Amer. J. Physiol. **211**, 487 (1966).
12. Gerber, J. G., Nies, A. B., Friesinger, G. C., Gerkens, J. F., Branch, R. A., and Oates, J. A., Prostaglandins **16**, 519 (1978).
13. Bolger, P. M., Eisner, G. M., Ramwel, P. W., and Slotkoff, L. M., Nature (London) **271**, 467 (1978).
14. Hill, T. W. K., and Moncada, S., Prostaglandins **17**, 87 (1979).
15. Folin, O. and Wu, H. J. Biol. Chem. **38**, 81 (1919).
16. Selkurt, E. E., Proc. Soc. Exp. Biol. Med. **155**, 605 (1977).
17. Sinclair, R. J., Bell, R. D., and Keyl, M. J., Amer. J. Physiol. **227**, 1062 (1974).

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