Effects of Diazoxide on Renal Function in the Dog.* (32095)

JAMES A. GREENE, JR.† (Introduced by J. M. Weller)
Department of Internal Medicine, University of Michigan, Ann Arbor

Diazoxide, a benzothiadiazine derivative, causes sodium retention when given orally or intravenously(1,2). It is a potent vasodilator and brings about an immediate increase in blood flow to kidney, hind limb, or myocardium when injected into renal, femoral, or coronary arteries, respectively(3,4). The following experiments were designed to study the effect on sodium excretion and renal hemodynamics when diazoxide is given directly into the renal artery.

Materials and methods. Mongrel dogs of both sexes weighing 14 to 22 kg were anesthetized with 30 mg/kg body weight sodium pentobarbital intravenously. Ureters were isolated through flank incisions and catheterized with polyethylene tubing tied securely in the renal pelvis. A 24-gauge needle was inserted into the left renal artery at its junction with the aorta at least 30 minutes before beginning observations. This renal artery was infused at a constant rate of 0.4 to 2 ml 0.85% sodium chloride/minute, first alone and later as the vehicle for diazoxide.

Renal clearance and stop flow experiments were done. Clearance experiments were carried out with saline or mannitol diuresis. For saline diuresis a priming infusion of isotonic saline containing 0.06 g creatinine/100 ml or 0.7 g inulin/100 ml was given intravenously at a rate of 25 ml/min for 20 minutes, then continued at 5 ml/min. Because of the possibility that p-aminohippurate (PAH) and diazoxide may compete for a common secretory site in the renal tubule, PAH was not used(3). Observations began after stabilization of diuresis occurred (approximately 2 hours). Consecutive control clearance periods were 15 minutes in duration with a blood sample obtained at the beginning and end of each. Following completion of 3 or 4 control clearances, diazoxide in isotonic saline solution was substituted for the saline infusion in the left renal artery. The drug was given at a rate of 0.08 to 0.20 mg/kg body weight/min for 30 minutes. Two to 7 clearances were obtained over 30 minutes beginning 5 minutes after diazoxide was started.

Mannitol diuresis was induced by infusing 20 g mannitol/100 ml isotonic saline containing 0.21 g creatinine/100 ml and/or 0.7 g inulin/100 ml at a rate of 10 ml/min intravenously following a priming dose of creatinine or inulin given intravenously. When the mannitol diuresis had stabilized, 2 or 3 control urine collections of 5 min each were obtained with blood taken at the middle of each. As in the saline diuresis experiments, diazoxide then was infused into the left renal artery at a rate of 0.18 to 0.36 mg/kg body weight/min for 30 minutes and 2 to 4 clearances were collected over 20 to 30 minutes beginning 2 minutes after the drug was started.

Stop flow experiments were done during diuresis with 20 g mannitol/100 ml isotonic saline given intravenously at a rate of 10 ml/min. Observations were begun when mannitol diuresis from that kidney stabilized at greater than 7 ml urine/min. Each stop flow ureteral occlusion was 5 to 8 minutes in duration. Diazoxide was infused into the left renal artery at a rate of 0.09 to 0.96 mg/kg body weight/min for 4 to 12 minutes before beginning the second ureteral occlusion, except in one experiment when it was given for 34 minutes. Following release of each occlusion, serial 0.5 to 1.0 ml urine samples were collected.

Sodium was determined by a Beckman direct reading flame photometer, creatinine by the method of Bonsnes and Taussky(5), inulin by the method of Schreiner(6), and osmolality by freezing point depression with a Fiske osmometer.

Results. When diazoxide is given directly into one renal artery of dogs, there is a prompt diuresis, natriuresis and increase in

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creatinine or inulin clearance in that kidney, while there is no comparable change in the opposite kidney. These results are illustrated in Table I which gives the data obtained from a typical clearance experiment during mannitol diuresis. When the left kidney was infused with diazoxide, there was an increase in sodium excretion (U_{Na} V) on that side. This continued throughout the period of drug infusion. The per cent of filtered sodium excreted (U_{Na} V/$P_{Na}C_{Cr}$), osmolal clearance, urine volume, creatinine and inulin clearances all increased on the left side while there was much less change in these values on the right side. Similar results were found in all clearance experiments. When these values are expressed as the individual ratio between the left and right kidneys during diazoxide infusion into the left renal artery, divided by the average of the same ratio observed in the initial control period during isotonic saline infusion, any change from unity represents the effect produced by diazoxide. Any increase in the ratio above one indicates a relative increase in the parameter measured in the left kidney during diazoxide infusion compared with the control period. The ratios for sodium excretion, urine volume, osmolal clearance, fraction of filtered sodium excreted and creatinine or inulin clearance were greater than one in all cases. The mean ratios for these were as follows: sodium excretion, 3.14; urine volume, 2.05; fraction of filtered sodium excreted, 2.55; osmolal clearance, 1.92; and GFR, 1.29.

The results of diazoxide infusion into the left renal artery on the renal function of that kidney are shown in Table II. In all dogs diazoxide produced a significant increase in sodium excretion (U_{Na} V) in the kidney in which diazoxide was given ($P<0.001$). The control kidney showed either no change or a slight increase in sodium excretion in 6 dogs and a slight decrease in 4. Urine volume and osmolal clearance (Cosm) were increased by diazoxide in all animals at all doses while values for TC_{H_2O} and C_{H_2O} showed no consistent change. Diazoxide in all animals produced moderate GFR increases as estimated by the clearance of creatinine or inulin. The fraction of the filtered sodium excreted (U_{Na} V/$P_{Na}C_{Cr}$) was increased by diazoxide in all animals. The increase in the amount of sodium filtered ($P_{Na}C_{Cr}$) was greater than the increase in the amount of sodium excreted (U_{Na} V) in all animals.

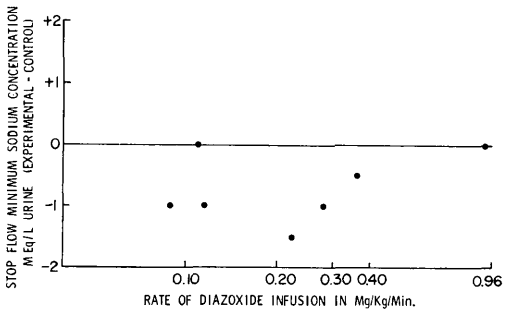

FIG. 1. The stop flow minimum sodium concentration in mEq/l urine during the diazoxide infusion minus the stop flow minimum sodium concentration developed during the control period is plotted against the rate of diazoxide infusion in mg/kg/min expressed logarithmically. Any increase above zero indicates a decrease in distal sodium reabsorption due to diazoxide.

In 7 of 10 experiments, during the renal artery infusion of diazoxide, the mean systemic arterial pressure remained within 5 mm Hg of control values. In 2 experiments a decrease of 7 to 13 mm Hg was observed while in one there was a rise of 10 mm Hg.

The lack of any significant effect of diazoxide on distal tubular sodium transport as evaluated by the stop flow technique is shown in Fig. 1. The data are expressed as the differences between control and diazoxide stop flow sodium minimum concentrations. In the one dog in which arterial pressure fell these values were corrected for the effect on the sodium minimum caused by the fall in arterial pressure using the formula previously described(7). No attempt was made to evaluate proximal sodium reabsorption by stop flow.

Discussion. These experiments demonstrate that diazoxide given directly into the renal artery causes natriuresis rather than sodium retention. The mechanism for this natriuresis is unknown; however there are several possible explanations. Diazoxide may directly cause inhibition of proximal tubular sodium reabsorption. This is not unreasonable since chlorothiazide, a close chemical relative of

TABLE I. *Effect of Diazoxide on Renal Function.*

EXPERIMENT D230B

Time min	$U_{Na}V$ μEq/min		$U_{Na}V/P_{Na}C_{Cr}$ %		Osmolal Clearance ml/min		Urine Vol. ml/min		Mean Arterial Pressure mm Hg	P_{Na} μEq/ml	Creatinine Clearance ml/min		Inulin Clearance ml/min	
	Right	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*			*Right*	*Left*	*Right*	*Left*
Before diazoxide. 20% mannitol infused at 10 ml/min for osmotic diuresis.														
275-280	270	575	7.75	14.15	5.49	7.80	6.0	9.0	128	153	22.8	26.6	22.0	
280-285	267	612	7.62	15.20	5.93	8.64	6.4	9.8	130	150	23.4	27.6	22.1	28.2
0.20 mg/kg/min diazoxide infused into left renal artery. Right kidney non-infused.														
292-322	325	1338	10.40	22.70	7.20	15.52	7.4	17.6	136	144	21.7	40.9	22.8	38.3
294-299	300	1462	9.96	28.20	6.98	16.81	7.0	18.5	138	144	20.9	36.0	21.6	38.4
299-304	239	1428	8.21	27.52	6.60	17.32	6.2	18.2	140	144	20.2	37.0	21.7	38.5
304-309	151	1380	6.40	26.20	5.24	16.78	4.8	17.8	142	146	16.2	36.2	17.4	36.1
After diazoxide terminated.														
332-337	186	918	8.50	23.55	5.97	13.22	5.4	13.2	138	145	15.1	27.9	16.4	28.5
337-342	204	836	9.80	21.82	6.00	12.23	5.6	12.3	136	144	14.3	26.6	14.9	24.4

diazoxide, has such an effect(8). Diazoxide might have a similar action which would be evident when it is given directly in the renal artery, but which might be masked by the sodium retention resulting from its hypotensive effect when given either by vein or orally.

A second possibility is that the increment in GFR which consistently occurred may be responsible for the natriuresis. Indeed, the increment in the amount of filtered sodium excreted following diazoxide could be entirely accounted for by the increase in filtered sodium. The hypothesis that diazoxide by altering GFR causes parallel changes in sodium excretion is attractive since intravenous diazoxide in the dog causes GFR reduction and sodium retention(2).

A third possibility is that the profound renal hemodynamic changes that diazoxide produces might be responsible for the natriuresis. It has been shown that diazoxide immediately causes renal vasodilation and an increase in renal blood flow when given directly into the renal artery(3). Renal perfusion studies were done and confirmed this. Acetylcholine similarly causes renal vasodilation and natriuresis when injected into the blood supply of the perfused kidney; however, larger doses decrease sodium excretion and systemic arterial pressure(9). When acetylcholine is infused directly into the renal artery of dogs in doses of 5 to 25 μg/min, there is an increase in urine volume, sodium excretion, RPF and relatively small increases in GFR(10). In many respects the renal effects of diazoxide are like those of acetylcholine(11) and other vasodilators given directly to the kidney(12) and their mechanisms of action may be similar.

One explanation for the alteration of electrolyte reabsorption which may occur with vasodilators is a redistribution of blood flow so that there is increased flow to outer cortical nephrons. These nephrons may promote sodium losing while inner cortical nephrons may be more sodium retainers(13). If this were true the *intravenous* administration of diazoxide would be expected to have similar effects; however, this renal hemodynamic action might be overcome by the direct effects

TABLE II. Effect of Diazoxide Infusion into the Left Renal Artery on Function of the Left Kidney.

Exp.		$U_{Na}V$ μEq/min	Volume ml/min	Cosm ml/min	GFR ml/min	$U_{Na}V/P_{Na}C_{Cr}$ %	$P_{Na}C_{Cr}$ μEq/min	Increase in $U_{Na}V$ μEq/min	Increase in $P_{Na}C_{Cr}$ μEq/min
220	Control	129 ± 10	.58 ± .08	.64 ± .08	16 ± .6	2.54 ± .57	2368		
	Diazoxide	234 ± 32	2.79 ± .39	2.03 ± .28	19.6 ± .3	7.98 ± 1.23	2940	105	572
221A	Control	1.2 ± 0.2	.18 ± .005	.48 ± .07	24.2 ± 1.1	.04 ± .004	3483		
	Diazoxide	6.7 ± 3.3	.32 ± .015	.64 ± .075	32.5 ± 6.7	.18 ± .1	4745	5.5	1261
221B	Control	3.5 ± 2.1	.32 ± .012	.49 ± .005	22.1 ± 1.2	.11 ± .076	3227		
	Diazoxide	16.3 ± 10	.65 ± .125	.64 ± .03	29.6 ± 2.4	.36 ± .21	4366	12.8	1139
224B*	Control†	376	8.8	5.58	21.4	15.4	2440		
	Diazoxide	1050 ± 60	15.6 ± .60	11.85 ± .95	26.8 ± 1.5	34.5 ± 4.15	3068	674	928
225*	Control†	320	9.4	—	28.6	8.86	3632		
	Diazoxide	765 ± 92	16.75 ± .75	—	32.7 ± 2.0	18.48 ± 1.08	4120	445	488
229	Control	47 ± 5.6	1.64 ± .20	—	22.3 ± 2.1	1.40 ± .06	3412		
	Diazoxide	229 ± 21	4.59 ± .24	—	23.4 ± 1.6	6.18 ± .13	3709	182	297
230A	Control	460 ± 5	3.74 ± .32	3.44 ± .0002	29.7 ± .6	9.98 ± .25	4648		
	Diazoxide	698 ± 95	5.62 ± 1.00	4.89 ± .65	33.6 ± 1.6	12.74 ± 2.49	5159	238	543
230B*	Control†	593	9.4	8.2	27.1	14.67	4106		
	Diazoxide	1402 ± 47	18.03 ± .35	16.6 ± .66	37.5 ± 2.0	26.16 ± 2.12	5419	809	1313
231A	Control	335 ± 29	3.37 ± .11	2.53 ± .17	33 ± .8	6.65 ± .60	5057		
	Diazoxide	796 ± 67	5.82 ± 1.10	5.43 ± .47	36.4 ± 2.0	14.24 ± .82	5587	461	530
231B*	Control	852 ± 70	10.8 ± .12	10.95 ± .36	33.5 ± 1.0	17.8 ± 1.35	4807		
	Diazoxide	978 ± 188	13.9 ± 1.91	12.14 ± 1.31	34.7 ± 3.8	19.0 ± 1.54	5084	126	277

* = experiments done with mannitol diuresis; others done with saline.
† = experiments having only 2 control periods. Values shown as mean ± S.D.

of the reduction in systemic arterial pressure(7).

The stop flow experiments present evidence that diazoxide does not act on the distal tubule to inhibit sodium transport since diazoxide did not elevate the distal stop flow sodium minimum concentration. It appears, therefore, that the natriuresis which occurs when diazoxide is given directly into the renal artery is probably due to renal vasodilation resulting in an increase in renal blood flow, glomerular filtration rate and alterations in intrarenal hemodynamics. That the hemodynamic alterations are the major cause for the natriuresis is supported by the fact that no distal tubular effect on sodium transport could be detected using the stop flow technique.

Summary. Renal clearance and stop flow studies were done in dogs before and after infusion of diazoxide directly into the left renal artery. In all animals the left kidney showed a prompt diuresis, natriuresis and increase in creatinine or inulin clearance when diazoxide was given. No inhibition of distal tubular sodium reabsorption following diazoxide administration was found using the stop flow technique. The natriuresis produced by infusing diazoxide directly into the renal artery is probably due to renal vasodilation and resulting changes in intrarenal hemodynamics.

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Uterine Metabolism Changes During Gestation in Rats of Different Age Groups.*† (32096)

A. B. SCHULTZE

Department of Dairy Science, University of Nebraska, Lincoln

Reduction of triphenyltetrazolium chloride (TTC) by uterine tissue of rats apparently measures the estrogen status of the uterus since the rate of reduction is increased in proportion to estradiol dosage in spayed females and is highest at pro-estrus in intact cyclic females. Furthermore, the effect of estradiol on uterine TTC reduction rate is diminished by progesterone in proportion to the progesterone dosage(1,2).

Using TTC reduction rate of uterine tissue as a metabolic index, Schultze reported that maximum litter size in rats was associated with a level of uterine metabolism that varied during different stages of gestation(3). Size of litter is influenced by many factors of which intrauterine mortality is an important one. Intrauterine mortality reportedly increases in old rats(4,5). If reduced litter size in old

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