The Effect of Beta Adrenergic Stimulation on Proximal Tubular Sodium Reabsorption[1] (36898)

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It has been suggested by Gill and Casper (1) that beta adrenergic stimulation may depress proximal tubular sodium reabsorption by a direct tubular effect. These investigators found in water diuresis studies in the dog a small but consistent increase in urinary flow rate during the intrarenal administration of isoproterenol at a dose which did not alter renal or systemic hemodynamics. Sodium excretion was not increased in these studies. Morimoto, Abe and Yamamoto (2) found that the intrarenal infusion of isoproterenol was associated with a significant increase in sodium excretion. However, in these studies there was also a significant increase in glomerular filtration rate and renal plasma flow during beta stimulation. Because of these experimental findings and their possible relevance to the regulation of sodium balance, micropuncture studies were performed to evaluate directly the effect of beta adrenergic stimulation on proximal tubular sodium reabsorption. The results indicate that at the maximum dose which has no effect on intrarenal or systemic hemodynamics, there is no inhibitory effect of isoproterenol on proximal tubular sodium reabsorption.

Methods. Studies were performed on mongrel dogs weighing between 12 and 22 kg. All animals were deprived of food and water for 18–24 hr before the study. The dogs were anesthetized with pentobarbital (30 mg/kg) and were subsequently given small maintenance doses as necessary. An endotracheal tube was inserted and the animals were ventilated with a Harvard respirator. Cannulas were inserted in a leg vein for infusions and in the femoral artery for blood pressure measurements and blood collection. Both ureters were cannulated through a suprapubic incision. A 23-gauge hooked needle was placed in the orifice of the left renal artery and kept open with an infusion of Ringer's solution at a rate of 0.4 ml/min. A catheter was placed in the left renal vein for measurement of para-aminohippurate (PAH) extraction. The dogs were prepared for micropuncture in the manner previously described (3).

An infusion was given to establish and maintain a plasma inulin concentration of 100 mg/100 ml and a PAH concentration in plasma of 2 mg/100 ml. The maintenance infusion was given at a rate of 1 ml/min. Samples of proximal tubular fluid were obtained with sharpened micropipettes containing colored mineral oil. Each tubule was blocked with a long column of oil and all fluid reaching the puncture site was collected in a precisely timed interval to permit the determination of tubular flow rate. In the majority of samples, the fluid was collected spontaneously after initial gentle suction to allow fluid to enter the pipette. The recollection technique was utilized in all studies. During the control period, 3–7 tubules were punctured and at the end of each collection an adjacent tubular segment was marked with nigrosine and a map was drawn of the area to facilitate repuncture during the experimental period. During the experimental period each of the previously punctured tubules was repunctured at the same site in a random sequence. No attempt was made to localize end proximal segments because of the unresolved question of the effect of the dyes

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used for this procedure on sodium transport. However, it should be noted that in previous studies from this laboratory major alterations in proximal tubular sodium reabsorption have been demonstrated with the identical methods used in the present work (3, 4).

Two groups of recollection experiments were performed: Group I: In five studies samples were collected before and during the intrarenal infusion of isoproterenol, 0.018 μg/kg/min (Winthrop Laboratories, New York, NY). Second period collections were obtained 40 min after the infusion had begun. Group II: In a second group of six studies, a continuous infusion of phenoxybenzamine (Smith, Kline and French Laboratories, Philadelphia, PA), 0.09 μg/kg/min, was given in the left renal artery throughout the entire study. After the control collections had been obtained, isoproterenol was given in the left renal artery at the same dosage as in the first group of studies and experimental period collections were obtained 40 min after the infusion had begun. In all studies, the renal artery infusion rate was kept constant at 0.4 ml/min with the drugs being added to this infusion in the appropriate amount.

In all experiments three 15-min clearance periods were obtained in both the control and experimental periods. Glomerular filtration rate was determined from the clearance of inulin and renal plasma flow from PAH clearance corrected for PAH extraction ratio. Para-aminohippurate was determined by the

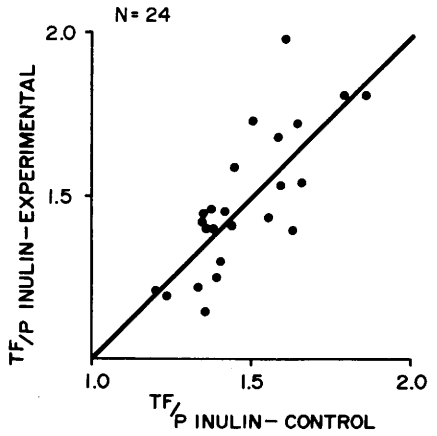

FIG. 1. Effect of isoproterenol on the tubular fluid to plasma inulin ratio.

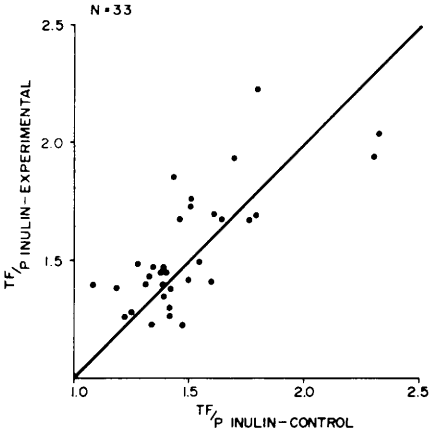

FIG. 2. Effect of isoproterenol during continued alpha blockade on the tubular fluid to plasma inulin ratio.

method of Bratton and Marshall (5). Plasma and urine inulin concentrations were determined by the diphenylamine method (6). The concentration of inulin in tubular fluid was measured by the fluorometric method of Vurek and Pegram (7). Tubular fluid volume was measured in constant-bore capillary tubes of known internal diameter. Sodium and potassium concentration in urine and plasma was measured by an Instrumentation Laboratory flame photometer.

Calculations. 1. Nephron-filtration rate $(V_0) = [(TF/P)_{In}\ (V_F)]$ where $(TF/P)_{In}$ is the tubular fluid-to-plasma inulin ratio and V_F equals the tubular flow rate in nanoliters per minute.

2. Absolute reabsorption (nl/ min) $= (V_0 - V_F)$. Standard statistical analysis was used and all data are presented as the mean ± SE.

Results. In preliminary studies, it was found that 0.018 μg/kg/min of isoproterenol was the maximum dose that could be given without alteration in systemic or intrarenal hemodynamics. In addition, this was the same amount given by Gill and Casper in their studies (1). Therefore, this dose was used in all of the following experiments.

A summary of these studies is shown in Table I and Figs. 1 and 2. In five Group I studies (isoproterenol alone), 24 recollection pairs were obtained. As shown in Fig. 1, there was no alteration in the fractional reabsorp-

TABLE I. Summary of Isoproterenol Micropuncture Studies.[a]

Expt no.	$(TF/P)_{In}$ ratio C	$(TF/P)_{In}$ ratio E	Nephron filtration rate (nl/min) C	Nephron filtration rate (nl/min) E	Absolute reabsorption (nl/min) C	Absolute reabsorption (nl/min) E	Glomerular filtration rate (ml/min) C	Glomerular filtration rate (ml/min) E	Sodium excretion (μEq/min) C	Sodium excretion (μEq/min) E	Fractional sodium excretion (%) C	Fractional sodium excretion (%) E	Renal plasma flow (ml/min) C	Renal plasma flow (ml/min) E	Filtration fraction C	Filtration fraction E	Mean blood pressure (mm Hg) C	Mean blood pressure (mm Hg) E
Isoproterenol alone																		
1	1.52 (7)	1.65	75	79	25	31	31	33	5	9	0.1	0.2	101	108	0.33	0.33	117	117
2	1.30 (5)	1.21	42	47	9	8	18	20	6	6	0.2	0.2	66	64	0.27	0.32	105	108
3	1.51 (4)	1.47	90	90	30	28	40	41	13	6	0.2	0.1	109	120	0.37	0.34	121	119
4	1.39 (3)	1.39	65	60	18	17	20	22	19	24	0.7	0.8	61	66	0.33	0.33	125	128
5	1.61 (5)	1.60	93	94	34	34	33	30	15	13	0.3	0.3	89	80	0.37	0.38	132	128
Mean	1.47	1.46	73	74	23	24	28	29	12	12	0.3	0.3	85	88	0.33	0.34	120	120
SEM	0.04	0.08	9	9	4	5	4	4	3	3	0.1	0.1	9	11	0.01	0.02	4	4
Phenoxybenzamine + Isoproterenol																		
1	1.48 (6)	1.57	97	96	32	35	41	45	6	7	0.1	0.1	104	119	0.39	0.38	121	123
2	1.32 (6)	1.48	140	153	34	49	46	48	10	17	0.2	0.3	122	120	0.38	0.40	105	109
3	1.48 (6)	1.46	86	75	28	24	30	30	15	11	0.3	0.2	120	130	0.25	0.23	121	117
4	1.34 (5)	1.32	89	84	23	20	31	27	38	40	0.9	1.1	101	93	0.31	0.29	115	115
5	1.37 (4)	1.39	81	79	22	22	21	23	12	15	0.4	0.5	65	73	0.34	0.31	105	108
6	1.90 (6)	1.93	91	90	43	43	34	33	20	18	0.4	0.4	100	95	0.34	0.35	119	119
Mean	1.48	1.52	97	96	30	32	34	34	17	18	0.4	0.4	102	105	0.34	0.33	114	115
SEM	0.09	0.09	9	12	4	5	3	4	5	5	0.1	0.2	8	9	0.01	0.02	4	3

[a] Numbers in parentheses indicate the number of recollection pairs in each period. C = control period; E = experimental period.

tion of sodium in the proximal tubule. The mean TF/*P* inulin ratio for the five experiments was 1.47 ± 0.04 in the control period and was unchanged at 1.46 ± 0.08 in the experimental period. Nephron filtration rate was unchanged at 73 and 74 nl/min, while absolute reabsorption was 23 and 24 nl/min in the control and experimental periods respectively.

As is shown in Fig. 2, there was also no alteration in fractional reabsorption of sodium in the proximal tubule during beta stimulation in six further animals treated throughout the study with phenoxybenzamine (Group II). The mean TF/*P* inulin ratio was 1.48 ± 0.09 in the control period and was unchanged at 1.52 ± 0.09 in the experimental period (33 recollection pairs). As in the Group I studies there was no alteration in nephron GFR or absolute reabsorption.

In addition, as is shown in Table I, there was no change in glomerular filtration rate, renal plasma flow, filtration fraction, sodium excretion, or mean arterial pressure during beta stimulation in either group of studies.

Discussion. The purpose of this study was to determine whether beta adrenergic stimulation had a direct effect on proximal tubular transport of sodium independent of its known intrarenal and systemic hemodynamic effects (8, 9). Therefore, we used a dose of 0.018 μg/kg/min of isoproterenol, the highest dosage tested which had no discernable hemodynamic action. The results indicate that beta adrenergic stimulation in the dosage utilized has no effect on proximal tubular sodium reabsorption in the hydropenic dog.

Gill and Casper (1) in hypophysectomized dogs undergoing water diuresis found a small but significant increase in urinary volume during the infusion of the same dose of isoproterenol as used in the present study and concluded that this was due to a decrease in proximal tubular sodium reabsorption. Further, the authors postulated this suppression of reabsorption was due to the increased production of cyclic AMP which occurs during beta stimulation (10).

There are several possible explanations for this apparent discrepancy between the results found during water diuresis and in the present micropuncture studies performed during hydropenia. First, a small change in proximal tubular sodium reabsorption may not be discerned by the recollection micropuncture technique. This is a possibility in any study of this type because of the various analytical and technical problems involved. Yet, even if beta stimulation did cause a small but immeasurable increase in delivery out of the proximal tubule, this would seemingly have little effect on overall sodium excretion since a number of recent studies have demonstrated models in which a marked diminution in proximal tubular sodium reabsorption is associated with a minimal increase in sodium excretion (11–14). Second, the increase in urine volume noted by Gill and Casper during water diuresis (1) may not be due to a decrease in proximal tubular reabsorption. The urinary flow rate during water diuresis is a function of the magnitude of back diffusion of water out of the descending limb of the loop of Henle and the collecting duct as well as delivery out of the proximal tubule (15). Therefore, if isoproterenol altered medullary tonicity or water permeability in the descending limb or collecting duct, an increase in urinary volume could occur without an effect on proximal tubular sodium reabsorption. Third, the proximal effect of beta stimulation might occur only during water diuresis. It is possible that in hydropenia, antidiuretic hormone may have so stimulated cortical adenyl cyclase as to render the effect of isoproterenol ineffective. This would imply that antidiuretic hormone may alter proximal tubular sodium reabsorption. Davis, Knox and Berliner found that ADH had no effect on reabsorption in the proximal tubule in micropuncture studies in hydropenic dogs (16), while Martinez-Maldonado *et al.* have data in water diuresis suggesting an inhibitory action of ADH on proximal tubular sodium and phosphorous reabsorption (17). Therefore, it is possible that the state of hydration may be a factor in determining the proximal tubular response to beta stimulation. However, if an effect is only noted during water diuresis, the physiologic significance of this finding would certainly be decreased.

In the studies of Morimoto, Abe and Ya-

mamoto (2) intrarenal isoproterenol caused an increase in urinary sodium excretion while none occurred in the present experiments. However, a higher dose was used in many of the former studies and all data were obtained during mild saline diuresis. In addition, in the work of Morimoto, Abe and Yamamoto there was a significant increase in both filtration rate and renal plasma flow, while no change occurred in these micropuncture studies.

Therefore, the results of the present work indicate that in the hydropenic dog beta adrenergic stimulation has no direct effect on proximal tubular sodium reabsorption or on overall sodium excretion. This obviously does not exclude a role for the hemodynamic effects of beta stimulation to participate in the regulation of sodium balance.

Summary. Studies were designed to determine whether beta adrenergic stimulation has a direct effect on proximal tubular sodium transport. The recollection micropuncture technique was utilized before and during the intrarenal infusion of isoproterenol, 0.018 μg/kg/min, the highest dose of the drug which had no renal or systemic hemodynamic effects. In the first group of five studies, the tubular fluid to plasma inulin (TF/*P* inulin) ratio was 1.47 ± 0.04 SEM and 1.46 ± 0.08 before and during beta stimulation, respectively. In a second group of six experiments in which alpha blockade was maintained throughout with phenoxybenzamine, 0.09 μg/kg/min, the TF/*P* inulin ratio was again unchanged at 1.48 ± 0.09 in the control period and 1.52 ± 0.09 in the experimental period. Nephron filtration rate and absolute reabsorption were unchanged in both groups of studies. In addition, total GFR, renal plasma flow, sodium excretion and mean arterial pressure were unchanged during isoproterenol infusion. From these data, there is no indication that beta stimulation has an inhibitory effect on proximal tubular sodium reabsorption.

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