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ATTEMPTS TO DEMONSTRATE A HORMONAL NATRIURETIC FACTOR BY MICROPUNCTURE TECHNIQUES¶

It is clear that acute expansion of the extracellular fluid volume produces an increased excretion of sodium by the kidneys, due at least in part to reduced absorption in the proximal tubule. When saline is infused rapidly, fractional reabsorption of the glomerular filtrate is reduced in the proximal tubules of dogs and rats.[1,2] Under the same circumstances a reduction of the reabsorptive capacity of blocked segments of proximal tubules of rats, measured during stopped flow, can also be demonstrated.[3,4]

Recent evidence has suggested that a hormonal inhibitor of renal tubular reabsorption is elaborated in response to expansion of extracellular fluid volume.[5] Prolongation of the rate of reabsorption of droplets of saline in oil-blocked tubules ($t_{1/2}$) and reduced fractional reabsorption during free flow were found when rats were infused intravenously with plasma from volume expanded animals but not when infused with plasma from hydropenic animals. In addition, when plasma dialysates from saline-infused animals were placed in the lumen of proximal tubules the reabsorptive half-time was found to be prolonged, suggesting a direct action on the renal tubule.

The present studies represent an attempt to confirm this demonstration of a circulating natriuretic factor by micropuncture techniques, using the same methods as previously described.[5]

METHODS

In the first set of experiments 20 ml. of heparinized blood was obtained through catheters placed in the internal jugular vein of four dogs, before and after acute volume expansion. After overnight fast the extracellular volume was expanded by infusing each dog with an amount of isotonic saline (150 mM NaCl) equal to 10 per-

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cent of its weight in kilograms, over a 60 minute period. The "natriuretic" sample was obtained at the end of the infusion when sodium excretion exceeded 500 μEq/min. The blood was centrifuged immediately after collection at 4,000 rpm for 10 minutes at 4°C. Ten milliliters of hydropenic and "natriuretic" plasma as well as 10 ml. solutions of isotonic bicarbonate-saline (Na 135 mEq/L, Cl 110 mEq/L, and HCO_3 25 mEq/L) were then dialyzed through cellophane membrane against equal volumes of isotonic bicarbonate-saline solution for 24 hours at 4°C. Dialysis was performed using seamless regenerated cellophane tubing with an inflatable diameter of 29 mm. and an average pore radius of 24 Å (Lapine). Dialysates were stored at 4°C. until experiments were performed within 1-7 days.

Rats were prepared for micropuncture as previously described.[3] In order to avoid the possibility of volume expansion in the assay animal, saline was not given to the rat to correct for fluid losses resulting from surgery. The dialysates of hydropenic and natriuretic plasma and isotonic bicarbonate-saline solution, chosen in a random manner, were placed in the lumen of proximal tubules using the split droplet method of Gertz.[6] Shrinking drop measurements were not performed more than twice in individual tubules. Photographs were taken at three-second intervals and the length of the droplet in consecutive enlarged prints was measured with a caliper. The percentage change of the length of each droplet was plotted semilogarithmically against time. A straight line was drawn through the points by eye and the reabsorptive half-time ($t_{1/2}$) estimated. Special care was taken to prevent dilution of the aqueous contents of the pipette with tubular fluid at the time of initial puncture.

One experimenter (J.H.) performed all the micropuncture manipulations and calculations. In order to reduce observer bias, the source of the sample solutions were unknown to him both during experiments and in calculation of reabsorptive half-times.

In the second group of experiments plasma was obtained from the internal jugular vein of two hydropenic dogs and two dogs in which extracellular fluid had been expanded. The volume-expanded animals were injected subcutaneously with 10 mg. of desoxycorticosterone in oil for four consecutive days before acute infusion of a volume of isotonic saline equal to 10 percent of body weight. As in the previous experiments, plasma was taken from the jugular vein when sodium excretion exceeded 500 μEq/min. Plasma was stored at 4°C. and assayed within one week after being obtained.

Rats were prepared in a manner similar to that used in the first group of experiments. In addition, a small polyethylene catheter (PE 10) was placed in the abdominal aorta, through the left femoral artery, with the tip placed slightly above the left renal artery. The position of the catheter tip was confirmed at the termination of each experiment. Plasma from hydropenic and volume-expanded dogs was infused into the abdominal aorta at a rate of 0.02 ml./min. in a random sequence in each rat studied. This rate of infusion was selected because it was used by Rector and associates.[5] Split droplet studies, using isotonic saline, were made during infusion of each sample of plasma, starting thirty minutes after each infusion was begun.

The identity of the plasma samples was unknown to the person (J.H.) doing the micropuncture experiments until the final tabulation of the results.

RESULTS

Intratubular injection of the test material

Table 1 shows the results of placing bicarbonate-saline solution and plasma dialysates from hydropenic and volume-expanded dogs within the

TABLE 1. INTRATUBULAR EFFECT OF DIALYSATES ON REABSORPTIVE HALF-TIME ($t_{1/2}$)

	*Reabsorptive half-time ($t_{1/2}$) sec.**		
Dog no.	*Dialysate bicarbonate-saline*	*Dialysate hydropenic plasma*	*Dialysate natriuretic plasma*
1	10.5 ± 0.6	10.4 ± 0.6	9.3 ± 0.8
	n = 12	n = 19	n = 8
	a = 3	a = 3	a = 3
2	10.5 ± 0.7	10.3 ± 0.9	14.0 ± 1.7
	n = 13	n = 13	n = 11
	a = 3	a = 3	a = 3
3	12.1 ± 1.7	8.3 ± 0.6	11.8 ± 0.9
	n = 12	n = 6	n = 11
	a = 3	a = 2	a = 3
4	9.9 ± 0.9	8.8 ± 0.6	13.0 ± 1.9
	n = 9	n = 8	n = 11
	a = 2	a = 2	a = 3
Total	10.8 ± 1.2	9.7 ± 0.4	12.2 ± 0.8
	n = 46	n = 45	n = 41
	a = 11	a = 10	a = 12
p value	<0.40	<0.001	

* Values represent means ± SE.
n = Number of observations.
a = Number of rats studied.
p value represents comparison with results using "natriuretic" plasma.

TABLE 2. DEMONSTRATION OF THE VARIATION IN SINGLE $t_{1/2}$ VALUES WHEN THE INTRATUBULAR EFFECT OF DIALYSATES OF BICARBONATE-SALINE SOLUTION AND PLASMA SAMPLES FROM ONE DOG WERE ASSAYED IN RATS

		*Reabsorptive half-time ($t_{1/2}$) sec.**					
Dog no.	*Rat no.*	*Dialysate bicarbonate-saline*		*Dialysate hydropenic plasma*		*Dialysate natriuretic plasma*	
3	8	24.0	10.5			12.0	18.0
		24.0				12.0	
3	9	7.5	14.5	9.0	9.0	10.5	7.5
		9.0		6.0	10.5	9.0	12.0
						15.0	
3	10	7.0	10.5	9.0		9.5	10.5
		7.5	9.0	6.5		13.5	
		9.0	13.0				
Total		12.1 ± 1.7**		8.3 ± 0.6		11.8 ± 0.9	

* Values represent $t_{1/2}$ values in single tubules.
** Values represent mean ± SE.

lumen of proximal tubules. When plasma dialysate from four volume-expanded dogs was placed in the tubular lumen, the mean reabsorptive half-time ($t_{1/2}$) in 12 rats was 12.2 ± 0.8 sec. (mean ± SE). This value was significantly prolonged over the mean $t_{1/2}$ (9.7 ± 0.4 sec.) using plasma dialysates from four hydropenic dogs ($p<0.001$). A longer $t_{1/2}$ was found with dialysate from volume-expanded dogs than with dialysate from hydropenic dogs in three of the four animals studied. The mean $t_{1/2}$ for bicarbonate-saline solution alone, however, was 10.8 ± 1.2 sec., not different from the value found with dialysates from expanded dogs. Comparison of dialysates from expanded dogs and bicarbonate-saline solution demonstrated a prolonged $t_{1/2}$ in rats tested with "natriuretic" dialysate in only two of four dogs. Considerable variation in the value for $t_{1/2}$ was found in different rats and even in different tubules from a single kidney when tested with the same dialysate, as shown in Table 2.

Intra-arterial infusion of the test material

In the second group of experiments, plasma from two hydropenic and two volume-expanded dogs was assayed in eight rats for natriuretic properties by direct infusion into the abdominal aorta. With this method the concentration of soluble substances in plasma reaching the kidney would presumably be greater than with intravenous infusion of plasma. Mean values of reabsorptive half-time for both control and experimental plasma samples are shown in Table 3. The $t_{1/2}$ was 8.5 ± 0.4 sec. when hydropenic plasma was infused. This value was not different ($p<0.20$) from the $t_{1/2}$ (9.2 ± 0.2 sec.) using plasma from expanded dogs.

DISCUSSION

The mechanism whereby expansion of the extracellular fluid volume inhibits the reabsorption of fluid in the proximal tubule may involve a single factor or a combination of several factors. Many investigators have suggested that the inhibiting influence may be mediated by a circulating hormone[7-9] but unequivocal evidence for such a substance has been difficult to amass.[10] Recent studies by Rector and his co-workers[5] suggested that with the use of micropuncture techniques, a factor inhibiting proximal tubular absorption of sodium could be readily identified in plasma and the dialysate of plasma from volume-expanded dogs and rats. Although rats that were infused intravenously with such samples of plasma did not increase their excretion of sodium, fractional reabsorption and reabsorptive capacity for sodium in the proximal tubule were sharply reduced to levels found in rats studied during a saline infusion. Subsequent attempts to confirm this initial report have met with indifferent success,[11] though results

TABLE 3. EFFECT OF THE AORTIC INFUSION OF HYDROPENIC AND NATRIURETIC PLASMA ON REABSORPTIVE HALF-TIME ($t_{1/2}$)

		*Reabsorptive half-time ($t_{1/2}$) sec.**	
	Rat no.	*Hydropenic plasma*	*Natriuretic plasma*
Dog no.		6	7
	23		8.8
			n = 6
	24	9.0	8.5
		n = 2	n = 3
	25	8.0	9.1
		n = 4	n = 5
	26	8.6	9.4
		n = 4	n = 5
Dog no.		8	9
	28	7.8	10.0
		n = 5	n = 3
	29		8.4
			n = 4
	30	9.1	9.5
		n = 6	n = 5
	31	8.8	9.8
		n = 5	n = 6
Total		8.5 ± 0.4**	9.2 ± 0.2
		n = 26	n = 37
		p<0.20	

* Values represent mean.
n = Number of observations.
** Values represent mean ± SE.

similar to Rector's were reported by Auld and co-workers.[12] It is clear that a simple and accurate method for identifying a natriuretic factor(s) in the plasma of volume-expanded subjects would have immense importance in elucidating the mechanisms normally controlling sodium diuresis.

The present studies were performed to explore the reliability of these techniques in detecting a hormonal natriuretic factor that might be released during saline diuresis. An important characteristic of these experiments was the attempt made to reduce observer bias since such bias may influence the investigator in a variety of ways both during micropuncture studies and in calculation of the $t_{1/2}$ value.

When plasma dialysate was placed directly into the tubular lumen, an inhibiting influence on sodium absorption was suggested in only two of the four dialysates from volume-expanded dogs. Although the mean $t_{1/2}$ for all

rats studied was prolonged when "natriuretic" plasma dialysate was compared to plasma from hydropenic dogs, the difference was less marked, and did not reach the level of significance, when bicarbonate-saline solution and "natriuretic" dialysate were compared. The split-droplet technique is subject to the uncertainties introduced by occasional widely discrepant values for half-time, as illustrated in Table 2. If observer bias is not eliminated, these values may be inadvertently selected or discarded.

Rector and his co-workers[5] suggested a correlation between the level of inhibiting substance and the magnitude of volume expansion and found that inhibiting effect was completely lost when natriuretic plasma infused intravenously was diluted 1:4 with Ringer's bicarbonate. Because a likely site of action of a natriuretic substance is the basal membrane of tubular cells, rather than their luminal border, plasma from natriuretic dogs was infused at the aortic orifice of the renal artery in the present studies to insure that a sufficient concentration of inhibitory substance reached the periluminal capillaries of the kidney. Disappointingly, no reduction in sodium absorption was found when "natriuretic" plasma was infused.

These experiments are sufficient neither to confirm nor deny the existence of a natriuretic factor affecting reabsorption by the proximal tubule. The tantalizing suggestion of a dialysate substance prolonging reabsorptive half-time can be discerned in dogs 2 and 4 of Table 1. Nevertheless, it appears that with the standard micropuncture techniques used in the present studies, a "natriuretic hormone" cannot be demonstrated reliably and reproducibly in the plasma of dogs given large amounts of saline intravenously so as to produce a sodium diuresis. Further refinements of assay techniques with fractionation and purification of plasma will probably be necessary if the presence and nature of a natriuretic substance is to be indubitably established.[13]

SUMMARY

An attempt was made to confirm the demonstration of a circulating natriuretic factor by micropuncture techniques. When dialysate of plasma from volume-expanded dogs was placed directly in the lumen of proximal tubules, sodium absorption was reduced, compared to the effect of dialysates from hydropenic dogs. No difference in sodium absorption, however, was found when dialysate of plasma from natriuretic dogs was compared to isotonic bicarbonate-saline solution.

In a second group of experiments, the effect on tubular function of plasma from hydropenic and volume-expanded dogs infused into the abdominal aorta was assayed. A natriuretic property of plasma from dogs with expansion of ECF was not found.

In these studies a hormonal "natriuretic" factor was not reliably and reproducibly demonstrated by standard micropuncture techniques. Further refinements of assay techniques with fractionation and purification of plasma will probably be necessary if the presence and nature of a natriuretic substance is to be indubitably established.

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