MICROPUNCTURE STUDY OF DISTAL TUBULAR FUNCTION OF CAT KIDNEY

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Micropuncture studies of the tubules accessible on the surface of the mammalian kidney have been performed usually in the rat, dog and monkey.

The tubular fluid from early distal tubule in the rat was always hypotonic to plasma (1), but it reached isotonic to plasma in the late portion of the distal tubule (2-5). In contrast to the observation in the rat, it has been demonstrated that the tubular fluid was markedly hypotonic to plasma along the entire length of the distal tubule in the dog during antidiuretic as well as diuretic state (6, 7). In the kidney of the rhesus monkey, the distal tubular fluid was also hypotonic to plasma during hydropenia (8). The fact mentioned above has suggested that the distal tubular function of the rat kidney was different from that of the dog and monkey kidney. The micropuncture study of the cat kidney has not been reported.

The present study was designed to investigate the pattern of the tubular function of the cat kidney.

METHODS

Renal micropuncture experiments were performed on twenty-five male and female cats weighing from 2 to 4 kg. Six cats were kept away from water 24 hours before experiments. The remaining cats were allowed free access to water before experiments, three of them were loaded water (10 ml/kg) via a gastric tube just prior to experiments, and one cat was injected 20 ml of 0.9% saline intravenously immediately before micropuncture.

Initially anesthesia was induced by ether. The trachea was exposed in order to insert an endotracheal tube and respiration was maintained by an artificial respirator. Polyethylene catheters were inserted into both hindlimb veins for injection and blood collection. A polyethylene catheter was inserted into the ureter of the experimental side in some cases for collection of urine samples.

Cats were immobilized by intravenous administration of Flaxedil (3 mg/kg) and this was added 1-1.5 mg/kg in case of need during experiment.

The left kidney was exposed, isolated from adipose tissues and decapsulated partially. The kidney was fixed in order to prevent respiratory movement with holders at the upper and lower sides of kidney. Care was taken not to disturb the renal blood flow. Warmed liquid paraffin oil was dropped on the surface of the kidney throughout the experiment.

Micropipettes of Pyrex glass were prepared by a microelectrode puller and sharpened by a small grinder. Usually the tips of the pipettes were less than 10 microns in outer diameter. Micropipettes were filled with liquid paraffin oil, colored by oil blue and previously saturated with water. Micropuncture was performed under a binocular microscope at a magnificant of 80-fold. The surface of the kidney was illuminated by a glass rod of Knisely type. One or two samples of proximal fluid and two to ten samples of distal tubular fluid were collected from the surface convolutions during experimental period.

The identification of distal tubules was facilitated by an intravenous injection of small amounts of 5% lyssamine green dissolved in 0.9% saline (9). Lyssamine green appeared in distal tubules about 50–80 seconds after injection. Blood was collected from hindlimb vein before, during and after experiment. In some cases, latex was injected into the tubules after the experiment and the kidney was removed and macerated with HCl and water, in order to isolate the punctured tubules and to identify the puncture sites. Their locations in their respective convolutions were measured with an eyepiece micrometer. On the case of the measurement the proximal tubule is defined to extend from the glomerulus to the thin segment of the loop of Henle and the distal convolution from the macula densa to the distal tubular junction to the collecting duct.

Measurement of osmolality and Na concentration:

After the collection of tubular fluid into the micropipette small amounts of liquid paraffin was sucked from the tip of the same micropipette, so the collected tubular fluid was put between liquid paraffin droplets in the micropipette. The tip of the micropipette was sealed. The osmolalities of tubular fluid, plasma and urine were measured by Ramsay's microcryoscopic technique (10).

After the measurement of osmolality, the tubular fluid in the micropipette was blown out into the siliconized plate filled with liquid paraffin. Na concentration was measured by Erma ultramicroflame photometer using 3–4 nanoliter in volume. The measurement was usually done in duplicate. Na concentration of plasma and urine was measured by Evans-flame photometer.

Determination of inulin concentration:

In some experiments 1% inulin in isotonic saline was administered intravenously at the rate of 20 ml/hr. Micropuncture was performed after inulin equilibration. The tubular fluid was applied for inulin determination following the measurement of osmolality and Na concentration. The inulin concentration of tubular fluid was determined by the ultramicrofluorometric method of Vurek and Pergam using sample about 10 nanoliter in volume (11, 12). Inulin concentration of plasma and urine was measured by the anthrone method (13).

RESULTS

Na and osmolality of distal tubular fluid:

Samples from distal tubules were analyzed for osmolality and Na concentration (Table 1, Fig. 1). Values of osmolality ranged from 91 to 334 m Osmol/kg water. Values of

TABLE 1. Osmolality and Na of distal fluid.

Cat No.	Osmolality (in Osm/kg water)	(TF/P)Osm.*	Na (m Eq/L)	(TF/P)Na**
1)	312	0.98	45.0	0.31
2)	312	0.95	32.0	0.24
3)	285	0.95	88.0	0.62
	242	0.80	83.0	0.56
4)	242	0.74	35.5	0.25
	188	0.57		
	269	0.82	40.0	0.28
5)	182	0.55	74.0	0.49
	317	0.95	15.0	0.10
	306	0.92	37.0	0.24
6) saline injected	102	0.44	32.0	0.17
	91	0.40	33.0	0.20
	145	0.63	62.0	0.38
	145	0.63	81.0	0.50
	264	0.77	70.0	0.43
7) water loaded	161	0.48	37.0	0.27
	188	0.57	60.0	0.43
	172	0.52	35.0	0.25
	269	0.96	90.0	0.62
8)	291	0.89	66.5	0.48
	334	1.02	79.0	0.57
	291	0.89	66.5	0.48
	291	0.89	69.5	0.50
9) hydropenic	242	0.76	73.0	0.46
10) hydropenic	285	0.90	63.5	0.43
	279	0.88	37.0	0.25
11) water loaded	312	1.00	19.5	0.13
	312	1.00	42.0	0.28
	301	0.97	39.0	0.26
12) hydropenic	312	0.89	110.5	0.77
	167	0.48	47.0	0.33
	318	0.91	100.0	0.70
	205	0.58	38.0	0.27
13) hydropenic	150	0.51	56.0	0.42
	102	0.35	23.5	0.17
	242	0.82	80.0	0.59
	172	0.58	66.0	0.49
	188	0.64	58.5	0.43
	199	0.67	89.5	0.67
14)	307	0.89	100.5	0.74
	323	0.94	67.0	0.47
15)	226	0.68	76.5	0.59
	221	0.66	80.0	0.62
	264	0.79	69.5	0.54
16)	307	0.95	104.5	0.72
17) hydropenic	269	0.85	56.0	0.37

	312	0.98	44.5	0.30
	258	0.81	57.5	0.38
	226	0.71	50.0	0.33
	231	0.73	48.0	0.32
18) hydropenic	209	0.65	30.0	0.20
	177	0.55	43.0	0.29
	177	0.55	58.0	0.39
	317	0.98	50.0	0.33
	209	0.65	75.0	0.50
	215	0.67	47.0	0.31
19) water loaded	253	0.81	25.0	0.19
	301	0.97	48.0	0.37
	296	0.95	40.0	0.31
	280	0.90	59.5	0.45
	285	0.91	50.0	0.38
	274	0.88	34.0	0.26
	242	0.78	43.0	0.33
	318	1.02	35.5	0.27
	301	0.97	48.3	0.37
	296	0.95	57.5	0.44

* (TF/P)Osm.=tubular fluid to plasma ratio of osmolality

** (TF/P)Na—tubular fluid to plasma ratio of Na concentration

Osmolality and sodium concentration in distal tubule of cat

FIG. 1. Relationship between osmolality and Na concentration of same sample.

Na concentration ranged from 15.0 to 110.5 m Eq/L. Tubular fluid to plasma ratios (TF/P) of Na concentration in distal tubules were within a range from 0.10 to 0.77. While, those of osmolality ranged from 0.35 to 1.02 (Fig. 2). Na concentration of each distal

Fig. 2. Relationship between tubular fluid to plasma ratio of osmolality and that of Na concentration of same sample.

Fig. 3. Relationship between site of micropuncture and tubular fluid to plasma ratio of osmolality and that of Na concentration.

sample was much lower than that of proximal fluid and plasma in many cases. Other solutes besides Na, e.g. urea and K, seemed to contribute much to the osmolality of distal tubular fluid. There was no significant difference of osmolality and Na concentration in the distal tubule between the hydropenic and the water loaded cats. These results sug-

gested that the tubular fluid might reach isotonic to plasma during the flow. In order to identify the micropuncture site, latex was injected into the tubular lumen and the microdissection of the tubular tissue was performed. In Fig. 3 the correlation between the osmolality as well as Na concentration and punctured sites of the distal tubule were shown.

The tubular fluid was hypotonic in the early distal tubule, and reached approximately isotonic in the late portion as shown in Fig. 3. On the other hand, Na concentration was lower than that of plasma along the entire distal length.

Na and osmolality of proximal tubular fluid:

One or two samples of proximal tubular fluid were collected in each cat. Na concentration of these samples were from 126 to 162 mEq/L. Proximal tubular fluid to plasma ratio (TF/P) of Na concentration was almost equal to unity. While, values of osmolality ranged from 285 to 361 m Osm/kg water and all samples were almost isotonic with plasma along the entire proximal length (Table 2, Figs. 4 and 5).

TABLE 2. Osmolality and Na of proximal fluid.

Cat No.	Osmolality (m Osm/kg water)	(TF/P)Osm.*	Na (m Eq/L)	(TF/P)Na**
1)	344	1.02	135.0	1.05
	323	0.95	135.0	1.05
2)	328	1.0	127.5	0.96
	312	0.95	127.5	0.96
	328	1.0	128.5	0.96
3)	318	1.05	138.5	0.98
4)	285	1.02	150.5	1.04
5)	318	1.0	152.0	0.99
6) saline injected	334	0.97	162.0	1.01
7) water loaded	323	0.97	137.0	0.99
8) hydropenic	317	1.0	143.0	0.98
9)	318	1.02	143.0	0.94
10) hydropenic	339	0.97	145.0	1.01
11) hydropenic	285	0.96	135.0	1.0
	296	1.0	130.5	0.97
12)	361	1.08	126.0	0.97
13)	328	1.02	142.0	0.97
14) hydropenic	307	0.97	150.5	1.0
15) hydropenic	312	0.97	151.5	1.01
16) water loaded	296	0.95	133.5	1.02

* (TF/P)Osm.=tubular fluid to plasma ratio of osmolality

** (TF/P)Na=tubular fluid to plasma ratio of Na concentration

Water reabsorption:

Net water reabsorption along the each segment of nephron was calculated from inulin concentrations in the tubular fluid. Plasma to tubular fluid ratio (P/TF) of inulin concentration represents the remained fraction of filtered water in the each portion of the nephron (Table 3, Fig. 6). About 50% of filtered water remained in the late surface convolu-

FIG. 4. Relationship between osmolality and Na concentration of same sample.

FIG. 5. Relationship between site of micropuncture and tubular fluid to plasma ratio of osmolality and that of Na concentration.

tion of proximal tubule, the remained fraction of filtered water in the early distal tubule was about 15 to 17% of filtered water, and this water decreased further along the distal tubule.

This result showed that water was reabsorbed significantly along the distal tubule.

TABLE 3.

Cat No.	Location	Osm. (mOsm/kg water)	(TF/P)Osm.	Na (mEq/L)	(TF/P) Na	(P/TF) In.	$\frac{(\text{TF/P}) \text{Na}}{(\text{TF/P}) \text{In.}}$
1)	D* 47%	280	0.93	72	0.47		
	D 65%	301	1.0	89	0.58		
2)	P** 37%	318	0.91	130	1.02		
	D 30%	242	0.69	73.5	0.58	0.057	0.033
	D 70%	334	0.95			0.033	
	U***	635	1.82	153.5	1.20	0.009	0.036
3)	P 52%	339	1.0	150	1.04		
	D 40%	301	0.89	95	0.66	0.094	0.062
	D 62%	323	0.95	116.5	0.80	0.063	0.05
	D 30%	280	0.83	77	0.53		
	U	963	2.84	249	1.72	0.021	0.037
4)	P 58%	318	0.97	150	0.98	0.400	0.392
	D 50%	307	0.93	107.5	0.71		
	U	872	2.66	240	1.57	0.014	0.022
5)	P 36%	312	0.98	138	0.99	0.390	0.387
	P 45%	312	0.98	141	1.01	0.456	0.459
	D 23%	237	0.75	70	0.50	0.156	0.078
	D 40%	269	0.85	106	0.76		
	U	667	2.10	165.5	1.19	0.052	0.062
6)	P 67%	339	1.03	143	1.02	0.450	0.459
	P 40%	339	1.03	144	1.03		
	D 35%	167	0.51	32	0.23		
	D 40%	237	0.72	30	0.21	0.135	0.028
	D 31%	156	0.48	27.5	0.20	0.170	0.034
	U	1981	6.03	256	1.83	0.007	0.013

* Distal tubule

** Proximal tubule

*** Ureteral urine

Location=Site of fluid collected

Osm.=Osmolality

(TF/P)Osm.=Tubular fluid to plasma ratio of osmolality

(TF/P)Na=Tubular fluid to plasma ratio of Na concentration

(P/TF)In.=Plasma to tubular fluid ratio of inulin

Na reabsorption:

The fraction of filtered sodium remained in the each portion of nephron is shown in Fig. 7. About 50% of filtered sodium was reabsorbed along the proximal tubule. The fraction of filtered sodium which entered the early portion of distal tubule was about 8%. Large fraction of sodium was presumed to be reabsorbed in the Henle's loop and some fraction of sodium might be reabsorbed along the distal tubule.

DISCUSSION

In the mammalian kidney, it has been known that there are two different types as for the function of the distal tubule. In the dog as well as in the monkey the tubular fluid

FIG. 6. Relationship between remained fraction of filtered water and site of fluid collection.

was always hypotonic along the entire distal convolution (6-8). While, in the rodents, e.g. the rat, distal tubular fluid was hypotonic only in the early portion but the osmolality elevated along the distal length to isotonic in the late portion (1-5). Micropuncture studies of the kidney have been performed generally in the rat, dog or monkey, and the function of the kidney of other animal, e.g. the cat, has not been studied. Therefore, the renal function of the cat, especially that of distal tubule, was investigated with micropuncture technique.

Both osmolality and Na concentration of proximal tubular fluid in the cat were roughly equal to respective value of plasma and the osmolality of distal tubular fluid was hypotonic in the early portion as well as in the rat, dog and monkey but reached isotonic in the late portion. In the dog, as well as monkey, fluid was always hypotonic along the entire distal tubule (6, 7, 14). The cat was clearly different from the dog and monkey with regards to this pattern of the distal tubular fluid. In the dog, the permeability of the distal tubule to water was low. About one-fourth of the water was reabsorbed in the distal tubule (7). In the monkey, the pattern of the water reabsorption in the distal tubule was almost same as in the dog (8, 14).

On the other hand, the distal tubular fluid in the rat was considerably reduced to roughly one-fourth of the water entered from loop of Henle. The fluid was passively

Fig. 7. Relationship between remained fraction of filtered Na and site of fluid collection.

reabsorbed and the fluid in the distal tubule became isotonic (4). The findings reported here showed that the permeability of water in the distal tubule of the cat was similar to that of the rat.

The distal tubular fluids were collected in this experiment under various conditions and the osmolalities of these samples were iso- or hypotonic. The correlation between the osmolality and the site of puncture in the tubule was analyzed.

These facts suggest that the fluid becomes isotonic in the late distal tubule under the various conditions experimented in this study.

It could be concluded that the nature of the tubular membrane of the water permeability in the cat was different from that of the dog or monkey, and was resembled to the rat.

SUMMARY

This experiment studied the distal tubular function of the cat kidney. Tubular fluid was collected by micropuncture technique from the surface convolutions of the proximal and distal tubules of the left kidney and the collected tubular fluid was analyzed for the osmolality and the concentration of sodium and inulin.

Tubular fluid from the early distal tubule was hypotonic but was approximately isotonic to the plasma in the late portion. The sodium concentration of the distal tubular fluid was much lower throughout this segment than that of the plasma and the proximal tubular fluid.

The plasma to distal tubular fluid ratio of inulin decreased in the late distal tubule as compared with that in the early portion. This suggested that water was reabsorbed in the distal tubule of the cat kidney.

This study showed that the distal tubular function in the cat was different from that in the dog and monkey, but was resembled to the function of the distal tubule in the rat.

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REFERENCES

- 1) WIRZ, H.: *Physiol. Pharmac. acta* **14**, 353 (1956)
- 2) GOTTSCHALK, C.W. AND MYLLE, M.: *Am. J. Physiol.* **196**, 927 (1959)
- 3) GOTTSCHALK, C.W.: *Physiologist* **4**, 35 (1961)
- 4) LASSILER, W.E., GOTTSCHALK, C.W. AND MYLLE, M.: *Am. J. Physiol.* **200**, 1139 (1961)
- 5) ULLRICH, K.J., SCHMIDT-NIELSEN, B., O'DELL, R., PEHLING, G., GOTTSCHALK, C.W., LASSITER, W.E., AND MYLLE, M.: *Am. J. Physiol.* **204**, 527 (1963)
- 6) CLAPP, J.R. AND ROBINSON, R.R.: *J. clin. Invest.* **45**, 1847 (1966)
- 7) BENNETT, C.M., CLAPP, J.R. AND BERLINER, R.W.: *Am. J. Physiol.* **213**, 1254 (1967)
- 8) BRENNER, B.M., BENNETT, C.M. AND BERLINER, R.W.: *Physiologist* **10**, 132 (1967)
- 9) STEINHAUSEN, M.: *Arch. ges. Physiol.* **277**, 25 (1963)
- 10) RAMSAY, J.A. AND BROWN, R.H.J.: *J. scient. Instrum.* **32**, 372 (1955)
- 11) VUREK, G.G. AND PERGAM, S.E.: *Analyt. Biochem.* **16**, 409 (1966)
- 12) SUZUKI, A., IKEZAWA, I. AND SAKAI, F.: *Igaku No Ayumi* **70**, 127 (1969)
- 13) FUHR, J., KACZMARCZYK, J. AND KRÜTTGEN, C.D.: *Klin. Wschr.* **33**, 729 (1955)
- 14) BENNETT, C.M., BRENNER, B.M. AND BERLINER, R.W.: *J. clin. Invest.* **47**, 203 (1968)