Characteristics of Salt and Water Transport in Superficial and Juxtamedullary Straight Segments of Proximal Tubules

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ABSTRACT The purpose of the present studies was to characterize the nature of salt and water transport out of the superficial (SF) and juxtamedullary (JM) straight segments of rabbit proximal tubules as examined by in vitro microperfusion techniques. When the perfusate consisted of a solution simulating ultrafiltrate of plasma, there were no differences between SF and JM straight tubules in either net reabsorption of fluid (SF = 0.47 nl/mm per min; JM = 0.56 nl/mm per min) or in transtubular potential difference (PD) (SF = − 2.1 mV; JM = − 1.8 mV). Removal of glucose and alanine from the perfusate had no effect on the magnitude of the PD in either straight segment. Ouabain decreased both the net reabsorptive rates and the PD. Isosmolal replacement of NaCl by Na-cyclamate (a presumed impermeant anion) in the perfusate and the bath caused an increase in luminal negativity in both segments whereas similar substitution of NaCl by choline-Cl (nontransported cation) changed the PD to near zero. These studies, therefore, suggest that sodium is transported out of the proximal straight tubules by an active noncoupled process that generates a PD (electrogenic process).

When the perfusate consisted of a solution with a high chloride concentration (resulting from greater HCO_3 than Cl reabsorption in the proximal convoluted tubule), different PDs in SF and JM tubules were generated: SF = + 1.6±0.2 mV; JM = − 1.3±0.3 mV. This difference in PD was attributed to relative differences in Na and Cl permeabilities in these two segments. Electrophysiological and isotopic estimates of the chloride to sodium permeability revealed that the SF tubule is about twice as permeant to chloride than to sodium, whereas the JM tubules are approximately twice as permeant to sodium than to chloride.

It is concluded that the mechanism of active sodium transport in the straight segment of proximal tubule differs from that of the convoluted segment and that both the SF and JM straight segments differ from each other with respect to sodium and chloride permeability.

INTRODUCTION

It is now generally accepted that at least two populations of nephrons exist in the mammalian kidney. These two types may be broadly classified as superficial nephrons (SF),[1] possessing short loops of Henle, and juxtamedullary nephrons (JM), possessing long loops that course into the inner medulla and papillary tip.

These two populations of nephrons have been studied almost exclusively with respect to filtration rate and regional renal plasma flow. In general, despite evidence to the contrary (1–5), recent studies in the rat and the dog have indicated that massive saline infusions cause a disproportionate increase in filtration rate in the SF nephrons (6–10) and in plasma flow to the JM nephrons (10–13); in the rat (9) and the dog (10) these changes are associated with a profound fall in deep nephron filtration fraction. These findings have given rise to the hypothesis that redistribution of filtrate, plasma flow, and filtration fraction may be an important factor in the regulation of sodium excretion.

Apart from hemodynamic studies, data are not available delineating the intrinsic tubular transport proper-

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[1] *Abbreviations used in this paper:* JM, juxtamedullary; PD, potential difference; SF, superficial.

ties of the two sets of nephrons. Moreover, although in the rat there are available abundant direct data concerning the transport characteristics of the convoluted portion of the SF proximal nephrons and inferential data concerning the convoluted portion of the JM nephrons, data detailing the intrinsic characteristics of salt and water in the straight tubule are of limited scope. Such published data describe only net rates for fluid reabsorption (14–16). Previous studies have not examined the possible differences in specific transport and permeability properties that might exist between these two sets of nephrons.

The purpose of the present studies was twofold. First, the transport properties with respect to sodium, chloride, and water of the SF and JM straight segments were compared with those of the convoluted segments by perfusing with a solution simulating ultrafiltrate of plasma. Second, the SF and JM nephrons were compared with each other by perfusing with fluid of a composition simulating that which normally issues out of the proximal convolution to determine whether the straight segments of these nephron populations have different intrinsic properties.

METHODS

Isolated straight segments of the proximal tubules obtained from female New Zealand rabbits were perfused by the same techniques previously described (17). All rabbits weighed between 1.5–2.5 kg, were fed a standard laboratory diet, and had free access to water before guillotine decapitation. The kidney was quickly removed, and a 1–2-mm slice was transferred into a chilled dish of rabbit serum that was kept at pH of 7.4 by continuous bubbling with 95% O_2–5% CO_2. Proximal straight segments of either SF or JM nephrons were then dissected out without the use of collagenase. During dissection, positive identification of the origin of the straight segment is not difficult. In each case the straight segment was dissected up to the convoluted portion of the proximal tubule. If the straight segment was connected to a convoluted portion that ran a horizontal course near the corticomedullary junction, it was assigned to the JM group. On the other hand, if the straight segment originated near the surface capsule, it was assigned to the SF group. JM straight segments were obtained exclusively from the outer medulla while straight segments of the proximal tubules from SF nephrons were obtained both from the cortex and the outer medulla. The results with the SF tubules obtained from the superficial cortex as contrasted to the outer medulla were identical, and thus, the results have been grouped together. All tubular segments were labeled prospectively before obtaining the result except in one specific series of 18 consecutive tubules where one investigator dissected out the tubules without telling the other investigator which segment he was perfusing. In this series of 18 tubules, the microperfuser was able to identify the origin of each segment correctly even though morphologically the two segments are indistinguishable.

TABLE I

Composition of Artificial Perfusion and/or Bathing Solutions

	A	B	C	D	E	F
NaCl	105	141	105	—	—	55
$NaHCO_3$	25	5	25	—	25	25
$KHCO_3$	—	—	—	5	—	—
KCl	5	5	5	—	—	5
K_2SO_4	—	—	—	—	2.5	—
K_2HPO_4	—	—	—	2	—	—
Na_2HPO_4	4	4	4	—	4	4
Na acetate	10	5	10	—	6.4	10
Ca acetate	—	—	—	2.5	1.8	—
$CaCl_2$	1.8	1.8	1.8	—	—	1.8
Choline Cl	—	—	—	148	—	—
Na cyclamate	—	—	—	—	108.6	—
Mg SO_4	1	1	1	1	1	1
Glucose	8.3	—	—	8.3	8.3	8.3
Alanine	5	—	—	5	5	5
Sucrose	—	—	13.3	—	—	92
Osmolality* *mosmol/liter*	300	301	299	310	296	300

* Osmolality measured by standard freezing point depression techniques.

Six different perfusion solutions were used. Table I summarizes the concentrations of constituents in the various perfusates. In all instances, the tubules were initially perfused with control solution A, which was constructed to simulate isosmolal ultrafiltrate of same rabbit serum as used in the bath.

Net reabsorption of fluid, C, was calculated by previously published techniques (18):

$$C = \frac{V_i - V_o}{L}, \tag{1}$$

where V_i is calculated by dividing the ^{125}I (Glofil-125, Abbott Laboratories, North Chicago, Ill.) counts per minute of the collected fluid by ^{125}I counts per minute per nanoliter of perfusion fluid and by the time of the collection period; V_o is obtained directly by previously calibrated constant volume collection pipette as originally described by Burg (19); L is the length of tubule in millimeters; and C is expressed in units of nanoliters per millimeter per minute.

The permeability coefficients for sodium and chloride were obtained both isotopically and electrophysiologically. The apparent passive permeability coefficients for ^{24}Na (New England Nuclear, Boston, Mass.) and ^{36}Cl (ICN Corp., Chemical and Isotope Div., Irvine, Calif.) were estimated simultaneously from the disappearance rate from the luminal fluid of the respective isotope added to the perfusate. In these experiments control isosmolal artificial solution A was used as the perfusate while rabbit serum was used as the bath. Lumen-to-bath apparent permeability coefficients (centimeters per second) for ^{24}Na and ^{36}Cl were estimated according to the following equation (20):

$$P = \frac{V_i - V_o}{A}\left[\frac{\ln C_i^*/C_o^*}{\ln V_i/V_o} + 1\right], \tag{2}$$

where A is the area of tubule calculated from the length of tubule and inside diameter (assuming ID = 20 μm); V_i equals the perfusion rate; V_o is the collection rate; C_i^* equals the counts per minute per milliliter of isotope in the perfusate; and C_o^* equals the counts per minute per milliliter of isotope in the collection fluid. In those experiments in which $V_i = V_o$ exactly, then the apparent permeability coefficients were calculated as described by Grantham and Burg (20):

$$P = \frac{V_i}{A} \ln \frac{C_i^*}{C_o^*}. \tag{3}$$

The relative permeability of sodium to chloride in both the SF and JM straight tubules was estimated from the magnitude of the diffusion potential when a 50-meq/liter NaCl concentration gradient was imposed across the respective epithelium. During control periods the base-line transtubular PD was measured using solution A as perfusate and regular rabbit serum as the bath. During experimental periods the bath NaCl was reduced by 50 meq/liter by dilution with isosmolal sucrose solution that contained physiological amounts of other electrolytes but was free of NaCl. The relative conductances were calculated by use of the Goldman equation:

$$PD = \frac{-RT}{F} \ln \frac{P_{Na}[Na]_l + P_{Cl}[Cl]_b}{P_{Na}[Na]_b + P_{Cl}[Cl]_l}, \quad (4)$$

where R is the gas constant; T is absolute temperature; F is the Faraday constant; P_{Na} and P_{Cl} are passive permeability coefficients; [Na] and [Cl] refer to concentrations of Na and Cl; and l and b refer to lumen and bath.

The same electrical circuit was used in these experiments as has been previously described from our laboratory (21). Equivalent bridges of 300 mosmol/liter Ringer's solution in 4% agarose were connected to the end of perfusion pipette and bath. The other end of the bridges were placed in saturated KCl solution that contained Beckman (Beckman Instruments, Inc., Fullerton, Calif.) calomel half-cells. The PD was measured by a battery operated Keithley model 602 electrometer (Keithley Instruments, Inc., Cleveland, Ohio) and was continuously recorded on a Rikadenki model B-261 multipen recorder (Rikadenki Kogyo Co., LTD). The stability of this system was excellent with base-line voltage drift of less than ±0.5 mV for the duration of the experiment. All of the transtubular PDs reported in the results section have been corrected by the liquid junction potential where applicable. The magnitude of the liquid junction potential was calculated by a general junction potential equation based on Nernst-Planck equation as derived by Barry and Diamond (22). There are a number of sources of error in the calculation of the exact liquid junction potential correction. However, these are considered minimal as previously discussed in detail (21). The magnitude of the liquid junction potential correction ranged from 0 mV, when the perfusate and the bath electrolyte concentrations were identical to −3.6 mV when 50 meq/liter sodium chloride was replaced in the bath by isosmolal volume of sucrose.

The radioactivity of ^{125}I and ^{24}Na was measured by a Packard model 3365 three-channel gamma spectrometer while ^{36}Cl activity was measured with a Packard model 2420 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). In those experiments where ^{24}Na and ^{36}Cl permeabilities were measured simultaneously, ^{24}Na was counted immediately after samples were obtained, whereas ^{36}Cl counts were measured 15 days after the initial ^{24}Na counting.

The data of each tubule are obtained as a means of three or four collection periods. The results are expressed as mean±SE of number of tubules studied. The statistics were calculated by either a paired or nonpaired t test analysis.

RESULTS

Net transport of fluid out of the SF and the JM straight tubules. Net transport of fluid out of these segments was measured using solutions A and B (Table I) as the perfusion fluid, with the bath consisting of isosmolal rabbit serum. The mean length of the SF tubules was 2.1±0.2 mm when solution A was used and 2.2±0.2 mm when solution B was used. The length of the JM tubules was somewhat shorter, the mean value being 1.4±0.1 mm when solution A was used and 1.5±0.1 mm when solution B was used. In all groups the perfusion rates were the same, ranging from 10.9 to 13.0 nl/min.

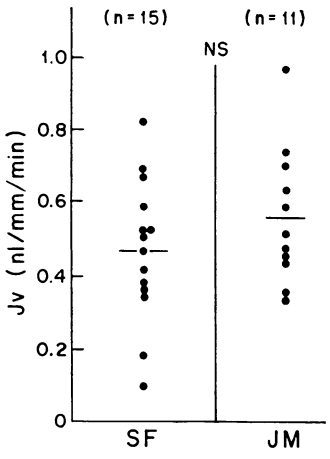

FIGURE 1 Net transport of fluid (J_v) by SF and JM straight segments of proximal tubules. Perfusate, solution A; bath, regular rabbit serum.

The results of these experiments are graphically depicted in Fig. 1 and partly summarized in Table II. When solution A (fluid simulating the ultrafiltrate) was perfused, net transport was 0.47±0.05 nl/mm per min in SF ($n = 15$) and 0.56±0.06 nl/mm per min in JM ($n = 11$) straight tubules. These values are not statistically different ($P > 0.2$). When solution B (fluid simulating that which normally is delivered to the straight segment) was perfused, net transport was 0.31±0.05 nl/mm per min in SF ($n = 10$) and 0.24 ±0.08 nl/mm per min in JM ($n = 7$) straight tubules. These values also are not statistically different ($P > 0.2$). However, when solution B was perfused instead of solution A, net reabsorption was less both in SF ($P < 0.02$) and in JM ($P < 0.01$) straight segments.

Contribution of active transport to SF and JM PDs. In the initial series of experiments, the transtubular PD in SF and JM straight tubules was measured at 37°C with a perfusion rate of over 10 nl/min using solution A (Table I) as the perfusate. The bath was isosmolal rabbit serum. The mean transtubular PD of the SF tubules was −2.1±0.9 mV ($n = 55$) whereas the mean PD of the JM tubules was −1.8±0.7 mV (Fig. 2). These results are not statistically different.

To determine the contribution of active transport to the negative PD, the PD was measured under identical circumstances as described above but with either cool-

TABLE II

Net Reabsorption of Fluid Using either Low or High‡ Chloride Solutions as Perfusate*

	Low chloride		High chloride	
	SF	JM	SF	JM
	$n = 15$	$n = 11$	$n = 10$	$n = 7$
Tubule length, *mm*	2.06±0.11	1.38±0.13	2.17±0.20	1.45±0.07
Perfusion rate, *nl/min*	12.72±1.07	13.00±0.82	11.81±0.54	10.92±0.63
Net reabsorption (J_v), *nl/mm/min*	0.47±0.05	0.56±0.06	0.31±0.05	0.24±0.08
	($P > 0.2$)		($P > 0.2$)	

* Low chloride solution, solution A, Table I.

‡ High chloride solution, solution B, Table I.

J_v in SF tubules using solution A vs. solution B as perfusate are different ($P < 0.02$).

J_v in JM tubules using solution A vs. solution B as perfusate are different ($P < 0.01$).

ing or addition of ouabain to the bath. Cooling to 27°C decreased the mean PD towards zero by approximately 1 mV in each case. The addition of 10^{-5} M ouabain to the bath reversibly decreased the PD towards zero (Fig. 3). These data constitute strong evidence that active transport contributes to the negative PD.

Glucose and alanine were removed from the perfusate (solution C, Table I) to determine whether the negative PD was the consequence of active transport of these substances, as it appears to be in the proximal convoluted tubule (21). Removal of glucose and alanine did not significantly alter the PD in either tubule. The PD decreased insignificantly in the SF tubules from −2.1±0.2 to −1.9±0.2 mV ($n = 17$) and in JM tubules from −1.9±0.2 to −1.7±0.2 mV ($n = 9$). However, when ouabain was added to the bath using solution C as the perfusion fluid, the PD again reversibly decreased toward zero (Fig. 3). It is therefore concluded that the negative PD is due to active transport but in a manner independent of glucose and alanine.

It is evident, therefore, that both straight tubules generate the same PD of approximately −2 mV which is presumably, in part at least, the consequence of active transport. Furthermore, the negative orientation of the PD suggested that outward sodium transport was responsible for the observed PD in both the SF and JM straight tubules. This hypothesis was tested in one group of experiments by substituting isosmolal amounts of choline for sodium in both the perfusates and bath

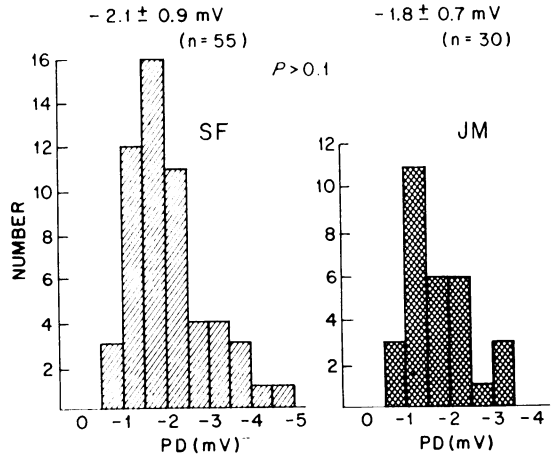

FIGURE 2 Transtubular PD in SF and JM straight segments of proximal tubules. Perfusate, solution A; bath, regular rabbit serum.

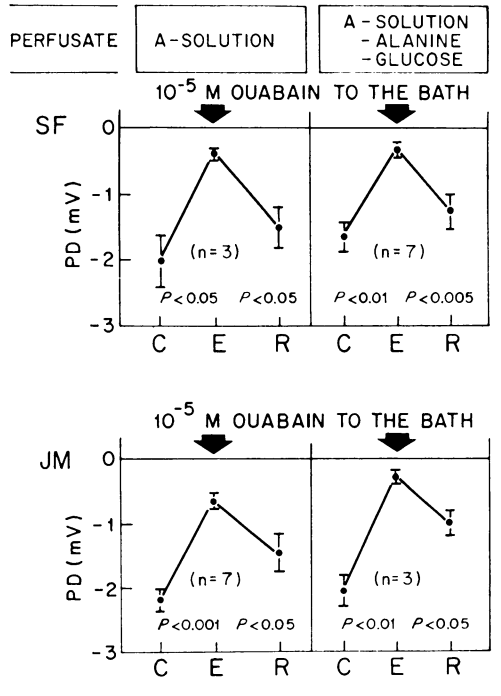

FIGURE 3 The response of transtubular PD in SF and JM tubules to addition of 10^{-5} ouabain to the bath and to removal of glucose and alanine from the perfusate.

TABLE III

Response of Transtubular PD (mV) in SF and JM Straight Segments of Proximal Tubules to Complete Removal of Sodium or Chloride from the Perfusate and Bath

Bath and perfusate	Control (Solution A, Table I)	Choline Cl (Solution D, Table I)	Control (Solution A, Table I)	Na cyclamate (Solution E, Table I)
SF	−1.00±0.14	+0.05±0.06	−1.71±0.36	−4.37±0.56
Proximal straight tubules	(n = 6)		(n = 7)	
	$P < 0.01$		$P < 0.001$	
JM	−2.40±0.32	+0.73±0.09	−1.98±0.40	−2.64±0.40
Proximal straight tubules	(n = 6)		(n = 10)	
	$P < 0.001$		$P < 0.002$	

Statistical significance by Student's paired t test.

(solution D, Table I). As Table III indicates, the PD promptly fell to zero. In the second group of experiments a large presumably nonreabsorbable anion, cyclamate, was substituted for chloride in perfusate and bath while the sodium concentration was kept unchanged (solution E, Table I). Under these circumstances, the transtubular PD became even more negative (Table III).

Contribution of passive transport to the SF and JM PDs. The increase in negative PD when cyclamate is substituted for chloride (Table III) suggests that passive diffusion of chloride reduces the magnitude of the negative PD generated by active outward sodium transport. The contribution of chloride to the PD in straight tubules can be examined when the chloride concentration simulates that which would normally reach the straight tubules (solution B, Table I). Perfusion of SF straight tubules with solution B changes the polarity of the potential from −2.4±0.2 mV (observed when solution A was used) to +1.6±0.3 mV. The left-hand panel of Fig. 4 illustrates the reversible nature of the potentials as the perfusate is changed from A to B and again back to A. The addition of 10^{-5} ouabain when solution B was perfused increased the PD from +1.2 ±0.4 to +2.4±0.4 mV ($P < 0.05$) in a separate series of experiments ($n = 6$). Since the composition of the fluid ordinarily reaching the straight tubule simulates solution B, not solution A, it seems likely that the net in vivo PD in this segment is positive despite the presence of active sodium transport.

In contrast, perfusion of JM tubules by solution B elicited a completely different response. When the perfusate in JM tubules was changed from A to B and back to A solution, the respective changes in PD were only minimal and of questionable significance (n = 15): −1.8±0.2 to −1.3±0.3 ($P < 0.05$) to −1.6±0.3 (NS), paired t test (right-hand panel, Fig. 4). Ouabain (10^{-5} M) changed the PD from −1.4±0.3 to +0.2±0.6 mV in JM tubules in a separate series of studies (not tabulated) using solution B as the perfusate ($n = 4$). This suggests that passive chloride diffusion is trivial in the JM straight tubules. Only when active transport is inhibited by ouabain can a small positive PD (presumably attributed to chloride diffusion) be detected.

Quantitative differences in passive permeability characteristics between SF and JM straight tubules. The PD results, as depicted in Fig. 4 using solution B as perfusate, suggest different passive permeability characteristics between SF and JM tubules. To measure the relative permeability coefficient of chloride to sodium, these two sets of tubules were perfused with solution A (Table I) against an isosmolal bath where the sodium chloride concentration was 50 meq/liter lower (sucrose replacement; solution F, Table I) than the perfusate. The control and recovery baths were regular isosmolal rabbit serum. The results of these experiments are shown in Fig. 5 and indicate that the transtubular PD in the SF tubules becomes positive, +3.4 ±0.3 mV ($n = 33$) when the sodium chloride concentration of the bath was lowered, whereas the PD of

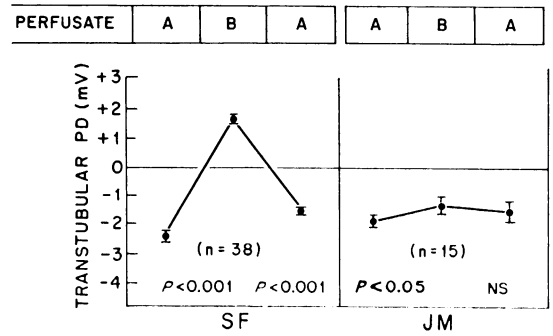

FIGURE 4 The response of transtubular PD of SF and JM straight tubules to changing perfusate from low chloride (solution A, Table I) to high chloride (solution B, Table I) solutions.

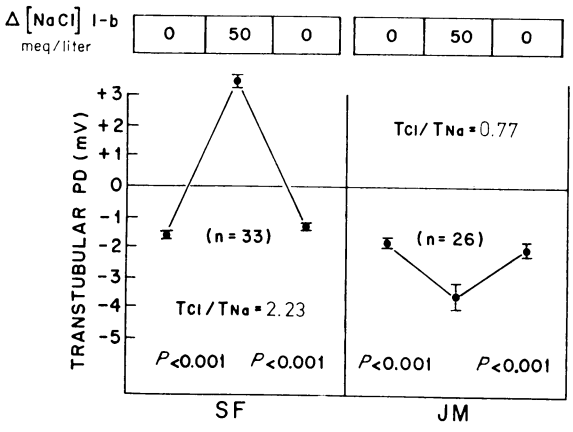

FIGURE 5 The effect of transtubular sodium chloride concentration gradient on transtubular PD of SF and JM straight tubules. NaCl of bath was diluted with isosmolal replacement with sucrose.

the JM tubules under similar conditions became more negative -3.7 ± 0.5 mV ($n=26$). Both of these changes were highly significant ($P<0.001$). The calculated relative permeability of chloride to sodium in the SF tubules was 2.23 and in the JM tubules was 0.77.

The apparent permeability to sodium (P_{Na}) and chloride (P_{Cl}) was also checked isotopically. The results of these studies indicate that the P_{Na} in SF tubules was $2.6\pm0.2\times10^{-5}$ cm/s, while P_{Cl} was $5.6\pm0.4\times10^{-5}$ cm/s ($n=15$). In contrast, the permeability coefficients measured in the JM tubules indicated a much greater permeability to Na than to Cl; $P_{Na}=5.8\pm0.9\times10^{-5}$ cm/s and the $P_{Cl}=2.1\pm0.1\times10^{-5}$ cm/s ($n=11$). Simultaneous electrophysiologically determined relative permeability coefficients were measured in these same tubules. The results of these experiments are shown in Fig. 6 where the relationship of an electrophysiologically determined permeability ratio is plotted against an isotopically determined permeability ratio. These two ratios are identical in the SF tubules. In the JM tubules the chloride to sodium permeability ratio was much lower, whether determined isotopically or electrophysiologically, than in SF tubules (Fig. 6).

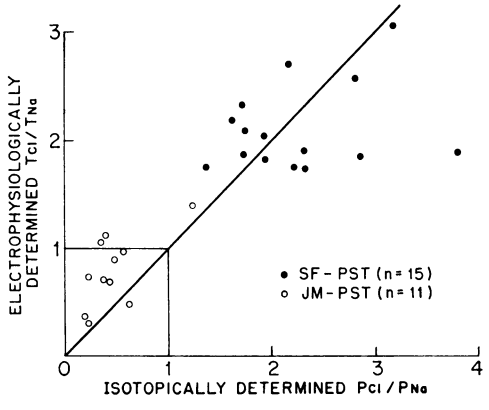

FIGURE 6 The relationship of simultaneously determined electrophysiologically determined transference numbers, T, (y axis) to isotopically determined permeability coefficients, P, (x axis) of chloride to sodium in SF and JM straight tubules. In SF tubules $T_{Cl}/T_{Na}=2.11\pm0.10$ while $P_{Cl}/P_{Na}=2.24\pm0.17$ ($n=15$). In JM $T_{Cl}/T_{Na}=0.80\pm0.10$ and $P_{Cl}/P_{Na}=0.46\pm0.09$ ($n=11$).

In vivo it is not unreasonable to postulate that effective osmolality gradients exist between the luminal fluid and peritubular capillary blood. To examine whether bulk movement of fluid as induced by effective osmotic gradients can influence the magnitude of the PD, these two populations of tubules were perfused with solution A (Table I) in a bath to which 92 mmol/liter sucrose was added. In SF ($n=8$) tubules the PD changed from -1.3 ± 0.2 to -0.3 ± 0.2 mV ($P<0.001$) when sucrose was added to the bath, while the PD in JM tubules became more negative ($P<0.001$), -1.9 ± 0.4 to -3.4 ± 0.9 ($n=7$) when sucrose was added to the bath. These streaming potentials again are consistent with the view that SF tubules are more permeant to chloride than to sodium whereas the JM tubules are more permeant to sodium than to chloride.

DISCUSSION

The present studies disclose that both the SF and JM straight tubules are capable of sodium transport. This is evidenced by the PD of approximately -2 mV when both types of straight tubules were perfused with solution simulating ultrafiltrate of plasma (solution A, Table I; Fig. 2) and by the disappearance of this potential when sodium salts are removed from the perfusate (Table III). The fact that ouabain added to the bath decreases the negative PD towards zero when solution A is perfused (Fig. 3) suggests that sodium transport is mediated by an active process.

At least two possibilities could be advanced to explain the electrogenic sodium transport in the straight tubules. First, it is possible that the active process involves removal of some constituents (such as glucose and amino acids) to which the transport of sodium is somehow coupled. Previous studies from this laboratory have, in fact, demonstrated that at least a fraction of sodium transport is by a coupled mechanism in the early proximal convoluted tubule studied both by in vitro microperfusion of rabbit tubules (21) and by in vivo micropuncture of the rat (23). Second, it is possible that sodium transport is accomplished by an active uncoupled ("direct") transport process that generates a PD (electrogenic process but a process in which the magnitude of the "pump coupling ratio" has not been measured). The demonstration that the perfusion of

straight tubules with a solution that simulates ultrafiltrate but from which glucose and amino acids have been removed (solution C, Table I) still results in the generation of negative PD of approximately 2 mV indicates that sodium reabsorption is not coupled to glucose and amino acid reabsorption.

This electrogenic sodium transport system in the straight tubules differs sharply from that identified in the proximal convoluted tubule (21). Previous studies from this laboratory have suggested the existence of at least two types of active sodium transport in the convoluted segment. A fraction of sodium reabsorption appears to be coupled to hydrogen secretion, thereby mediating sodium bicarbonate reabsorption (24). We have not examined sodium bicarbonate transport in the straight tubules. A second mechanism involves the reabsorption of sodium coupled to active transport of glucose and amino acid (21). By contrast, the straight tubule has a direct electrogenic sodium transport system without coupling to glucose and amino acids, a system which has not been demonstrated to exist in the proximal convoluted tubule where perfusion with chloride-free fluid (chloride substituted by methyl sulfate) did not generate a negative PD (21). When the straight tubules were perfused with a solution containing sodium cyclamate (solution E, Table I) instead of sodium chloride, the transtubular PD became even more negative (Table III). This result would be expected if active sodium transport were generating a negative PD which could not be shunted by passive outward diffusion owing to the nonreabsorbable nature of cyclamate. We therefore conclude that, at least with respect to active sodium transport, both straight tubules differ from the proximal convolution.

Perfusion of the straight tubules with a solution simulating ultrafiltrate of plasma permits examination of the transport properties when the straight tubules are presented with a fluid of identical composition to that entering the proximal convoluted tubules. This solution is used to compare the transport characteristics of the convoluted and straight tubules when these segments are presented with identical substrates. Under normal circumstances, however, the fluid reaching the straight tubules has a very low concentration of bicarbonate and a commensurately high concentration of chloride. Solution B (Table I) simulates the composition of the fluid which ordinarily reaches the proximal straight tubules. When solution B was perfused through the SF and JM straight tubules, markedly different responses were noted from those elicited when solution A was perfused. The SF tubules changed their polarity from -2.4 mV during perfusion with solution A to $+1.6$ mV during perfusion with solution B (Fig. 4). By contrast, in JM tubules there was no significant difference in the transtubular potential when solution A or B was perfused (Fig. 4).

These findings suggest that whereas both populations of straight tubules are similar with respect to sodium transport, the SF tubules are far more permeable to chloride than are the JM tubules. Two lines of evidence support the conclusion that passive chloride movement is responsible for the positive PD during perfusion with solution B in the SF straight tubules. First, in the group of experiments in which a large presumed nonreabsorbable anion cyclamate was substituted for chloride in both perfusate and bath, the transtubular PD became even more negative as compared with the control experiments in which chloride rather than cyclamate was present (Table III). This indicates that chloride is more permeant than cyclamate and that chloride has the capacity to shunt part of the transtubular PD generated by the active electrogenic sodium pump. Second, when ouabain was added to the bath of SF tubules using solution B as perfusate, the transtubular PD became even more positive, which again is consistent with the view that part of the generated PDs can be shunted by chloride (Results). However, other electrolytes, which we have not examined, might also contribute to the magnitude of the transtubular PD. It is, therefore, concluded that despite the similar types of active sodium transport in both sets of straight tubules, the greater intrinsic passive permeability to chloride in the SF than in JM tubules is responsible for the positive potential.

The quantitative aspects of the permeability differences was examined both electrophysiologically (Fig. 6) and isotopically (Fig. 6). Both groups of studies indicated that chloride is approximately twice as permeable as sodium in the SF tubules whereas sodium is approximately twice as permeable as chloride in the JM tubules. Thus, electrophysiologically determined transference numbers ($T_{Cl}/T_{Na} = 2.23$ in SF tubules, $T_{Cl}/T_{Na} = 0.77$ in JM tubules), isotopically determined permeability coefficients ($P_{Cl}/P_{Na} = 2.24$ in SF tubules, $P_{Cl}/P_{Na} = 0.46$ in JM tubules), and the streaming potentials in response to osmotic gradients all indicate that chloride is more permeable than sodium across SF straight segments while sodium is more permeable than chloride across JM straight segments.

These studies, therefore, indicate that both populations of straight tubules differ from the convoluted segments with respect to sodium transport mechanisms and differ from each other with respect to the relative sodium to chloride permeabilities.

While there is little difficulty in distinguishing the straight tubules from proximal convoluted tubules, it is impossible after dissection to establish the identity of a straight SF tubule as part of either a SF or JM

nephron. In the absence, therefore, of morphological criteria to distinguish these straight segments, it might be argued that the results somehow indicate that the differences are artifactual. Actually the tubules were distinguished by their anatomical location and connections before the actual dissection. Two criteria were used to identify the origin of the straight tubules. First, the straight segment of the SF tubules originated at the surface of the kidney whereas the straight segment of the JM tubules originated near the corticomedullary junction. If the straight segment was connected to a convoluted segment when the horizontally coursing convolutions began near the corticomedullary junction, then this segment was judged to be a JM tubule. Second, JM straight tubules were connected to loops that penetrated deep into the medulla whereas the loops of the SF tubules were much shorter and ended in the outer part of the medulla. These two criteria served to identify SF and JM tubules. In 18 consecutive experiments the origin of tubules (dissected by one investigator) prepared for study was not disclosed (to the perfuser) until the experiment was completed. In all instances, the perfuser, after obtaining his results, was able to identify and assign the tubule correctly either to the SF or JM group. The homogeneity of results lends support to the view that there are two groups of tubules with different intrinsic properties rather than a random distribution of a single population.

The demonstration of sharp differences of SF and JM straight tubules represents the first demonstration that there can be intrinsic differences in transport properties of the same segment of tubules derived from different nephron populations. This finding has great physiological significance, since the principal segment of the proximal tubule accessible to micropuncture has been the surface portion. The present findings raise serious question as to whether the reabsorptive properties derived from the experiments on SF nephrons alone can be extrapolated to the kidney as a whole. Clearly, studies are required to characterize the transport properties of the JM as well as SF convoluted portion, in a manner similar to the present studies in straight tubules, to ascertain whether the proximal convolution has homogeneous or different transport properties in different nephron population.

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