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Harry R. Jacobson, Howard L. Baer Jr.

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Research Article

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HARRY R. JACOBSON with the technical assistance of HOWARD L. BAER, JR.,
Microperfusion Laboratory, U. S. Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

ABSTRACT Segments of superficial and juxtamedullary proximal convoluted tubules of the rabbit were perfused in vitro to examine the mechanisms responsible for net volume reabsorption. The very early postglomerular segments were not studied. Fluid reabsorptive rates and transepithelial potential differences were compared under various conditions: (*a*) with perfusate that simulated glomerular filtrate; (*b*) with perfusate that lacked glucose, amino acids, and acetate and that had HCO_3 and Cl concentrations of 5 and 140 mM, respectively; (*c*) with perfusate that lacked glucose, amino acids, and acetate but with 20 meq of $NaHCO_3$ replaced with 20 meq of Na cyclamate; (*d*) with the same perfusate as in *b* but in the presence of ouabain in the bath; (*e*) with ultrafiltrate of rabbit serum titrated with HCl to final HCO_3 and Cl concentrations of 2 and 134 mM, respectively. Tubules were perfused with this titrated ultrafiltrate at 37°C, 21°C, and in the presence of 0.1 mM ouabain in the bath. Bath fluid in all experiments was regular rabbit serum. Under conditions *a* and *b* superficial proximal convoluted tubule (SFPCT) and juxtamedullary proximal convoluted tubule (JMPCT) behaved similarly with the exception that SFPCT exhibited a lumen-positive and JMPCT a lumen-negative electrical potential under condition *b*. However, under condition *c* SFPCT failed to exhibit net volume reabsorption, whereas reabsorption in JMPCT continued unchanged. Ouabain did not affect volume reabsorption in SFPCT under condition *d*, whereas neither ouabain nor hypothermia affected SFPCT under condition *e*. In contrast, ouabain and hypothermia totally inhibited volume reabsorption in JMPCT under conditions *d* and *e*. These studies document heterogeneous mechanisms responsible for volume reabsorption in the major portions of SFPCT and JMPCT with passive forces predominating in SFPCT and active forces in JMPCT.

INTRODUCTION

As evidenced by two recent reviews (1, 2) the subject of structural and functional heterogeneity of nephrons has become a major interest of many investigators of renal physiology. Stimulated by earlier studies documenting anatomical differences between nephrons originating in the superficial and deep cortex (3–5), recent studies have been aimed at determining whether functional counterparts of these anatomic differences exist (6–10). In a previous in vitro microperfusion study (7) significant intrinsic functional differences were found to exist both between proximal convoluted tubule segments from superficial and juxtamedullary nephrons and between early and later segments of superficial convolutions. Electrophysiologically determined relative permeabilities to Na and Cl revealed that the early superficial proximal convoluted tubule (SFPCT)[1] was more permeable to Na than Cl, whereas the reverse was true for later portions of the SFPCT. In contrast, the juxtamedullary proximal convoluted tubule (JMPCT) exhibited a greater relative Na permeability throughout its entire length. Whether these differences in relative ion permeability are associated with, or responsible for, differences in volume reabsorption in these various

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[1] *Abbreviations used in this paper*: JMPCT, juxtamedullary proximal convoluted tubule(s); J_v, net fluid reabsorption; PD, potential difference(s); SFPCT, superficial proximal convoluted tubule(s).

nephron segments is unknown. The present studies were designed to determine whether SFPCT and JMPCT display heterogeneity with respect to volume reabsorptive characteristics.

METHODS

Segments of rabbit proximal convolutions were perfused in vitro by methods originally described by Burg et al. (11). Briefly, female New Zealand white rabbits that weighed between 1.5 and 2.5 kg were sacrificed by decapitation. After sacrifice a kidney was quickly removed and cut into 1–2 mm thick coronal slices. One of these slices was placed in a chilled Petri dish that contained rabbit serum (type 2-UC rabbit serum from Pel-Freeze Biologicals Inc., Rogers, Ark.) and kept at pH 7.4 by continuous bubbling with a 95% O_2 and 5% CO_2 gas mixture. Proximal tubule segments were dissected free hand with the aid of a dissecting microscope. The anatomic origin of the tubule segments studied is depicted in Fig. 1. The very early portion, ≅1 mm, of both SFPCT and JMPCT was avoided because of their previously demonstrated electrophysiological similarities (7). Segments were designated as superficial only if a portion of their length reached the immediate subcapsular region of the cortex, whereas segments were designated as juxtamedullary only if they originated from those convolutions closest to the corticomedullary junction. After dissection, tubule segments were transferred to a thermostatically controlled perfusion chamber that held a volume of ≅1 ml and that contained several ports to permit continuous bubbling with 95% O_2, 5% CO_2, and continuous exchange of bath fluid (at 0.5 cm^3 min in these experiments). In all studies the original bathing fluid was rabbit serum. The two ends of a tubule segment were sucked into holding pipettes and sealed into place by an elastomeric silicone resin, Sylgard 184 (Dow Corning Corp., Midland, Mich.), which had been placed into the tips of the holding pipettes. The perfusion pipette, which had been centered in one of the holding pipettes, was advanced into the tubule lumen ≅100 μm. This pipette also served as intraluminal electrode because it was connected, via a Ringer's bicarbonate-agarose bridge, to a calomel electrode. To complete the electrical circuit and allow for measurement of transtubular potential differences (PD), the bath fluid in the perfusion chamber was also connected to a calomel electrode via an identical agarose bridge. The circuit used to measure PD has been diagramed previously (7). Perfusate that had coursed through the tubule was collected under oil in the second holding pipette and sampled at timed intervals by a constant volume pipette. Perfusion rate was regulated by adjusting the height of a water column attached to the rear of the perfusion pipette.

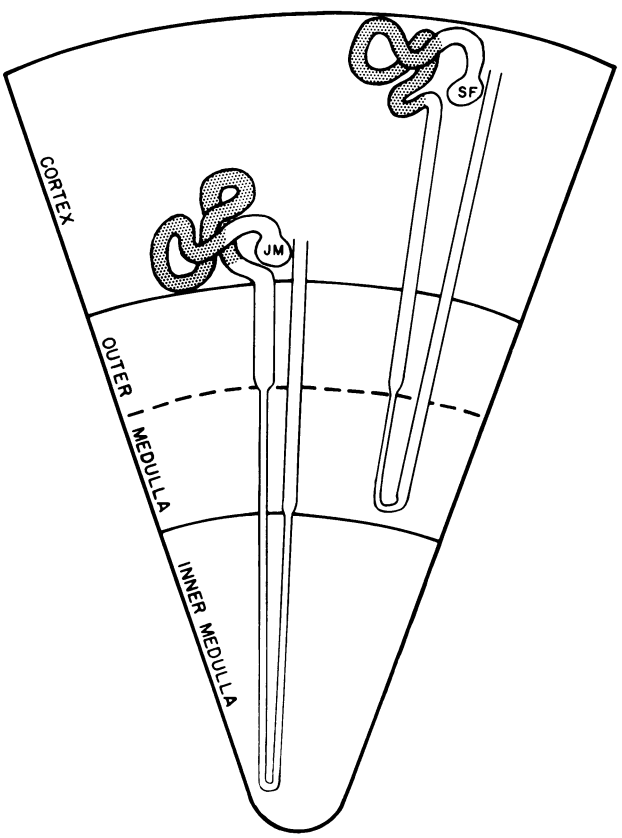

FIGURE 1 This figure is a schematic representation of the anatomic origin of the tubule segments examined in the present studies. Those portions of the superficial (SF) and juxtamedullary (JM) proximal convolutions that are stippled were studied.

Depending on the experimental protocol, tubule segments were perfused with one or more perfusates. Before any tubule was studied, it was perfused for 30 min at 37°C with the initial perfusate used in that protocol. After this equilibration period the bath was transiently changed to a solution that allowed imposition of a 50-meq NaCl gradient across the tubule (Table I). The polarity of the diffusion potential generated by this maneuver was used to verify if the tubule segment had greater relative Na or Cl permeability (7). All superficial segments in these studies exhibited greater relative Cl and all juxtamedullary greater relative Na permeability. In those studies where the effects of more than one perfusate were determined in a single tubule, perfusate was changed by entering the rear of the perfusion pipette with PE-10 tubing, which was advanced as far as possible towards the tip of the perfusion pipette. The original perfusate was then flushed out with an air bubble and replaced with new perfusate. This process was repeated at least three times. Isotope recovery studies without a tubule in place verified that this technique allows for complete change of perfusate.

The four perfusates used in these studies are listed in Table I and were designed as follows. Solution A was designed to simulate an ultrafiltrate of rabbit plasma. Solution B was designed to simulate proximal tubule fluid as it exists in vivo beyond the very early convolutions. Glucose and amino acids were replaced with NaCl, Na acetate was replaced by Na cyclamate, and 20 meq of $NaHCO_3$ was replaced with 20 meq NaCl. Tubules perfused with B solution were thus exposed to a lumen-to-bath Cl gradient and bath-to-lumen HCO_3 gradient. In addition, if the reflection coefficient for NaCl is less than those for glucose, amino acids, and $NaHCO_3$, an effective osmotic pressure gradient existed across tubules perfused with B solution. As will be discussed later, both the Cl gradient and effective osmotic gradient have been proposed as passive driving forces for volume reabsorption (12–17).[2] Solution C was designed to omit those factors that are currently

[2] Hierholzer, K., H. R. Jacobson, S. Kawamura, D. W. Seldin, and J. P. Kokko. Reflection coefficients of various substrates across superficial and juxtamedullary proximal convoluted segments of rabbit nephrons. Submitted for publication.

TABLE I

Composition of Solutions Used in Present Studies

	Perfusates				Baths	
	A	B	C	†Cl ultrafiltrate	Rabbit serum	A-50
				mM		
Na	145	149	143	144	144	95
K	5	5	5	5.1	5.1	5
Cl	112	140	112	134	108	62
HCO_3	25	5	5	2	22	25
Ca	1.8	1.8	1.8	1.6	2.8	1.8
PO_4	2.3	1.2	1.2	1.9	2.3	2.3
Mg	1.0	1.0	1.0	*		1.0
SO_4	1.0	1.0	1.0			1.0
Acetate	10	—‡	—			10
Cyclamate	—	10	30			—
Glucose	8	—	—	7	7	8
Alanine	5	—	—			5
Raffinose	—	—	13			90
Creatinine, *mg/100 ml*	—	—	—	1.1	1.2	—
Urea nitrogen, *mg/100 ml*	—	—	—	22	22	—

Osmolality of all solutions 298–302.

* Blank space means not measured.

‡ —, not present.

proposed to support volume reabsorption. Glucose and alanine were replaced by the nonreabsorbable solute raffinose. Na acetate was replaced by Na cyclamate and 20 meq of $NaHCO_3$ were replaced with 20 meq of Na cyclamate. The fourth perfusate used in these studies was made from an ultrafiltrate of the same rabbit serum used as the bathing fluid. Ultrafiltrate was prepared by pressure dialysis of rabbit serum through Aminco PM-30 membranes (American Instrument Co., Silver Spring, Md.) and then titrated with HCl to a final HCO_3 concentration of 2 mM (Table I).

Four sets of experiments were performed. First, because a major part of these studies deals with comparison of SFPCT and JMPCT exposed to lumen-to-bath Cl gradients, it was necessary to document that a Cl gradient could be generated by JMPCT. Thus, a group of JMPCT was perfused with an unadulterated ultrafiltrate of rabbit serum and bathed in rabbit serum. Perfused and collected fluid Cl concentrations were determined by the micromethod of Ramsay et al. (18).

In the second group of studies segments of SFPCT and JMPCT were perfused sequentially and in random order with solutions A, B, and C. Although transepithelial PD and net volume reabsorption were measured, it should be noted that the transepithelial potentials reported in this paper are the observed potentials corrected, when appropriate, for liquid junction potentials. As recently pointed out by Schafer et al. (19) a significant transepithelial Donnan voltage exists across in vitro perfused tubules when the bathing medium contains protein. This Donnan voltage may be counterbalanced by a liquid junction potential between the bath fluid and bath electrode. If this is the case, the Donnan voltage is not recorded in the observed potential. The observed potential thus differs from the actual transepithelial potential by the magnitude of the Donnan voltage. For the purposes of this paper, because we are interested only in comparing SFPCT and JMPCT with various perfusates, observed potentials are quite sufficient. However, the observed potentials with B solution and titrated ultrafiltrate as perfusate were corrected by a liquid junction potential of +1 mV as previously measured (7). Volume reabsorption was measured with [*methoxy*-^{3}H]inulin, exhaustively dialyzed by the method of Schafer et al. (20), and added to the perfusates to a final activity of 50–75 μCi/ml as volume marker. Collected fluid was sampled at least three times with each perfusate. In addition, 15 min elapsed after each perfusate change before new collections were taken.

In the third group of studies tubules were perfused with solution B and bathed in rabbit serum. After at least three control collection periods, ouabain was added to the bath to a final concentration of 0.1 or 0.01 mM. These studies were performed to determine the active and passive components of volume reabsorption with solution B as perfusate. It was assumed that ouabain inhibited all active transport and did not significantly affect any other epithelial characteristics.

In the final group of studies tubules were perfused with an ultrafiltrate of rabbit serum which had been titrated with HCl such that the perfusate HCO_3 concentration was 20 meq/liter less than the bath and the perfusate Cl concentration 26 meq/liter higher than the bath. After three control collections with regular rabbit serum in the bath (at 37°C), the bath was either changed to one that contained ouabain at 0.1 mM or cooled to 21°C. The order of these maneuvers, i.e., ouabain or cooling first, was randomized. Cooling to 21°C was accomplished by continuous bath exchange at from 0.5 to 0.6 cm^3/min with precooled rabbit serum. At least two determinations of net volume reabsorption were made in each tubule at 21°C and during exposure to 0.1 mM ouabain. In all studies the bath was changed to regular rabbit serum at 37°C between the periods of hypothermia and exposure to ouabain. The rationale behind the last group of experiments was two-

fold. First, the use of titrated ultrafiltrate avoids interpretive problems that may be confronted when artificial solutions with varying ion and solute substitutions are used. For example, substitution of Na cyclamate for Na acetate and $NaHCO_3$ in solutions B and C was made under the assumption that cyclamate behaves as a nonreabsorbable anion and that it neither supports net sodium transport nor induces any change in other epithelial transport characteristics. In addition, it was assumed that the reflection coefficient for Na cyclamate was not significantly different from Na acetate and $NaHCO_3$. In the micropuncture studies of Neumann and Rector (16) where cyclamate was used in similar fashion, the same assumptions were made. That these assumptions may be valid is supported by their data showing that substitution of two other anions, sulfate and methylsulfate, gave similar results to cyclamate. However, the use of titrated ultrafiltrate combined with active transport inhibition, permits one to look at the effects of the oppositely poised Cl and HCO_3 gradients without concern for possible artifacts induced by ion substitutions. The second rationale for these studies was to use two independent methods of active transport inhibition in the same tubule, hypothermia and ouabain. Similar results with both methods permit one to conclude more strongly that the observations are related to inhibition of active transport and not some nonspecific effect of the method used.

Calculations. Net fluid reabsorption (J_v) was calculated as described previously (22). J_v in nanoliters per millimeter per minute = $V_i - V_o/L$, where V_i = perfusion rate in nanoliters per minute, V_o = collection rate in nanoliters per minute, and L = tubule length in millimeters. V_o was measured directly and V_i calculated from $V_i = V_o$ cpm_o/cpm_i, where cpm_o = ^3H counts per minute per nanoliter of collected fluid and cpm_i = ^3H counts per minute per nanoliter of perfused fluid. Collected fluid was counted to at least 0.7% accuracy (at least 50 min) in a Beckman LS-250 Liquid Scintillation Counter (Beckman Instruments Inc., Fullerton, Calif.). [^3H]Inulin counts were always >30 times background. Tubule length was measured at the end of each experiment via an eyepiece micrometer. In those experiments with hypothermia, the constant volume pipette used to collect all samples was calibrated at both 37°C and 21°C by filling it at least three times at each temperature with perfusate that contained ^3H at a known specific activity. The calibration volume at 21°C was slightly larger (by a mean of 0.5%) presumably because of differences in the density of water at 21°C and 27°C. In the calculation of volume reabsorption at 21°C the calibration volume at 21°C was used.

Statistical analysis. Because evaluation of each of the three major studies presented here requires multiple, nonindependent comparison of group means, analysis was performed by the method of Scheffe (22). This technique, which permits grouping of common means, is stringent but affords excellent protection against type 1 error. The null hypothesis for all comparisons was rejected at the 5% significance level.

Individual means were assessed as differing from zero when their 95% confidence intervals failed to include zero.

RESULTS

Collected fluid chloride in JMPCT. Five JMPCT were perfused with ultrafiltrate and bathed in rabbit serum. Samples of perfused and collected fluid were analyzed for Cl concentration. Table II shows the results of these experiments. With a mean perfusion rate of 10.8 nl/min and a mean tubule length of 1.16 mm, the tubular fluid chloride concentration increased by a mean±SEM of 14.5±1.5 meq/liter. Bath chloride in these experiments was 108 meq/liter. These studies indicate that the JMPCT, like the SFPCT, is capable of generating a lumen-to-peritubular chloride gradient. It should be remembered that the purpose of these experiments was only to validate the use of high Cl perfusate in later segments of JMPCT and not to compare quantitatively the ability of SFPCT and JMPCT to establish Cl gradients. It is conceivable that in vivo the magnitude of the lumen to peritubular Cl gradient may differ in SFPCT and JMPCT.

Perfusion with A, B, and C solutions (Table III). When perfused with solution A, which simulates glomerular filtrate, SFPCT reabsorbed 0.98±0.06 nl/mm per min, whereas JMPCT reabsorbed 1.14±0.06 nl/mm per min ($0.10 > P > 0.05$). Transepithelial PD differed significantly between SFPCT and JMPCT, −4.1±0.3 mV in SFPCT vs. −6.3±0.9 mV in JMPCT. Perfusion rates were not different, 14.6±0.9 nl/min in SFPCT and 16.3±0.9 nl/min in JMPCT.

When perfused with B solution, high Cl, low HCO_3 solution, both SFPCT and JMPCT displayed similar reabsorptive rates of 0.56±0.05 nl/mm and 0.68±0.04 nl/mm per min, respectively ($0.10 > P > 0.05$). Transepithelial PD were significantly different. The Cl permselective superficial segments all developed lumen positive potentials ranging between +2.0 and +3.5 mV with a mean±SEM of +2.6±0.2 mV. In contrast, the Na permselective juxtamedullary segments

TABLE II

Comparison of Perfused and Collected Cl Concentrations in JMPCT

Experiment	Perfusion rate	Tubule length	Perfused (Cl)	Collected (Cl)	Δ (Cl)
	nl/min	*mm*		*meq/liter*	
1	10.5	0.9	113.9	125.9	12.0
2	11.0	0.9	105.5	119.7	14.2
3	7.5	1.4	108.3	123.5	15.2
4	13.0	1.3	105.2	124.8	19.6
5	12.2	1.3	109.7	121.1	11.4
Mean±SEM	10.9±0.9	1.16±0.1	108.5±1.6	123.0±1.1	14.5±1.5

TABLE III
Comparison of J_v and PD in SFPCT and JMPCT

Perfusate	Superficial PCT		Juxtamedullary PCT	
	J_v	PD	J_v	PD
	nl/mm/min	*mV*	*nl/mm/min*	*mV*
A Artificial ultrafiltrate	0.97±0.06*	−4.1±0.3‡	1.14±0.06	−6.3±0.9
B High Cl, low HCO_3 (glucose, alanine free)	0.56±0.05	+2.6±0.2‡	0.68±0.04	−0.6±0.2
C Normal Cl, low HCO_3 (glucose, alanine free)	0.04±0.03‡	−0.7±0.04	0.53±0.08	−0.8±0.2
B High Cl, low HCO_3 (glucose, alanine free)				
Control	0.47±0.04‡	+2.2±0.5‡	0.49±0.05	−1.9±0.1
Ouabain	0.30±0.01	+2.3±0.5	−0.15±0.07	−0.7±0.2
Titrated ultrafiltrate				
Control	1.51±0.10‡	−4.4±0.4	0.59±0.03	−5.6±0.9
Ouabain	0.34±0.03‡	+2.8±0.4‡	−0.05±0.01	−0.3±0.2
Hypothermia	0.37±0.04‡	+2.7±0.4‡	−0.04±0.02	−0.3±0.2

PCT, proximal convoluted tubules.
* All values of J_v and PD are expressed with SEM.
‡ SFPCT significantly different from JMPCT at least at the 5% level.

displayed lumen-negative potentials ranging from 0 to −2.0 mV (one segment had a PD of +0.6 mV) with a mean±SEM of −0.6±0.2 mV. These electrical findings are consistent with previous in vivo and in vitro studies of later SFPCT perfused with either naturally occurring later proximal tubule fluid or solutions simulating later proximal tubule fluid (13, 23–25) and with a previous in vitro study of JMPCT (7). Of interest is the finding that in spite of the differing electrical charge orientation of the lumen PD, fluid reabsorption was similar in SFPCT and JMPCT.

When C solution, low HCO_3, normal Cl, was used as perfusate, further differences between SFPCT and JMPCT were discovered. Whereas transepithelial PD were similar, −0.7±0.04 mV in SFPCT and −0.8±0.2 mV in JMPCT, fluid reabsorptive rates were markedly different. The SFPCT reabsorbed 0.04±0.03 nl/mm per min, a volume not significantly different from zero, whereas JMPCT reabsorbed 0.53±0.08 nl/mm per min. Thus, when perfused with a solution designed to eliminate factors favoring both active organic solute and HCO_3-linked transport and passive transport linked to transtubular Cl, HCO_3, and organic solute gradients, SFPCT ceased net reabsorption, whereas JMPCT continued to reabsorb at a substantial rate not significantly different than during perfusion with B solution. The findings in the SFPCT are quite similar to those of Neumann and Rector (16) who perfused the in vivo superficial convolution of the rat with a perfusate similar to solution C.

Perfusion with B solution and active transport inhibition by ouabain (Table III). In this set of studies tubule segments were perfused only with solution B (high Cl, low HCO_3). During the control periods regular rabbit serum was used as bath fluid. SFPCT again exhibited a lumen-positive potential of +2.2±0.5 mV, similar to the previous group of superficial tubules. The JMPCT exhibited a lumen-negative potential of −1.9±0.1 mV, which was slightly more negative than that seen in the previous juxtamedullary tubules. Although slightly lower than the rates seen in the previous groups of tubules, fluid reabsorptive rates were similar in both SFPCT and JMPCT, 0.47±0.04 nl/mm per min vs. 0.49±0.05 nl/mm per min, respectively. When the bath contained 0.1 or 0.01 mM ouabain (there were no differences between the two concentrations), the lumen-positive potential difference in SFPCT remained unchanged at +2.3±0.5 mV, whereas the lumen-negative potential in JMPCT decreased to −0.7±0.2 mV ($0.10 > P > 0.05$). Fluid reabsorption in the SFPCT decreased slightly but statistically insignificantly to 0.30±0.01 nl/mm per min whereas it decreased dramatically in JMPCT to −0.15±0.07, a value not significantly different than zero. This marked difference in the fluid reabsorptive response to ouabain between SFPCT and JMPCT constitutes strong evidence for major differences in the mechanisms responsible for volume reabsorption in these segments. Volume reabsorption in the SFPCT, except for its very early portion which was not studied here, when perfused with a solution mimicking in vivo conditions, is ouabain insensitive. In contrast, volume reabsorption in the corresponding region of the JMPCT, when perfused under identical conditions, is completely ouabain sensitive.

Perfusion with titrated ultrafiltrate in addition to active transport inhibition by hypothermia and ouabain (Table III). When segments of SFPCT were perfused with an ultrafiltrate of rabbit serum that had been titrated with HCl to a final Cl concentration of

134 meq/liter and a final HCO_3 concentration of 2 meq/liter, J_v was 1.51±0.10 nl/mm per min, whereas transepithelial potential difference was −4.4±0.4 mV. In contrast, J_v in the JMPCT was much lower with this perfusate, 0.59±0.03 nl/mm per min, whereas the PD was slightly but not significantly higher at −5.6±0.9 mV.

When SFPCT were exposed to a bath temperature of 21°C (shown previously by Schafer et al. (17) to inhibit active volume reabsorption in pars recta) the transepithelial PD changed significantly from −4.4 mV lumen negative to +2.7±0.4 mV lumen positive. Net volume reabsorption decreased to 0.37±0.04 nl/mm per min. When these same segments were exposed to ouabain the resultant potentials and fluid reabsorptive rates were not significantly different than the values observed with hypothermia. The PD was +2.8±0.4 mV and net volume reabsorption was 0.34±0.03 nl/mm per min. These reabsorptive rates are identical to those seen in SFPCT with the artificial, high Cl, low HCO_3 perfusate and ouabain.

The responses of the JMPCT to hypothermia and ouabain differed significantly from those of the SFPCT. With hypothermia and PD decreased to −0.3±0.2 mV and J_v decreased to −0.04±0.02 nl/mm per min (both values not significantly different from zero). Transepithelial potential and fluid reabsorption with ouabain in the bath were not significantly different than during hypothermia, −0.3±0.2 mV and −0.05±0.01 nl/mm, respectively.

The results of this last group of studies with titrated ultrafiltrate are significant for several reasons. First, the marked difference in volume reabsorption in SFPCT and JMPCT under control conditions, high Cl, low HCO_3 perfusate and rabbit serum as bath, demonstrates that volume reabsorption in the JMPCT is more sensitive to the removal of HCO_3. Second, when active transport was inhibited by either of two means, SFPCT continued to exhibit significant volume reabsorption in the presence of a lumen-positive transepithelial PD, whereas JMPCT ceased to reabsorb fluid and exhibited a PD not different from zero. The difference in volume reabsorption and potentials between SFPCT and JMPCT in the presence of active transport inhibition must be a result of a difference in the response to luminal fluid constituents. Finally, the fact that two different methods of active transport inhibition yielded identical results lends further support to the differences observed between the two populations of proximal tubules.

DISCUSSION

The mechanisms by which the proximal tubule reabsorbs salt and water are still a matter of dispute. One of the major areas of disagreement is centered around the quantitative significance of active vs. passive transport. Some authors report that the major portion of volume reabsorption in the proximal tubule is accomplished by passive mechanisms (12–17),[2] whereas others contend that a minor or insignificant amount is caused by passive driving forces (26, 27). It must be made clear that the controversy does not involve the total proximal tubule. Indeed, as will become evident, it is probably no longer appropriate to discuss the proximal tubule as if it was a functionally homogeneous segment of the nephron. However, there is general agreement over certain observations on transport in the very early postglomerular segment of the superficial proximal convolution (≅ the first 15–20% of the convolution). Filtered glucose (28–32) and amino acids (33–37) are mostly reabsorbed in the early segment. In addition there is early rapid HCO_3 reabsorption (38, 39) resulting in a reciprocal rise of the intraluminal Cl concentration. Thus, the remaining and major portion of the superficial convolution is exposed to luminal fluid with a composition different from an ultrafiltrate of plasma, a composition similar to solution B of the present studies.

Those investigators favoring a major role for passive driving forces in proximal volume reabsorption (12–17)[2] argue that the early proximal convolution, by actively altering the luminal fluid composition, establishes two important passive driving forces that may be responsible for volume reabsorption in the more distal regions of the proximal tubule. First, the lumen-to-peritubule Cl gradient generates a lumen-positive PD, which could serve as a driving force for Na movement out of the lumen. Second, if the reflection coefficient for NaCl is less than $NaHCO_3$, glucose, and amino acids, an effective osmotic pressure difference would exist across the tubule. The luminal fluid, which consists mostly of NaCl, would have a lower effective osmotic pressure than peritubular fluid. Water would thus flow from lumen-to-peritubular fluid and via solvent drag produce NaCl movement in the same direction.

However, because the JMPCT is not directly accessible to in vivo study, it is not certain that luminal fluid undergoes compositional changes in the early postglomerular segment identical to those seen in the SFPCT. Although, there is no available data quantitating glucose and amino acid reabsorption in early JMPCT, there is electrophysiological evidence showing that the early JMPCT and early SFPCT exhibit the same PD response to the removal of glucose and alanine from the perfusate (7). With respect to whether or not a lumen to perituble Cl gradient is generated in the JMPCT, the first group of studies reported on here (Table II) clearly document that such a gradient does exist. Thus, it is felt to be valid to compare SFPCT and JMPCT (beyond their most early portions) perfused with solutions that lack glucose and amino

acids and that have high Cl and reciprocally low HCO_3 concentrations.

The results of the present studies are felt to be an accurate quantitation of the role of active and passive transport in proximal convoluted tubule volume reabsorption for three major reasons. First, the anatomic origin of the tubule segments studied was identified. Thus the issue of heterogeneity could be addressed. Second, certain solutions used as perfusate were designed to mimic in vivo conditions (specifically with respect to anion gradients). Finally, several methods were used to examine the contributions of active and passive transport: solute substitutions, ouabain, and hypothermia. Agreement of the results obtained with each of these methods is strong evidence for the validity of the conclusions derived.

First, in the studies with perfusates of varying compositions (A, B, and C solutions) it is clear that heterogeneity exists between SFPCT and JMPCT. Although reabsorptive rates with solution A (artificial ultrafiltrate) and B (high Cl, low HCO_3) are similar in both populations of tubules, perfusion with solution C (low HCO_3, normal Cl, absent organic solutes) demonstrates significant heterogeneity. Volume reabsorption in the SFPCT ceases in the absence of a lumen-to-bath Cl gradient and luminal organic solutes, whereas volume reabsorption in the JMPCT is unaffected.

Second, the volume reabsorptive rates with B solution (high Cl, low HCO_3) as perfusate in the presence and absence of ouabain confirm the results seen with the multiple perfusate experiments. Ouabain caused a significant reduction in volume reabsorption only in JMPCT indicating that the theoretically favorable passive driving forces do not play a major role in this segment. Although there are certain significant differences in methodology, these results are consistent with the volume reabsorptive data of Cardinal et al. (27) who perfused randomly dissected segments of proximal convolutions. In contrast, the finding of significant volume reabsorption under these conditions in SFPCT conflict with the results of Cardinal et al (27). Although there is no data available to make a definite statement, the differing results of the two studies can be explained if (*a*) only the most superficial proximal convolutions exhibit significant passive transport and (*b*) Cardinal et al. studied tubules dissected largely from below the most superficial cortical layer.[3]

Third, the findings in SFPCT and JMPCT perfused with titrated ultrafiltrate and exposed to hypothermia and ouabain are entirely consistent with the results with artificial perfusates. The persistence of significant volume reabsorption in SFPCT despite two independent methods of inhibiting active transport and the absence of volume reabsorption in JMPCT under similar conditions document that a lumen-to-bath Cl gradient and the reverse gradient for HCO_3 play an important role in SFPCT volume reabsorption but a negligible role in JMPCT. It should be noted that control reabsorptive rates with titrated ultrafiltrate were significantly lower in JMPCT than SFPCT. Although further studies will be required to determine the exact significance of this finding, it is consistent with the other data presented here in that it suggests that, with respect to supporting volume reabsorption, Cl cannot replace luminal HCO_3 as efficiently in JMPCT as in SFPCT.

Finally, the rationale behind the present studies was to examine volume reabsorption in those regions of the SFPCT and JMPCT that had previously been shown to have differing relative Na and Cl permeabilities and probably different in vivo PD (7). Although significant differences were found in the volume reabsorptive characteristics of SFPCT and JMPCT, this heterogeneity cannot be attributed solely to permeability differences. Indeed, none of the experiments presented here can explain the reasons behind the differences that were observed. However, it is possible that the absence of a lumen-positive PD in JMPCT perfused with high Cl, low HCO_3 solution may in part explain why no evidence for passive reabsorption was found in these tubules. In addition, it is possible that even though solute asymmetry exists in JMPCT between luminal (high Cl, low HCO_3, absent glucose and amino acids) and bath (normal rabbit serum) fluids, no passive reabsorption will be observed if the reflection coefficients for these solutes are similar.

In summary, segments of superficial and juxtamedullary proximal convoluted tubules (beyond the first postglomerular millimeter of tubule) were perfused in vitro to determine various characteristics of volume reabsorption. When SFPCT and JMPCT were perfused with a solution simulating in vivo conditions (high Cl, low HCO_3 concentrations, absent glucose and amino acids) fluid reabsorptive rates were similar in spite of the fact that SFPCT exhibited lumen-positive PD, whereas JMPCT exhibited small lumen-negative PD. However, when the Cl gradient was obliterated, SFPCT ceased net volume reabsorption and exhibited a small lumen-negative PD, whereas JMPCT was unaffected. When active transport was inhibited by ouabain or hypothermia in the presence of a lumen-to-peritubule Cl gradient, volume reabsorption and the lumen-positive PD were unaffected in the SFPCT, whereas volume reabsorption was abolished in JMPCT with little or no effect on the small lumen negative PD. These results are

[3] It is the experience of this author that random dissection of proximal convolutions is least difficult in the midcortical and juxtamedullary regions. Considerably greater effort with a lower success rate is experienced when one specifically dissects tubules whose convolutions reach the cortical surface of the kidney.

best interpreted by a model predicting mostly passive forces being responsible for volume reabsorption in the major portion of the SFPCT and active transport being responsible for the bulk of volume reabsorption in the JMPCT.

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