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Research Article

To examine the renal tubular sites and mechanisms involved in the effects of hyponcotic volume expansion (VE) on renal electrolyte excretion, we performed clearance and isolated tubular perfusion studies using intact and thyroparathyroidectomized (TPTX) rabbits. We also examined the effect of VE on luminal brush border transport. In the microperfusion studies, proximal convoluted (PCT) and straight (PST) tubules were taken from rabbits without prior VE or after 30 min of 6% (body wt) VE. Acute VE increased the percentage excretion of Na, Ca, and P in TPTX animals and the percentage and absolute excretions of these ions in intact rabbits. In PST from VE animals, fluid flux (Jv) was depressed compared with Jv in PST from nonVE rabbits: Jv = 0.18 +/- 0.03, (VE) vs. 0.31 +/- 0.03 nl/mm.min, (nonVE) P less than 0.02. Phosphate transport (Jp) in the PST from VE animals was also depressed: JP = 1.58 +/- 0.10 (VE) vs. 2.62 +/- 0.47 pmol/mm.min, (nonVE) P less than 0.05. Similar results were obtained with TPTX animals. In the PCT from VE animals, Jv was decreased (0.49 +/- 0.10 (VE) vs. 0.97 +/- 0.14 nl/mm.min, (nonVE) P less than 0.02), but JP was not affected significantly. Transport inhibition was stable over approximately 90 min of perfusion. In the brush border vesicle studies, sodium-dependent phosphate transport was inhibited compared [...]

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Inhibitory Effects of Volume Expansion Performed In Vivo on Transport in the Isolated Rabbit Proximal Tubule Perfused In Vitro

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Abstract

To examine the renal tubular sites and mechanisms involved in the effects of hyponcotic volume expansion (VE) on renal electrolyte excretion, we performed clearance and isolated tubular perfusion studies using intact and thyroparathyroidectomized (TPTX) rabbits. We also examined the effect of VE on luminal brush border transport. In the microperfusion studies, proximal convoluted (PCT) and straight (PST) tubules were taken from rabbits without prior VE or after 30 min of 6% (body wt) VE. Acute VE increased the percentage excretion of Na, Ca, and P in TPTX animals and the percentage and absolute excretions of these ions in intact rabbits. In PST from VE animals, fluid flux (J_V) was depressed compared with J_V in PST from nonVE rabbits: $J_V = 0.18 \pm 0.03$, (VE) vs. 0.31 ± 0.03 nl/mm · min, (nonVE) $P < 0.02$. Phosphate transport (J_P) in the PST from VE animals was also depressed: $J_P = 1.58 \pm 0.10$ (VE) vs. 2.62 ± 0.47 pmol/mm · min, (nonVE) $P < 0.05$. Similar results were obtained with TPTX animals. In the PCT from VE animals, J_V was decreased (0.49 ± 0.10 (VE) vs. 0.97 ± 0.14 nl/mm · min, (nonVE) $P < 0.02$), but J_P was not affected significantly. Transport inhibition was stable over ~ 90 min of perfusion. In the brush border vesicle studies, sodium-dependent phosphate transport was inhibited compared with that in control animals, at the 9-, 30-, and 60-s time points. These findings indicate that the inhibition of renal ionic transport by VE occurs in both PCT and PST and is, in part, the result of a direct effect of VE on tubular transport mechanisms.

Introduction

The natriuretic and phosphaturic effects of hyponcotic extracellular fluid volume expansion (VE)¹ have been well-charac-

terized in several species (1–6). However, the mechanisms responsible for the actions of VE on renal ionic excretion remain a matter of considerable debate (7). In some circumstances, it is clear that the secretion of parathyroid hormone (PTH) contributes to the inhibition of renal tubular reabsorption observed during VE (8–10). Nonetheless, it is also evident that proximal renal tubular (8, 11, 12) and whole kidney (3, 4, 6) phosphate reabsorption are inhibited by VE in animals that have undergone prior parathyroidectomy, implicating the influence of inhibitory factors other than PTH during VE. Despite the substantial number of studies which suggest that VE has a direct effect on the renal tubule, inhibition of tubular transport during VE has not previously been demonstrated in the absence of the various hemodynamic and peritubular physical factors that are inherent to in vivo micropuncture and microperfusion techniques.

The present studies were designed to examine the effects of VE performed in vivo on the absorption of fluid and phosphate in proximal renal tubules isolated from volume-expanded animals and studied in vitro. This approach was adopted in order to preserve any alterations possibly induced by a circulating factor released in response to VE in vivo, but to allow the direct observation of transport in the absence of hemodynamic influences. Additionally, employment of the tubular microperfusion technique allows observation of the effects of VE on transport in the proximal straight tubule (PST), a segment that is inaccessible with micropuncture techniques and in which the effects of VE have not been previously described. The results of these experiments were then compared with those obtained utilizing the brush border membrane (BBM) vesicle technique. The data reveal that in vivo VE inhibits transport in both the proximal convoluted (PCT) and straight tubule (PST) segments, and in BBM vesicles in a manner consistent with the actions of a humoral agent, which, in these studies, is probably not PTH.

Methods

All experiments described in this report utilized 1.2–2.0-kg female New Zealand White rabbits (Green Meadows Rabbitry, Murraysville, PA). The animals were fed standard rabbit chow (Ralston-Purina Co., St. Louis, MO) containing 1.2% Ca and 0.5% P, and were given free access to food and water. Lighting in the animal housing was arranged on a 12-h cycle and all experiments were begun at approximately the same time of the day to avoid diurnal variations.

16–24 h before their use in some of the in vitro microperfusion experiments to be described, and 3–4 h before studies of BBM vesicle transport, the animals were subjected to thyroparathyroidectomy (TPTX). Because techniques yielding adequate TPTX for the rabbit had not been well described previously, we undertook a preliminary series of experiments to demonstrate the feasibility of TPTX in this species. Initially, the parathyroid and thyroid glands were identified by standard surgical and histologic means. Subsequently, a series

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1. *Abbreviations used in this paper:* BBM, brush border membrane; %ECa, percentage excretion of calcium; %EP, percentage excretion of phosphorus; J_p , lumen-to-bath phosphate absorption; J_V , fluid flux; PCT, proximal convoluted tubule; PST, proximal straight tubule; TPTX, thyroparathyroidectomy; VE, volume expansion.

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of experiments was performed to examine the changes in the serum calcium concentration following the surgical procedure, to provide biochemical evidence for adequacy of the TPTX. The animals were anesthetized with an intravenous infusion of sodium pentobarbital (30–45 mg/kg) and the neck was opened with a midline incision. The thyroid gland was identified by its characteristic bilobed structure overlying the trachea. Single parathyroid glands were identified bilaterally in or adjacent to the carotid sheath, immediately inferolateral to the inferior border of each lobe of the thyroid gland. Thyroid and parathyroid glands thus identified were then gently resected; the neck was irrigated with saline or povidone-iodine and closed. The animals were then fasted overnight. In a series of control experiments, rabbits were subjected to an almost identical procedure in which the thyroid and parathyroid glands were identified, gently manipulated, but left in place. The total serum calcium concentration was determined in all animals before and ~ 16 h after the procedure for the rabbits studied with microperfusion and at 3–4 h after TPTX in animals studied with the BBM vesicle transport technique. Comparisons of the two values within each group were made using a *t* test for paired variables.

Clearance studies. Standard clearance experiments as previously described from this laboratory were performed for the dog (13), with adaptation to the rabbit. Briefly, animals were anesthetized with pentobarbital sodium (30–45 mg/kg) and an 8 Fr. urinary catheter was placed in the bladder of each urethra. The carotid artery and jugular vein were cannulated with polyethylene tubing (PE-90). Standard loading and maintenance doses of inulin (10% inulin in 0.155 M NaCl) were administered by an infusion pump (Harvard Apparatus Co., Inc., S. Natick, MA). Arterial blood pressure was monitored continuously from the carotid artery cannula via a pressure transducer and electronic chart recorder (Hewlett-Packard Co., Palo Alto, CA). Blood and urine losses were replaced throughout the course of the experiments with a modified Ringer's solution containing 155 mM, Na 4 mM, K 134 mM, Cl and 25 mM acetate. After a 90-min equilibration, urine collections were begun. In all experiments an initial control period consisted of two to three 30-min urine collections, after which animals were subjected to either a second set of control collections (time-course controls) or VE. In the latter group, 6% (body wt) VE was induced over a 30 min period and subsequently maintained by the infusion of the previously described modified Ringer's solution, to which 5 mM CaCl₂ had been added. A second set of four to six 20–30-min urine collections was then completed. In animals serving as time course controls, VE was omitted and collections were continued to a time period equivalent to that of the VE studies. Throughout the course of all experiments, blood samples of ~ 1 ml each were obtained at regular intervals to allow the determination of the arterial blood pH; carbon dioxide tension and bicarbonate concentration; and the plasma concentrations of Na, K, Cl, total and ionized Ca, P, and inulin as described previously (13). Clearance results were calculated by standard formulas. The data were analyzed by comparing the means of the initial control collections with those of the second set of collections using a *t* test for paired variables.

Four groups of clearance studies were performed in animals studied with the *in vitro* microperfusion technique. In group 1 (*n* = 9), intact animals did not receive VE and served as time controls. In group 2 (*n* = 8), intact rabbits underwent VE after the initial control period. All animals in groups 3 and 4 underwent TPTX on the day before their use in experiments. Only animals that demonstrated a total serum calcium concentration of < 9.0 mg/dl at the initiation of the clearance study were included in the analyses. TPTX rabbits in group 3 (*n* = 7) served as time controls. TPTX animals in group 4 (*n* = 9) underwent VE in a manner identical to that described above, except that the calcium concentration of the fluid used for expansion was increased to 10 mM to prevent the occurrence of tetany during the procedure.

In studies performed utilizing the BBM vesicle transport technique, only two groups of animals were studied: time control rabbits (group 1) and rabbits subjected to VE (group 2). To assess the success of TPTX, the serum calcium in the animals subjected to this procedure was compared with that obtained in a group of animals in which sham

TPTX had been performed as outlined above. At the end of the clearance experiment, the kidneys were removed, the cortices were rapidly separated, and were placed in ice-cold 154 mM NaCl, 1 mM Tris-Hepes (pH 7.5). They were then processed at once for studies of BBM transport as described below.

Isolated tubular perfusion studies. The technique of isolated tubular perfusion was carried out by the method of Burg et al. (14) as modified in our laboratory (15). Fluid flux (J_V) and lumen-to-bath phosphate (J_P) transport were determined in all experiments. Each experiment was divided into three time phases: a 30-min equilibration, and two sequential collection periods of ~ 45 min each, consisting of four to six samples per period. To examine the stability of transport throughout the course of the studies, transport values obtained during the first collection period were compared with those obtained in the second collection period.

Seven groups of perfusion experiments were performed.

PSTs. Three groups of experiments were performed using cortical PST from intact rabbit. The animals used in VE (*n* = 8) experiments were anesthetized as described previously; the jugular vein was catheterized with polyethylene tubing. VE was induced as previously described over 30 min. The animal was then decapitated and the kidneys were harvested for isolation and perfusion of PSTs. To serve as controls, sham VE animals (*n* = 9) underwent anesthesia and surgery in a manner identical to those described above, except that VE was replaced by an infusion of 1–2 ml isotonic saline over 30 min before killing. PSTs isolated from these animals were then perfused identically to those taken from expanded animals. The third group of studies was performed as a control (*n* = 8) to examine the possibility that anesthesia and surgery as described above might alter transport in tubules subsequently perfused *in vitro*. Animals for these experiments were decapitated without prior anesthesia or surgery; PSTs were then isolated and perfused as described.

Two groups of experiments were performed using PSTs taken from rabbits that had undergone TPTX 16–24 h earlier. Animals for control tubules studies (*n* = 5) were handled as described above for control PSTs for intact rabbits, and animals used in VE experiments (*n* = 6) underwent VE as described above for the intact VE PST group.

PCTs. We studied two groups of superficial, early PCTs. For VE (*n* = 9) experiments (*n* = 9), tubules were dissected from the kidneys of intact rabbits that had received anesthesia and VE before sacrifice as described above. (*n* = 9): To serve as controls for the PCT volume expansion group, the final studies were performed using PCTs taken from intact rabbits that did not receive anesthesia or VE before killing. Because anesthesia had no effect on transport in the PSTs, a sham VE group was omitted for the PCT.

Statistical analyses. Within each group of tubules studied with the isolated tubular perfusion technique, a *t* test for paired variables was used to compare the means of transport values between the two sequential collection periods. An independent *t* test and an analysis of variance were used to compare the mean transport values of a given collection between two or more groups of tubules. To avoid any possible influence of genetic and/or seasonal variations in renal ionic transport, VE and control experiments were carried out contemporaneously.

Proximal tubular BBM vesicle transport. These experiments were performed to determine if the inhibitory effect of VE on transepithelial transport is also evident as a change in sodium gradient-driven phosphate uptake by proximal tubular BBM vesicles. One pair of rabbits, TPTXed 3–4 h before use, was used for each experiment. Both animals underwent anesthesia and placement of jugular venous and urinary catheters as described earlier. One animal, as a control, received only 1–2 ml of isotonic saline solution, while the other was volume-expanded over 30 min as described earlier. At the completion of the expansion period, the kidneys of both rabbits were harvested separately for use in BBM vesicle transport studies.

Proximal tubular BBM vesicles were prepared from the kidneys of each animal using a calcium precipitation method (16). Sodium-dependent phosphate transport was measured as previously described

from this laboratory (17). The vesicles, suspended in 100 mM mannitol, were incubated with a reaction mixture that contained 100 mM NaCl, Tris-Hepes, pH 8.5, and trace quantities of ^{32}P (New England Nuclear, Boston, MA). The reaction was stopped at predetermined time points (5, 9, 30, and 60 s, and 120 min) by the addition of an ice-cold arsenate solution. The vesicles were collected on filter paper and ^{32}P was counted in a liquid scintillation counter. Phosphate uptake by the vesicles prepared from the control kidneys was compared with that measured in the vesicles prepared from the kidneys of VE animals at the corresponding time point using a dependent *t* test.

Results

Effect of TPTX on the serum calcium concentration. The mean serum calcium concentration of the sham TPTX rabbits was 12.5 ± 0.6 mg/dl before TPTX and rose slightly to 13.1 ± 0.1 after TPTX ($P < 0.05$). In contrast, in the animals TPTXed 16–24 h before study, serum calcium concentration before surgery was 11.9 ± 0.4 mg/dl and fell to 7.3 ± 0.6 the following day ($P < 0.01$). In animals examined 3 h after TPTX, the mean total calcium values before and after TPTX were 11.6 ± 0.2 and 8.8 ± 0.6 mg/dl, respectively ($P < 0.001$); ionized calcium fell from 5.37 ± 0.10 to 4.64 ± 0.19 meq/liter ($P < 0.005$). In the sham TPTX rabbits, total calcium fell slightly but significantly from 12.0 ± 0.22 to 11.4 ± 0.14 mg/dl ($P < 0.05$), but ionized calcium was unchanged: from 5.76 ± 0.07 to 5.95 ± 0.16 meq/liter ($P > 0.10$).

Clearance studies. Table I shows the results of time control clearance studies in intact rabbits (group 1). There were no differences in any of the measured parameters between the two time periods, thus demonstrating the stability of the clearance procedure in intact rabbits.

Table II demonstrates the effects of 6% VE, induced after the initial control period in intact rabbits (group 2). While the mean arterial pressure, inulin clearance, and serum concen-

Table II. The Effects of 6% VE in Intact Rabbits, $n = 8$

	Control	6% Volume expansion	P
MAP (mmHg)	88±3	90±3	NS
C_{in} (ml/min)	15.1±2.1	16.4±2.2	NS
UpV ($\mu\text{mol}/\text{min}$)	0.6±0.2	1.8±0.6	<0.05
%EP	2.3±0.8	6.2±1.4	<0.02
U_{CaV} ($\mu\text{mol}/\text{min}$)	6.8±1.5	21.3±2.6	<0.001
%ECa	13.2±3.0	40.7±6.2	<0.005
%ENa	1.4±0.3	6.5±0.4	<0.001
$[\text{Ca}]_t$ (mg/dl)	11.5±0.2	11.6±0.4	NS
$[\text{Ca}]_i$ (mg/dl)	6.08±0.15	6.63±0.43	NS
[P] (mg/dl)	4.9±0.2	5.3±0.4	NS
Plasma pH	7.49±0.01	7.46±0.02	NS
Plasma PaCO ₂ Torr	35.6±3.6	35.2±2.1	NS
$[\text{HCO}_3^-]$ (mM/liter)	27.1±3.0	24.8±2.0	NS

Values shown are mean±SEM. *P*, level of significance, determined by *t* test. Abbreviations are as shown in Table I.

trations of phosphorus and total and ionized calcium did not change, the absolute and fractional excretions of calcium and phosphorus rose in response to VE. Thus, VE augmented the excretion of these ions in the absence of changes in filtered load. In addition, the effectiveness of VE is shown by the approximately fourfold increase in the percent excretion of sodium.

Tables III and IV show the results of clearance studies in TPTX rabbits. From Table III it can be seen that all of the measured parameters are stable over the time course of these studies in TPTX rabbits maintained under control conditions (group 3). In group 4 animals, (Table IV), volume expansion significantly increased the percentage excretion of phosphorus, calcium, and sodium, but did not alter other parameters. The absolute rates of excretion of calcium and phosphorus in group 4 trended upward but these increases failed to reach statistical significance ($P > 0.1$).

Table I. Time Course Control Clearance Studies in Intact Rabbits, $n = 9$

	Control 1	Control 2
MAP (mmHg)	89±7	89±6
C_{in} (ml/min)	10.9±1.4	10.6±1.4
UpV ($\mu\text{mol}/\text{min}$)	1.5±0.6	1.5±0.5
%EP	8.3±3.2	8.4±3.0
U_{CaV} ($\mu\text{mol}/\text{min}$)	7.3±2.4	9.6±2.9
%ECa	21.7±6.3	23.3±6.3
%ENa	2.08±0.59	1.66±0.57
$[\text{Ca}]_t$ (mg/dl)	11.2±0.2	10.9±0.3
$[\text{Ca}]_i$ (mg/dl)	6.09±0.11	6.00±0.11
[P] (mg/dl)	5.2±0.4	5.5±0.3
Plasma pH	7.48±0.02	7.49±0.02
Plasma PaCO ₂ Torr	31.8±1.6	31.0±1.7
$[\text{HCO}_3^-]$ (mM/liter)	23.6±1.3	23.2±1.4

Values shown are means±SEM. There were no differences between the two collection periods. Abbreviations: MAP, mean arterial blood pressure; C_{in} , clearance of inulin; %E, percentage excretion; UV, absolute rate of excretion; $[\text{Ca}]_t$ and $[\text{Ca}]_i$, total and ionized plasma calcium concentrations, respectively; PaCO₂, partial pressure of carbon dioxide; [P] and $[\text{HCO}_3^-]$, plasma phosphorus and bicarbonate concentrations, respectively.

Table III. Time Course Control Clearance Studies in TPTXed Rabbits, $n = 7$

	Control 1	Control 2
MAP (mmHg)	88±4	88±5
C_{in} (ml/min)	12.1±0.9	12.9±2.4
UpV ($\mu\text{mol}/\text{min}$)	2.0±0.4	2.8±1.0
%EP	10.2±2.4	11.1±2.7
U_{CaV} ($\mu\text{mol}/\text{min}$)	5.0±1.2	4.6±1.2
%ECa	24.0±6.0	21.3±4.8
%ENa	0.38±0.13	1.03±0.63
$[\text{Ca}]_t$ (mg/dl)	6.3±0.5	5.7±0.5
$[\text{Ca}]_i$ (mg/dl)	3.35±0.33	2.92±0.30
[P] (mg/dl)	5.7±0.5	6.1±0.4
Plasma pH	7.49±0.03	7.49±0.02
Plasma PaCO ₂ Torr	39.4±2.0	38.4±3.2
$[\text{HCO}_3^-]$ (mM/liter)	29.5±1.6	28.1±2.5

Values are mean±SEM. There were no differences between the two collection periods. Abbreviations are as shown in Table I.

Table IV. The Effects of 6% VE on Clearance Parameters in TPTXed Rabbits, n = 9

	Control	6% Volume expansion	P
MAP (mmHg)	84±2	87±2	NS
C _{in} (ml/min)	15.1±1.8	15.6±1.8	NS
U _P V (μmol/min)	2.4±0.9	3.3±0.7	NS
%EP	7.6±2.5	12.8±3.8	<0.05
U _{Ca} V (μmol/min)	7.5±1.8	16.4±3.7	NS
%ECa	32.2±7.2	70.2±18.0	<0.05
%ENa	0.49±0.13	6.98±2.66	<0.05
[Ca] _i (mg/dl)	6.2±0.6	6.0±0.4	NS
[Ca] _o (mg/dl)	3.47±0.32	3.27±0.41	NS
[P] (mg/dl)	6.1±0.4	6.3±0.6	NS
Plasma pH	7.45±0.02	7.48±0.01	NS
Plasma PaCO ₂ Torr	37.8±2.8	34.8±2.5	NS
[HCO ₃ ⁻] (mM/liter)	25.7±1.0	26.8±1.9	NS

Values shown are means±SEM. P denotes level of significance as determined by t test for paired variables. Abbreviations are as shown in Table I.

Perfusion experiments. Fluid flux (J_V) and lumen-to-bath phosphate (J_P) transport, measured over two sequential time periods in superficial PSTs from control, sham volume-expanded, and volume-expanded intact rabbits, are shown in Table V. Within each group of tubules, there were no changes in transport or differences in perfusion rate between the two collection periods. There were no differences in J_V or J_P between tubules taken from control and from sham volume-expanded animals, demonstrating the ineffectiveness of anesthetic and surgical manipulations on these measurements. Notably, however, J_V and J_P were significantly lower for tubules isolated from volume-expanded animals compared with J_V and J_P of the tubules from control and sham volume-expanded rabbits. Moreover, this inhibition of transport in tubules from volume-expanded animals was present throughout the course of both perfusion collection periods. Perfusion rate during both collection periods was equivalent among the groups.

Table VI demonstrates the results of perfusion experiments in superficial PCTs taken from intact control and VE rabbits. Within each group, transport and perfusion rates were stable over the two collection periods. In tubules of VE animals, J_V was significantly depressed during each collection period when compared with J_V observed in tubules from controls. In both

collection periods, the mean numerical values of J_P were lower in tubules from VE rabbits than controls, but this difference failed to reach statistical significance ($P > 0.1$).

The data from the perfusion studies in PSTs taken from TPTX rabbits are shown in Table VII. The mean 24-h serum calcium concentration after TPTX was 7.36 ± 0.54 mg/dl in controls and 7.38 ± 0.44 in the VE group ($P > 0.9$). Like the results obtained with tubules from intact animals, both mean J_V and J_P were lower in the tubules taken from the VE animals. This inhibition of transport persisted throughout the time of the perfusion. Rates of perfusion were similar for the two groups of tubules.

BBM vesicle phosphate transport. Table VIII shows data obtained in the experiments examining the uptake of phosphate by BBM vesicles prepared from paired control and VE TPTXed rabbits. At 9, 30, and 60 s, the uptake of inorganic phosphate by vesicles from the VE animals was lower than that by vesicles from the controls. At 120 min, representing the equilibrium phase, the mean phosphate uptake values of the two groups did not differ ($P > 0.2$), demonstrating the equality of vesicle size in the two groups.

Discussion

This study demonstrates that transport is decreased after in vivo VE when proximal tubules are isolated and perfused in vitro. Moreover, transport inhibition occurs not only in the PCT, as has been demonstrated by micropuncture studies (12), but also in the PST. The latter observation has not been reported previously due to the limitations of the micropuncture technique. Although our PST data cannot be compared with prior findings, the degree of VE-induced inhibition of J_V that we observed in the early, superficial PCT appears similar to the 40–60% inhibition of fluid reabsorption seen in the micropunctured PCT of the dog and rat (2, 12, 18). However, in contrast to micropuncture studies (11, 12), we did not observe an inhibition of phosphate transport in the PCT after VE. The reasons for this disparity are not clear; Species-related differences between the rabbit and other mammals might contribute to it. Although no micropuncture studies have examined the effects of VE in the rabbit, the peritubular physical factors and renal hemodynamic changes that contribute to the inhibition of reabsorption during VE in other species would most likely be present in the rabbit as well. Thus, the lack of J_P depression in the PCT after VE does not preclude the inhibition of phosphate reabsorption in that segment during VE in vivo. Finally, the data comparing J_P in the PCT between control and ex-

Table V. Fluid (J_V) and Lumen-to-bath Phosphate (J_P) Transport and Perfusion Rate Measured during Two Sequential Collection Periods in PSTs Taken from Intact Rabbits under Control Conditions, after sham VE and after 6%

	J_V		J_P		Perfusion rate	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
	nl/mm·min		pmol/mm·min		nl/min	
Control (n = 8)	0.31±0.03	0.33±0.04	2.62±0.47	2.61±0.49	12.35±0.16	12.28±0.11
Sham VE (n = 9)	0.33±0.04	0.35±0.04	2.51±0.12	2.40±0.16	11.94±0.16	11.91±0.16
VE (n = 8)	0.18±0.03*	0.21±0.02*	1.58±0.10‡	1.38±0.09‡	12.36±0.23	12.64±0.30

* $P < 0.02$ vs. sham VE and vs. control. ‡ $P < 0.001$ vs. sham VE and $P < 0.05$ vs. control.

Table VI. Fluid (J_v) and Lumen-to-bath Phosphate (J_p) Transport and Perfusion Rate Measured during Two Sequential Collection Periods (period 1, period 2) in Early PCTs Harvested from Intact Rabbits under Control Conditions and after 6% VE

	J_v		J_p		Perfusion rate	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
	nl/mm · min		pmol/mm · min		nl/min	
Control ($n = 9$)	0.97±0.14	1.00±0.14	7.08±1.23	7.12±1.27	12.47±0.31	12.29±0.19
VE ($n = 9$)	0.49±0.10*	0.54±0.12‡	4.85±0.61	4.96±0.54	11.86±0.28	11.85±0.16

There were no differences between periods within each group. * $P < 0.02$ vs. corresponding control; ‡ $P < 0.025$ vs. corresponding control. Values shown are means±SEM.

panded animals may have failed to reach statistical significance because of the high variability of J_p in the PCT within groups of animals.

Because the effects of VE in the rabbit had not been described by others, we felt it important to examine, using clearance techniques, the suitability of the rabbit as a model for proximal sodium and phosphate handling during VE. In the intact rabbit, VE increased both the absolute and fractional excretion of phosphate. In the TPTXed rabbit, however, the fractional but not absolute excretion of phosphorus rose after VE, suggesting that the phosphaturia in these animals is somewhat blunted in comparison with that seen in the intact animals. Some degree of variability in the basal phosphorus excretion of the TPTXed animals may have contributed to this observation. Notably, the magnitude of the phosphaturia produced by VE in the rabbit was somewhat less than that seen in other species (3–5, 8), and was not major. In intact animals, the percentage phosphate excretion rate almost tripled (Table II), whereas in the TPTXed rabbits it rose by ~70% (Table IV). However, the tubular transport of phosphate was inhibited by ~40–50% in the PST by VE. This disparity between proximal transport inhibition and final urinary phosphate excretion suggests that there is a significant amount of distal tubular phosphate reabsorption in the rabbit that is not substantially inhibited by VE. The finding that the rabbit nephron is also resistant to the phosphaturic effects of PTH (19), suggests that rabbit phosphate transport physiology differs from that of the dog, rat, and man. Alternatively, since we studied only superficial tubules, in vitro proximal transport data obtained in superficial nephrons and whole kidney clearance observations may not be strictly comparable, especially given the possibility that there exists nephron heterogeneity. Nonetheless, the rabbit appears to be a suitable model for these studies,

as our animals responded to VE in a manner qualitatively similar to other mammals.

The TPTX in our animals appears to have been adequate. Indeed, there was such a marked tendency to hypocalcemia within 16 h after the procedure that tetany occurred frequently. Few other workers have reported their experience with TPTX in the rabbit. Berndt and Knox (20) noted a 6% fall in the total serum calcium concentration 2 h after TPTX. In our own studies, there was a fall of ~24% at 3–4 h. Recently, Jao et al. (21) in a preliminary communication, reported a 40% reduction in the total serum calcium concentration up to 24 h after TPTX. Our method of TPTX also yielded a 40–50% reduction of both total and ionized calcium concentrations, that occurred in < 24 h after the procedure. Thus, we conclude that TPTX in the rabbit is a technically achievable and reproducible maneuver.

Although our data do not reveal the mechanism by which VE inhibited transport in our studies, it is apparent that glomerular, hemodynamic, and peritubular physical factors are not involved, at least as principal factors. In both TPTXed and intact animals, the filtered load of phosphorus and the mean arterial blood pressure were unaffected by VE. Note, however, that transport inhibition was evident when the proximal tubules were removed and perfused in vitro. Furthermore, BBM vesicles prepared from expanded animals demonstrated reduced phosphate uptake when compared with those obtained from control animals. Therefore, alterations in net interstitial pressure, peritubular capillary blood flow and oncotic pressure, and other intrarenal physical factors cannot explain the observed transport inhibition. Moreover, paracellular back-leak, which is thought to contribute to the excretory response to VE (22, 23), is most likely not a factor in our observations. Enlargement of the paracellular spaces induced by VE (24, 25)

Table VII. Fluid (J_v) and Lumen-to-Bath Phosphate (J_p) Transport and Perfusion Rate Measured during Two Sequential Collection Periods in PST Taken from TPTX Rabbits under Control Conditions and after 6% VE

	J_v		J_p		Perfusion rate	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
	nl/mm · min		pmol/mm · min		nl/min	
Control ($n = 5$)	0.52±0.09	0.56±0.09	2.14±0.24	2.03±0.22	11.61±0.13	11.50±0.19
VE ($n = 6$)	0.22±0.06*	0.22±0.07*	1.26±0.22*	1.19±0.22*	11.61±0.24	11.46±0.15

* $P < 0.025$ vs. corresponding control.

Table VIII. Phosphate Transport

	0.08 min	0.15 min	0.5 min	1.0 min	120 min
	<i>pmol/mg protein</i>				
Control	840.0±137.2	1,262±177.6	2,429±311.8	2,806±353.4	1,761±304.1
VE	570.1±122.0	806.9±118.9*	1,503±230.7*	1,882±264.6*	1,180±226.7

The uptake of inorganic phosphate by BBM vesicles prepared from the kidneys of paired control and volume-expanded TPTX rabbits, $n = 7$.
* $P < 0.02$ or better vs. corresponding control value.

has recently been shown to result primarily from increased luminal hydrostatic pressure rather than from volume flow across the membrane (26). Our tubules were perfused at identical, physiologic rates and, thus were subjected to identical perfusion pressures. Also, transport measurements were not made for 30–60 min after killing the animal. Therefore, pressure-induced alterations in the paracellular pathways occurring during *in vivo* VE should have completely resolved before transport measurement. The stability of the observed inhibition of transport over ~ 90 min of perfusion in our studies supports this concept.

Our data also indicate that PTH secretion is not necessary for the inhibition of transport by VE that we observed. Although intact animals were expanded with a calcium-containing solution to avoid dilution of the plasma-ionized calcium, the phosphaturia in these rabbits was greater than that in the TPTX animals. Therefore, PTH secretion may have contributed to the phosphaturia seen in intact rabbits. However, VE inhibited transport in tubules from TPTX animals, thus clearly dissociating the inhibition of transport from PTH effects.

The persistent inhibition of transport *in vitro* and its stability over ~ 90 min of perfusion suggest the possibility that a humoral factor, released *in vivo* in response to VE, altered transport by action on the tubular membrane. Endogenous volume-responsive natriuretic agents are good candidates for this role. Several previous studies support the possibility of a direct tubular action of purported natriuretic hormones. Natriuretic principles isolated from the urine or serum of uremic humans inhibited distal tubular transport *in vitro* (27) and proximal tubular reabsorption *in vivo* (28). The intravenous infusion and intratubular injection of plasma or plasma dialysates from expanded animals inhibited proximal tubular reabsorption in the micropunctured rat (29). Finally, in studies using the split-drop micropuncture technique, proximal tubular fluid harvested from acutely volume-expanded rats inhibited the transluminal Na concentration gradient in the proximal tubule of hydropenic rats when injected directly into the lumen of the PCT (30). Our results add to an increasing body of evidence supporting the role of humoral factors in the regulation of sodium and volume homeostasis through their actions on renal tubular transport.

The experiments presented in this report demonstrate phosphate transport inhibition in the PST of both intact and TPTX animals, but no effects of VE in the early PCT. However, membrane vesicle phosphate uptake was inhibited by volume expansion. Since the BBM vesicle preparation contains a preponderance of PCTs, these findings deserve comment. Although a completely satisfactory explanation recon-

ciling these two kinds of observations is not available, we presume that the magnitude of the transport inhibition was great enough in the PSTs so that it was reflected in the BBM vesicle phosphate uptake studies. Furthermore, similar observations to those obtained in this study with VE have been reported previously with PTH. Thus, the hormone does not alter phosphate transport in the early PCT (15, 31, 32); although several groups of workers have shown that PTH inhibits phosphate uptake in the BBMV preparation (16, 33–35).

In summary, our studies have demonstrated that VE results in the inhibition of the transport of fluid in the PCT and of fluid and phosphate in the PST, effects that persist when the tubules are removed and perfused *in vitro*. PTH secretion, peritubular physical factors, paracellular backleak, and hemodynamic changes cannot be implicated as causes of the inhibition observed in these studies. The findings are consistent with the presence of a humoral factor (or factors) released in response to VE and acting directly on tubular transport mechanisms.

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