Insulin Stimulates Volume Absorption in the Rabbit Proximal Convoluted Tubule

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Abstract

The present in vitro microperfusion study examined whether insulin affects volume absorption (J_v) in the proximal convoluted tubule (PCT). PCT were perfused with an ultrafiltrate-like solution and were bathed in a serum-like albumin solution. Addition of a physiologic concentration of 10^{-10} M insulin to the bathing solution resulted in a stimulation of J_v and a more negative transepithelial potential difference (PD). There was a progressive stimulation of the lumen negative PD and J_v with higher insulin concentrations. Maximal stimulation occurred at 10^{-8} M bath insulin. The insulin-induced stimulation of volume reabsorption was also observed when glucose and amino acids were removed from the luminal perfusate. Direct examination of the effect of insulin on glucose, chloride, and bicarbonate absorption demonstrated that the transport of all these solutes was stimulated by insulin. Addition of insulin to the luminal perfusate had no affect on J_v. These data show that insulin has a direct effect to stimulate J_v in the proximal tubule.

Introduction

Several studies have provided evidence that insulin decreases urinary sodium excretion (1–5). Withdrawal of insulin in humans with diabetes mellitus results in a diuresis and natriuresis that may not be due solely to the observed glucosuria and ketonuria. Readministration of insulin causes sodium retention, suggesting a possible role for insulin in sodium reabsorption by the kidney (1, 3). Administration of insulin to isolated dog kidneys where the glomerular filtration rate and filtered load of sodium remained constant results in a decrease in sodium, potassium, and water excretion (2). Further evidence for a direct effect of insulin on sodium excretion was demonstrated in human studies where insulin was administered and blood glucose was maintained at a constant level. Although glomerular filtration rate and renal blood flow remained unchanged, urinary sodium, potassium, and phosphate excretion decreased with insulin administration (4).

The nephron segments involved in the stimulation of sodium reabsorption are unknown. Although there are insulin receptors on the proximal tubule (6–11), previous studies have provided evidence against insulin-stimulating volume absorption (J_v)[1] in the proximal tubule (4, 5, 12). The purpose of the present in vitro microperfusion study was to examine directly whether insulin affects transport in the proximal tubule. The data show that both physiologic and pharmacologic concentrations of insulin stimulate volume reabsorption in the proximal tubule. These data provide the first direct evidence that the decrease in sodium excretion observed with hyperinsulinemia can, in part, be explained by a stimulation in proximal tubule volume reabsorption.

Methods

Isolated segments of randomly dissected midcortical and juxtamedullary rabbit proximal convoluted tubules (PCT) were perfused as previously described (13–16). Briefly, kidneys from female New Zealand white rabbits were cut in coronal slices. Individual tubules were dissected in cooled (4°C) ultrafiltrate-like solution containing 115 mM NaCl, 25 mM $NaHCO_3$, 2.3 mM Na_2HPO_4, 10 mM Na acetate, 1.8 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KCl, 8.3 mM glucose, and 5 mM alanine.

Tubules were perfused with the above ultrafiltrate-like solution and bathed in a similar solution containing 6 g/dl albumin. All solutions were bubbled with 95% O_2 and 5% CO_2 and had a pH of 7.40. The osmolality of the bath and perfusate were adjusted to 295 by the addition of either H_2O or NaCl. To maintain the pH and bath osmolality constant, bath fluid was continuously changed at a rate of at least 0.5 ml/min. All tubules were perfused at ~ 10 nl/min at 38°–39°C in a 1.2 ml temperature-controlled bath. The first period began after an equilibration time of 30–60 min. Subsequent periods were separated by an equilibration time of at least 30 min.

Net J_v (in nanoliters per millimeter per minute) was measured as the difference between the perfusion (V_0) and collection (V_L) rates (nanoliters per minute) normalized per millimeter of tubular length (L). Exhaustively dialyzed [methoxy-^{3}H]inulin was added to the perfusate at a concentration of 50 μCi/ml so that the perfusion rate could be calculated. The collection rate was measured with a 60-nl constant-volume pipette. The length, in millimeters, was measured with an eyepiece micrometer.

Net total CO_2 flux (J_{TCO_2}, picomoles per millimeter per minute) and net chloride flux (J_{Cl}, picoequivalents per millimeter per minute) were calculated according to the equation: J_{Cl} or $J_{TCO_2} = (V_0C_0 - V_LC_L)/L$, where C_0 and C_L represent the concentration of chloride or TCO_2 in the perfused and collected fluid, respectively. TCO_2 measurements were performed using microcalorimetry (Picapnotherm, model GVI; World Precision Instruments, Inc. New Haven, CT) (17). Chloride measurements were performed using the electrometric titration technique of Ramsay (microtitrator model FT-2230; World Precision Instruments, Inc.) (18).

To examine whether insulin affected glucose transport, 50 μCi/ml of [^{14}C]glucose (ICN Pharmaceuticals, Inc., Irvine, CA) was added to the perfusate. Glucose transport was calculated according to the equation: $J_{Glu} = [(V_0C_0^* - V_LC_L^*)/L](G_0/C_0^*)$, where $[G_0]$ is the glucose concentration in the perfusate and C_0^* and C_L^* are the concentration of [^{14}C]glucose in cpm per nanoliter in the perfused and collected fluid, respectively. In experiments where chloride, bicarbonate, and [^{14}C]glucose were measured, alternate samples were taken for the measurement of J_v and [^{14}C]glucose, bicarbonate, and chloride.

The transepithelial potential difference (PD, in millivolts) was measured by using the perfusion pipette as the bridge into the tubular lumen. The perfusion and bath solutions were connected to the recording and reference calomel half-cells, respectively, via a bridge containing perfusion

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1. *Abbreviations used in this paper:* J_v, volume absorption; PD, potential difference; PCT, proximal convoluted tubule.

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Table I. Effect of Insulin on the PCT

Experimental protocol	n	V_0			J_v			PD		
		Control	Experimental	Recovery	Control	Experimental	Recovery	Control	Experimental	Recovery
		nl/min	*nl/min*	*nl/min*	*nl/mm · min*	*nl/mm · min*	*nl/mm · min*	*mV*	*mV*	*mV*
Time control	4	10.34±0.49	10.00±0.45		0.86±0.06	0.87±0.08		−4.3±0.4	−4.5±0.5	—
10^{-11} M Bath insulin	5	10.23±0.13	10.45±0.18	10.46±0.07	0.79±0.06	0.80±0.04	0.79±0.04	−3.5±0.6	−3.6±0.6	−4.0±0.6
10^{-10} M Bath insulin	6	9.84±0.27	9.97±0.17	10.27±0.15	0.69±0.06	0.78±0.08*	0.71±0.08§	−3.3±0.6	−4.0±0.7†	−4.2±0.8
10^{-9} M Bath insulin	6	10.05±0.36	10.71±0.48*	10.76±0.24	0.73±0.09	0.87±0.09*	0.83±0.09§	−4.9±0.5	−5.9±0.5†	−5.8±0.5
10^{-8} M Bath insulin	4	12.66±0.45	13.28±0.73	13.33±0.37	0.65±0.10	0.88±0.13*	0.92±0.12	−4.2±0.4	−5.4±0.2*	−5.5±0.2
10^{-7} M Bath insulin	3	9.93±0.43	10.94±0.08	11.16±0.36	0.76±0.11	0.97±0.10†	0.93±0.12	−4.3±0.6	−5.8±0.9*	−5.7±1.0
10^{-6} M Bath insulin	5	9.99±0.48	10.43±0.36	10.48±0.42	0.77±0.13	0.95±0.13*	0.99±0.12	−4.4±0.3	−5.7±0.4†	−5.8±0.5

Values are means±SEM. *Mean paired difference from control significant at the 0.05 level. †Mean paired difference from control significant at the 0.01 level. §Mean paired difference from experimental significant at the 0.05 level.

or an ultrafiltrate of the bathing solution in series with a 3.6 M KCl/0.9 M KNO_3 agarose bridge. This arrangement avoided direct contact of KCl/KNO_3 agarose bridges with the solution that bathed the tubule. In addition, this arrangement eliminates the Donnan potential when the bath contains protein (14). The recording and reference calomel half-cells were connected to the high and low impedance side, respectively, of an electrometer (model 602; Keithley Instruments, Inc., Cleveland, OH).

To examine whether insulin has an effect on J_v and transepithelial PD in the PCT, tubules were perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. During the experimental period either 0, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6} M insulin was added to the bathing solution. Insulin was either added directly to the bathing solution, or from a 10^{-6} M stock solution mixed with isotonic NaCl. Insulin was removed during the recovery period. Glucose and amino acids were removed from the perfusate and replaced with NaCl to examine if the effect of insulin was due solely to a stimulation of transport by these solutes. During the experimental period 10^{-8} M insulin was added to the bathing solution. To examine directly the effect of insulin on glucose, chloride, and bicarbonate absorption, transport of these solutes was measured directly in the control period and after the addition of 10^{-8} M bath insulin. In experiments designed to examine if insulin stimulates active NaCl transport, tubules were perfused with a high chloride solution that contained 138.5 mM NaCl, 5 mM Na HCO_3, 4 mM Na_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, and 5 mM urea. The tubules were bathed in a similar solution that contained 6 g/dl albumin. In the bathing solution, 5 mM urea and 1 mM $CaCl_2$ were replaced by 5 mM glucose and alanine and 2 mM $CaCl_2$, respectively. In this setting the perfusate and bathing solutions are in approximate Donnan equilibrium and anion gradients responsible for passive NaCl transport are eliminated. High chloride solutions were bubbled with 99% O_2 and 1% CO_2 and had a pH of 7.40. During the experimental period, 10^{-8} M insulin was added to the bathing solution. In the final series of experiments designed to examine if insulin has an effect if added on the apical membrane, 10^{-8} M insulin was added to the luminal perfusate during the experimental period.

There were at least four measurements of each parameter in a given period for each tubule. The mean values for individual periods in a given tubule were used to calculate the mean value for that period.[2] Data are expressed as a mean±SEM. The *t* test for paired data was used to determine statistical significance.

Results

The first group of 33 experiments examined whether bath insulin has an effect on J_v and PD in the PCT. The mean tubular length was 1.5±0.1 mm. Table I shows the results of these experiments. A time control confirmed previous studies that have shown that J_v and PD are stable in the PCT under these conditions (15). 10^{-11} M bath insulin had no effect on J_v and PD. A physiologic concentration of 10^{-10} M insulin resulted in a significant stimulation of J_v and PD (Fig. 1). 10^{-9} M insulin resulted in a greater stimulation in both J_v and PD. Maximal stimulation of J_v and PD occurred at 10^{-8} M insulin with no further increase at higher concentrations. Half-maximal stimulation of J_v and PD occurred at a physiologic concentration of 10^{-10} M. The stimulation of J_v by 10^{-10} and 10^{-9} M bath insulin was reversed upon removal of bath insulin. The stimulation in J_v persisted in the recovery period when higher concentrations were examined. The stimulation of the PD persisted at all concentrations. Figs. 2 and 3 depict the percent stimulation of various concentrations of insulin on J_v and PD, respectively. Because most concentrations were not reversible, the difference depicted reflects that between the control and experimental period.

At 10^{-9} M insulin there was a small, but statistically significant increase in perfusion rate. To examine if this increase in perfusion rate affected the data, I perfused five tubules at 9.97±0.25 nl/min in the control period and increased the perfusion rate to 11.39±0.35 ($P < 0.01$) in the experimental period. J_v was 0.86±0.11 in the control period and 0.84±0.12 at the

2. Samples were counted for an average of 27 min with an average of 2,380±117 cpm/sample. The average mean counts per sample was 64,000 making the counting error, a square-root function of the total counts, ~ 0.4% per sample. In tubules perfused without organic solutes the rate of volume absorption and the differences between the control and experimental period were smaller. In these experiments there were five collections per period and an average of 129,000 cpm per sample. The counting error per sample was ~ 0.3%.

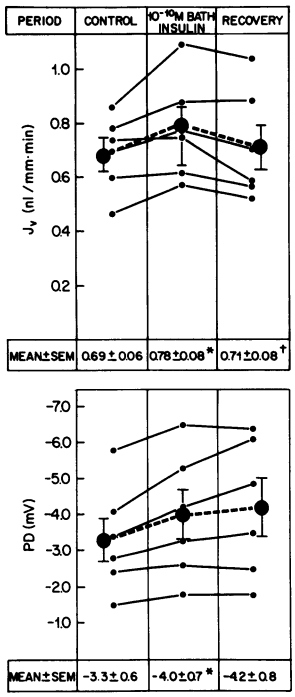

Figure 1. Effect of 10^{-10} M insulin on J_v and PD in the PCT. *Mean paired difference from control at 0.05 level. †Mean paired difference from experimental at 0.05 level.

faster rate. PD was -3.8 ± 0.4 at the slower rate and -3.9 ± 0.4 at the faster rate. Thus, small changes in perfusion rate did not affect the data.

In the next series of experiments, glucose and amino acids were replaced by NaCl to examine if the stimulation of J_v was due solely to a stimulation of glucose and amino acid transport. The mean tubular length was 1.6 ± 0.1 mm and the perfusion rate was 9.51 ± 0.23, 9.92 ± 0.17, and 9.89 ± 0.13 nl/min in the control, experimental, and recovery period, respectively. Fig. 4 shows the results of these experiments. There was a small lumen positive PD probably secondary to the anion concentration gradient resulting from the additional NaCl in the perfusate which was not affected by insulin (16). Addition of insulin produced a significant stimulation in J_v consistent with insulin stimulating the transport of other solutes besides glucose and amino acids.

The next series of experiments was designed to examine the effect of insulin on glucose, bicarbonate, and chloride transport in the PCT. The mean tubular length was 2.0 ± 0.1 mm and the perfusion rate was 8.60 ± 0.09, 9.46 ± 0.14, and 9.42 ± 0.11 nl/min in the control, experimental, and recovery periods, respectively. The difference in the perfusion rate between the control and experimental period, although small, was statistically significant. Table II and in Fig. 5 show the results. In these six

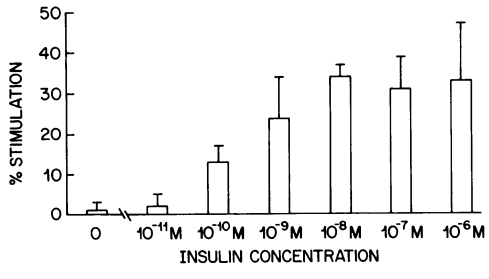

Figure 2. Percent stimulation of J_v by bath insulin.

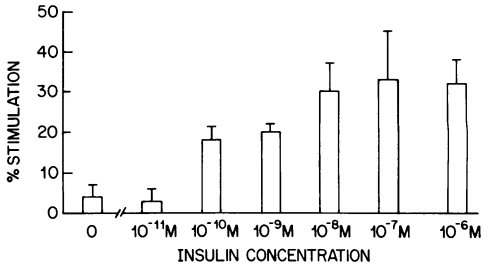

Figure 3. Percent stimulation of PD by bath insulin.

tubules, the perfusate bicarbonate concentration was 24.7 ± 0.1 milliequivalents per liter and decreased to 17.7 ± 0.8, 16.3 ± 1.3, and 16.9 ± 1.2 meq/liter in the control, experimental and recovery periods, respectively. The perfusate chloride concentration was 115.8 ± 0.8 and increased to 130.1 ± 2.1, 126.6 ± 2.2, and 128.6 ± 1.6 meq/liter in the control, experimental, and recovery periods, respectively. These data show directly that insulin stimulates bicarbonate, chloride, and glucose absorption in the PCT.

The next series of experiments was designed to examine if insulin stimulates active NaCl transport. In this series tubules were perfused with a high chloride solution simulating late proximal tubular fluid and bathed in a similar solution containing albumin. The mean tubular length was 1.7 ± 0.1 mm. The perfusion rate was 8.80 ± 0.14, 9.42 ± 0.21, and 9.49 ± 0.14 in the control, experimental, and recovery periods, respectively. Fig. 6 shows the results. These data are consistent with insulin-stimulating neutral active NaCl transport in the PCT.

The final group of experiments examined whether the addition of 10^{-8} M insulin to the lumen affected J_v and PD. The mean tubular length was 1.5 ± 0.1 mm and the perfusion rate was 10.92 ± 0.39 and 10.51 ± 0.29 nl/min in the control and experimental periods, respectively. Fig. 7 shows the results. Addition of insulin to the luminal perfusate had no affect on J_v or PD.

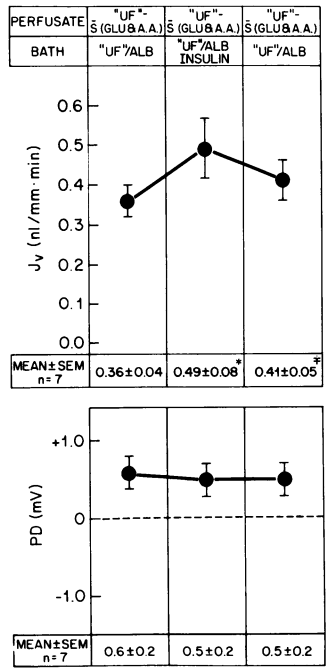

Figure 4. Effect of 10^{-8} M bath insulin on J_v and PD in PCT perfused without glucose and amino acids. *$P < 0.05$; ‡$0.05 < P < 0.10$.

Table II. Effect of 10^{-8} M Bath Insulin on PD, J_v, J_{Glu}, J_{Cl} and J_{TCO_2}

	Control	Experimental	Recovery
PD (mV)	−4.4±0.4	−5.7±0.5*	−5.7±0.5
J_v (nl/mm · min)	0.86±0.04	1.07±0.06*	1.04±0.06
J_{Glu} (pmol/mm · min)	39.9±2.1	46.9±3.4*	46.4±2.8
J_{TCO_2} (pmol/mm · min)	53.2±3.7	65.9±4.1*	62.9±5.0
J_{Cl} (pmol/mm · min)	39.2±6.3‡	68.6±11.6‡	62.3±8.1

*Mean paired difference from control at the 0.01 level.
‡Mean paired difference from control at the 0.05 level.

Discussion

The present in vitro microperfusion study examined whether insulin has a direct effect on the proximal tubule. Addition of a physiologic concentration of 10^{-10} M insulin to the bathing solution resulted in a stimulation in J_v in the PCT. Pharmacologic concentrations of insulin produced a greater stimulation of J_v with a maximum response occurring at about 10^{-8} M insulin. This stimulation in J_v was accompanied by an increase in the transepithelial PD. The stimulation of J_v by insulin was not dependent on luminal glucose and amino acids. Insulin stimulates glucose, chloride, and bicarbonate transport. Addition of insulin to the luminal perfusate had no effect on J_v and PD. These data demonstrate that insulin stimulates J_v in the proximal tubule.

The interaction of insulin with the renal proximal tubular cell has recently been extensively reviewed (19). Renal cortical membranes have high affinity receptors that bind insulin at physiologic concentrations (6–11). Insulin receptors are present on both the apical and basolateral membranes, but binding is much greater in basolateral membranes (8). Insulin binding not only affects solute transport as demonstrated in this study, but also is important for insulin degradation (7–9). Although binding is more avid on the basolateral membrane, insulin degradation occurs at a greater rate on the apical membrane (8).

Insulin binding is not homogeneous along the nephron. Specific binding is greatest in the medullary thick ascending limb, distal convoluted tubule and proximal tubule when expressed as a function of tubular length. However, insulin binding is greatest in the medullary and cortical thick ascending limb and distal convoluted tubule and similar in other segments when expressed per milligram of protein (9). Direct examination of the proximal tubule reveals high affinity, high specificity binding with significant binding at 10^{-10} M insulin. 50% inhibition of ^{125}I-labeled insulin binding occurred with 10^{-9} M unlabeled insulin and did not increase with insulin concentrations > 10^{-8} M (11). These binding studies are in good agreement with the concentration of insulin resulting in a stimulation in J_v. In the present study 10^{-11} M insulin had no significant effect on J_v.

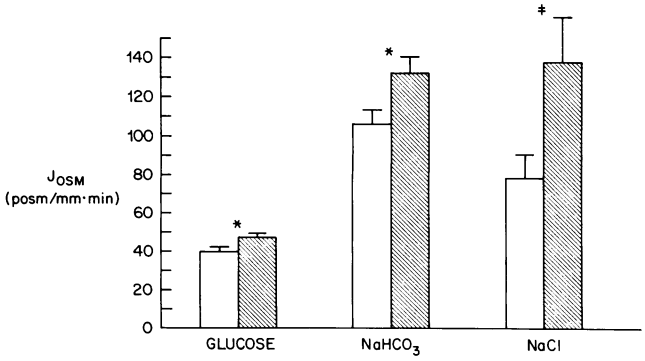

Figure 5. Effect of 10^{-8} M bath insulin on glucose, bicarbonate, and chloride transport. *Mean paired difference from control at the 0.01 level. ‡Mean paired difference from control at the 0.05 level.

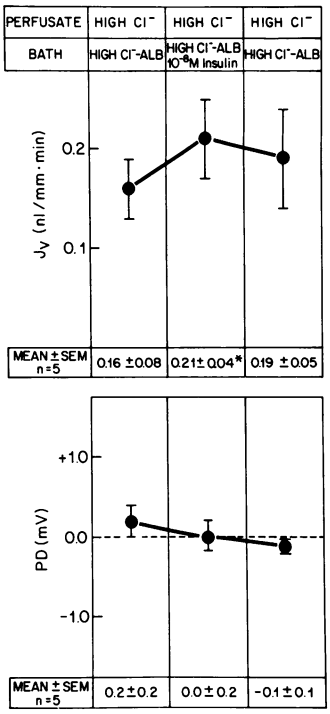

Figure 6. Effect of 10^{-8} M bath insulin on active NaCl transport. *Mean paired difference from control at the 0.05 level.

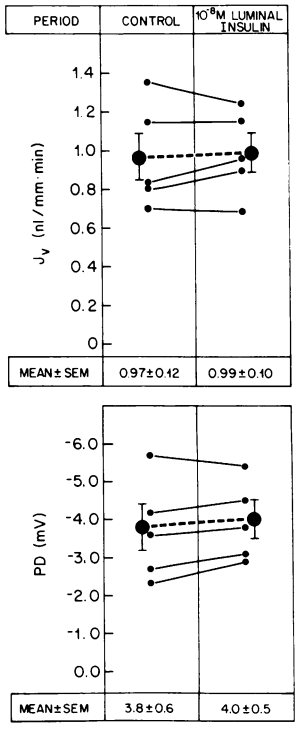

Figure 7. Effect of 10^{-8} M luminal insulin on J_v and PD in the PCT.

Addition of 10^{-10} M insulin caused a 13% increase in J_v. 10^{-9} M insulin increased J_v by 20%. Higher concentrations resulted in a 30–35% increase in J_v, which remained after removal of insulin from the bathing solution. Insulin also resulted in a dose-dependent stimulation in the transepithelial PD. This increase in the lumen negative PD remained after bath insulin was removed at concentrations > 10^{-10} M. The reason for the continued stimulation in J_v with higher concentrations of insulin and the persistent stimulation in the transepithelial PD observed is unclear. It is possible that insulin has a long term tonic effect, which allows for the acute expression of another factor in vivo. For example, physiologic concentrations of insulin increase the binding of insulin-like growth factors to adipocytes (20, 21). Insulin may play a permissive role to allow the expression of another hormone in vivo. However, this study clearly demonstrates that insulin has a direct effect to stimulate transport in the PCT in vitro.

Previous studies have demonstrated that insulin increases whole kidney sodium reabsorption (2, 4, 5). The mechanism whereby insulin stimulates sodium transport and the nephron segments involved is unclear. Earlier studies have provided evidence against insulin-stimulated J_v in the proximal tubule and have suggested that insulin stimulates sodium transport in the distal nephron (4, 5). Insulin stimulated free water excretion in clearance studies performed on water loaded normal human subjects who were in a state of water diuresis (4). The study suggests that insulin increases solute transport in the thick ascending limb. Micropuncture studies have also suggested that insulin increases sodium transport in the distal nephron. In euglycemic hyperinsulinemic dogs whose glomerular filtration rate and renal blood flow remained constant, there was a decrease in the fractional excretion of sodium. Proximal tubule J_v was inhibited under these conditions, suggesting that the stimulation of sodium transport was by the distal nephron (5). In volume-expanded parathyroidectomized rats, somatostatin infusion resulted in a decrease in plasma insulin concentration and an increase in the fractional excretion of sodium. Under these conditions there was no change in J_v by the proximal tubule and no detectable change in J_v by the early and late distal tubule. These studies provide evidence against a stimulation of J_v by the proximal tubule. The reason for the difference in the results between these studies and the current study is unclear. Although the above studies found no change in glomerular filtration rate, recent data suggests that insulin may increase glomerular filtration rate. Addition of physiologic concentrations of insulin to isolated perfused kidneys from male Sprague Dawley rats resulted in an increase in glomerular filtration rate (22). Small changes in glomerular filtration rate can have substantial effects on the filtered load delivered to the proximal tubule and obscure an increase in J_v. It is also possible that under the conditions used to alter insulin concentration in vivo other hormones may have also been affected which could have suppressed J_v by the proximal tubule. The current study was able to directly examine whether insulin affects J_v independent of filtered load or other hormones. The data show that insulin has a direct epithelial effect to stimulate J_v in the proximal tubule.

The mechanism for the observed stimulation in J_v is unknown. The fact that insulin caused an increase in the lumen negative transepithelial PD in tubules perfused with an ultrafiltrate-like solution suggests that insulin stimulates amino acid and glucose transport. To examine whether insulin affected the transport of other solutes, glucose and amino acids were removed from the luminal perfusion solution. Insulin resulted in an increase in J_v under these conditions consistent with insulin stimulating the transport of other solutes besides glucose and amino acids. To examine this directly, the effect of insulin on glucose, chloride, and bicarbonate absorption was examined. Insulin stimulated the transport of each of these solutes. The stimulation of chloride transport may be greater than that of bicarbonate and glucose. The more negative PD and the larger anion gradient resulting from the insulin-induced stimulation of organic solute and bicarbonate transport will increase passive chloride transport (16). Active chloride transport may also be stimulated. To examine this, tubules were perfused with a high chloride solution simulating late proximal tubular fluid and bathed in a similar solution containing albumin. Under these conditions NaCl transport is entirely active (14, 16). Insulin stimulated J_v under these conditions consistent with insulin-stimulating neutral active NaCl transport as well as bicarbonate and glucose transport.

Previous studies have demonstrated that insulin stimulates renal phosphate reabsorption (4, 5, 11, 12, 23). Infusion of insulin in humans where the plasma glucose concentration was maintained at a constant level resulted in a decrease in phosphate excretion (4). In parathyroidectomized rats insulin abolished the phosphaturia induced by parathyroid hormone infusion (23). Micropuncture studies have provided evidence that phosphate transport in the proximal tubule is enhanced by insulin. Proximal tubule phosphate transport was stimulated in hyperinsulinemic euglycemic rats (5). Hypoinsulinemia induced by somatostatin infusion in parathyroidectomized rats results in a decrease in proximal tubule phosphate transport (12). Brush border membrane vesicles prepared from dog isolated proximal tubules had an increase in sodium-dependent phosphate transport when insulin was added to the incubation media (11). This effect was enhanced by increasing the concentration of insulin. A significant stimulation occurred at 10^{-10} M insulin and a maximal stimulation occurred at concentrations > 10^{-8} M.

Insulin has been shown to stimulate transport in other epithelia (22–27). Insulin increases short-circuit current and sodium transport in amphibian skin and toad bladder (24–27). Exposure of renal proximal tubular cells grown in culture to insulin results in an increase in Na^+/H^+ antiporter activity (28). In many tissues insulin stimulates Na^+/K^+-ATPase (29).

The present study demonstrates that insulin has a direct dose-dependent effect to stimulate transport in the PCT when added to the bath but not the lumen. The insulin-induced stimulation in transport is present at physiologic concentrations. Insulin stimulates glucose as well as bicarbonate and chloride absorption in the PCT.

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