Effect of parathyroid hormone on renal tubular permeability

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Lorentz, William B., Jr. Effect of parathyroid hormone on renal tubular permeability. Am. J. Physiol. 231(5): 1401-1407. 1976—The effect of parathyroid hormone (PTH) on renal tubular permeability has been studied utilizing micropuncture techniques in the rat kidney. After microinjection into superficial nephrons during control conditions, inulin (98.8 ± 2.7%) and mannitol (97.2 ± 2.4%) recovery from the experimental kidney was essentially complete. During intravenous infusion of PTH, inulin (99.3 ± 2.9%) recovery was again complete. Mannitol recovery decreased significantly after both early-proximal (84.7 ± 5.8%, P < 0.001) and late-proximal (89.7 ± 2.8%, P < 0.001) injections. There was no loss of other mannitol or inulin following distal tubular injection. Late-proximal TF/P inulin ratios during control conditions were 2.10 ± 0.20 and decreased insignificantly to 1.99 ± 0.21 during PTH infusion. Late-proximal TF/P mannitol ratios were 2.09 ± 0.21 during control periods and during PTH infusion decreased significantly to 1.78 ± 0.19 (P < 0.001). These results indicate that PTH induces a change in proximal tubular permeability to a usually impermeable nonelectrolyte, mannitol. The effects of PTH on proximal tubular transport could be partially explained by this alteration in permeability, which would increase passive backflux of actively transported species and decrease net transport while having no effect on active transport.

IT IS NOW APPARENT that parathyroid hormone (PTH) influences many aspects of renal tubular function. Not only does parathyroid hormone decrease reabsorption of phosphate (13), but it also decreases the reabsorption of sodium (1), bicarbonate (9), and amino acids (20). These various actions of parathyroid hormone are mediated through stimulation of membrane-bound adenyl cyclase (4, 8) which subsequently increases intracellular levels of adenosine 3',5'-cyclic monophosphate (cAMP). Although the major site of action of parathyroid hormone is the proximal convoluted tubule, there is now increasing evidence that it may also affect transport from the distal nephron (2, 14). A recent study suggests that there are several portions of the nephron other than the proximal convoluted tubule which contain a PTH-responsive adenyl cyclase system (7). There seems to be no doubt that PTH broadly affects renal tubular transport. However, there is little information as to how PTH exerts its effect on these various processes. Parathyroid hormone could inhibit specifically multiple active transport systems. However, it is also possible that PTH, through stimulation of increased intracellular levels of cAMP, could alter membrane permeability to allow increased passive backflow of these substances from the peritubular capillary into the tubular lumen. The result would be decreased net transport with no effect on the active tubular lumen to peritubular capillary unidirectional flux. To evaluate the permeability characteristics of the nephron, we have studied the effects of PTH administration in thyroparathectomized rats utilizing microinjection, micropuncture, and clearance techniques. These studies indicate that PTH causes a change in tubular permeability to mannitol but has no effect on the simultaneously determined permeability to inulin.

METHODS

Male Sprague-Dawley rats weighing 225-320 g were anesthetized with sodium pentobarbital (50 mg/kg body wt ip). The animals were prepared for micropuncture as previously described (16). Throughout all experiments the animals were maintained on a heated table, and body temperature was maintained by a servo-control mechanism which was designed to maintain body temperature as monitored by rectal probe at 37.5 ± 0.5°C. Polyethylene catheters were placed in the right jugular vein for infusion of fluids and injection of lissamine green. The left carotid artery was cannulated for arterial sampling and monitoring of blood pressure. This tubing was connected to a Statham pressure transducer (model P23 DZ, Statham Instruments, Inc., Oxnard, Calif.), and blood pressure was recorded on a Grass polygraph (Grass Instrument Co., Quincy, Mass.). Both ureters were catheterized with PE-50 polyethylene tubing.

For the microinjection and micropuncture experiments, the kidney surface was illuminated with a fiberoptic light source (American Optical Corp., South Bridge, Mass.). The kidney was bathed with heated mineral oil delivered by a gravity system. The animals were made diuretic by the infusion of 5% mannitol and 0.85% sodium chloride, at a rate of 75 µl/min. Early-proximal, late-proximal, and distal convolutions were identified by the intravenous injection of 0.05 ml of 5% lissamine green. The total amount of lissamine green administered in the animal did not exceed 0.5 ml, and
no microinjections or tubular fluid collections were performed until at least 30 min had elapsed after the last injection of lissamine green. Intratubular pressures were measured by the method of Landis as modified by Gottschalk and Mylle (11).

A thyroparathyroidectomy was performed on each animal from 7 to 14 days prior to study. After thyroparathyroidectomy, the animals were maintained with 1% CaCl₂ in their drinking water. Urinary inorganic phosphate and cAMP excretion was monitored continuously throughout all experiments in order to be certain that there was evidence of renal end-organ response to the exogenous PTH. Urinary cAMP was measured by the protein-binding method of Gilman (10). Urinary inorganic phosphorus was measured by the malachite green micromethod (12).

In half the experiments, PTH was administered first and a series of microinjections or collections of tubular fluid performed during PTH infusion. Reinjections or recollections were performed at the same puncture site after cessation of PTH. A minimum of 45 min was allowed to elapse before initiating reinjections or punctures to allow the PTH effect to dissipate. In half the animals, control microinjections or tubular fluid collections were performed initially, and reinjection or recollection was performed beginning 45 min after initiation of PTH infusion. Highly purified parathyroid hormone was obtained from the Wilson Pharmaceutical and Chemical Co., Chicago. During experimental periods, a priming dose of 1 U of parathyroid hormone was given as an intravenous bolus, and a sustaining infusion of PTH was obtained from the Wilson Pharmaceutical and Chemical Co., Chicago. During experimental periods, a priming dose of 1 U of parathyroid hormone was given as an intravenous bolus, and a sustaining infusion of PTH was obtained from the Wilson Pharmaceutical and Chemical Co., Chicago. During experimental periods, a priming dose of 1 U of parathyroid hormone was given as an intravenous bolus, and a sustaining infusion of PTH was obtained from the Wilson Pharmaceutical and Chemical Co., Chicago.

During each clearance period, late-proximal tubular fluid collections were performed utilizing sharpened, siliconized micropipettes with tips of 8-10 μm (OD). Light mineral oil stained with Sudan black was injected in a length of 4-5 tubular diameters to block the tubule distal to the site of collection. Late-proximal puncture sites were identified by the passage of lissamine green. Controlled suction was used to keep the oil block in place and the tubular diameter constant if possible. An aliquot of the fluid collected was taken with previously calibrated volumetric pipettes and deposited into scintillation fluid. Measurements of levels of radioactivity of [¹⁴C]mannitol and [³H]inulin and [¹⁴C]mannitol in 5% mannitol and 0.85% sodium chloride at a rate of 75 μl/min. After a 30-min equilibration, three 10-min control or experimental collections were made, and then a period of 45 min was allowed to elapse for reequilibration, and an additional three 10-min collections of urine were obtained. In half the animals, PTH was infused during the first three collection periods and in half during the last three collection periods. PTH was administered during the experimental periods at the same rate during the microinjection experiments. Blood samples were drawn from the carotid artery at the midpoint of each clearance period. Radioactive levels in blood and urine were determined as described above.

During each clearance period, late-proximal tubular fluid collections were performed utilizing sharpened, siliconized micropipettes with tips of 8-10 μm (OD). Light mineral oil stained with Sudan black was injected in a length of 4-5 tubular diameters to block the tubule distal to the site of collection. Late-proximal puncture sites were identified by the passage of lissamine green. Controlled suction was used to keep the oil block in place and the tubular diameter constant if possible. An aliquot of the fluid collected was taken with previously calibrated volumetric pipettes and deposited into scintillation fluid. Measurements of levels of radioactivity of [¹⁴C]mannitol and [³H]inulin and [¹⁴C]mannitol in tubular fluid were performed as described above. Late proximal collection sites punctured during the initial clearance periods were reentered, and another tubular fluid collection was performed during the second series of clearances.

Results are presented as means ± 1 SD. The Student t test was used for evaluation of statistical significance.

RESULTS

A total of 202 satisfactory microinjections were performed in 29 animals. A microinjection was not considered satisfactory if there was visible leakage at the puncture site or retrograde flow. The results presented are recoveries measured in the urine from the injected kidney. All injections were performed with a test solution which contained both inulin and mannitol.

The recovery of mannitol and inulin after microinjection into early-proximal convolutions during control conditions and during infusion of PTH is shown in Fig.
1. During control conditions, mannitol recovery averaged 97.5 ± 2.4%. However, during infusion of PTH, there was a significant loss of mannitol. Average mannitol recovery was 84.7 ± 5.8%. This was significantly less than control (P < 0.001). Mannitol recovery during PTH infusion as the first experimental maneuver was 85.8 ± 6.9%. When PTH was infused after the control microinjections, mannitol recovery was 83.9 ± 5.0%. This difference was not significant. Inulin recovery was essentially complete after both control and experimental microinjections. Inulin recovery averaged 99.0 ± 2.8% during control conditions and 99.3 ± 2.3% during PTH infusion. The transit time was significantly prolonged after reinjection at the same puncture site. The transit time for each microinjection is defined as the time from the beginning of injection until the first appearance of the nigrosin-stained test solution in the distal convolution of the nephron under study. The transit time was 29 ± 11 s after control injections and 46 ± 19 s after experimental injections (P < 0.001).

Recovery of inulin and mannitol after microinjections into late-proximal convolutions is shown in Fig. 2. Mannitol recovery after late-proximal injections during control periods was 97.9 ± 2.1%. During infusion of PTH, mannitol recovery averaged 89.7 ± 2.8%. This difference is highly significant (P < 0.001). Inulin recovery was again essentially complete. Inulin recovery averaged 99.3 ± 2.4% during control conditions and 98.9 ± 1.9% during PTH infusion. Mannitol recovery averaged 90.6 ± 2.5% when PTH was infused initially. Mannitol recovery when PTH was infused after the control microinjections was 88.6 ± 2.9%. This difference was not significant. The transit time from the start of the late-proximal injections until the injectate appeared in its distal convolution was 24 ± 14 s during control microinjections and 29 ± 19 s during experimental injections. This difference was not significant.

In contrast to the proximal convolution microinjections, after microinjection into distal convolutions, no loss of inulin or mannitol was demonstrable. Recovery after distal microinjection is shown in Fig. 3. Mannitol recovery averaged 98.9 ± 2.3% during control conditions and during experimental periods averaged 99.0 ± 2.5%. Inulin recovery was 99.2 ± 1.8% during control periods and averaged 98.9 ± 2.3% during experimental periods.

In an additional six animals, late-proximal micropuncture tubular fluid collections and simultaneous clearance studies of inulin and mannitol were performed. The results of the late-proximal micropuncture collections are shown in Fig. 4. Note in Fig. 4A that during control conditions the TF/P mannitol ratio was 2.09 ± 0.21 while the simultaneously determined TF/P inulin ratio was 2.11 ± 0.20. During infusion of PTH, the average TF/P mannitol ratio was 1.78 ± 0.19 while the TF/P inulin ratio was 1.99 ± 0.21. The ratio of TF/P mannitol to TF/P inulin during control periods was 0.98 ± 0.03 and declined during infusion of PTH to 0.89 ± 0.02. This difference was statistically significant (P < 0.001). In Fig. 4B note that the TF/P values for both inulin and mannitol were lower during infusion of PTH than during control periods. The decrease in the TF/P mannitol ratio from 2.09 ± 0.21 during control periods to 1.78 ± 0.19 during infusion of PTH was highly significant (P < 0.001). Although the average TF/P inulin ratio during control periods was 2.11 ± 0.20 and decreased during infusion of PTH to 1.99 ± 0.21, this difference was not statistically significant (0.10 > P > 0.05).

The simultaneous clearances of inulin and mannitol are shown in Table 1. There was no difference in inulin and mannitol clearance during control periods, and the average clearance was 1.29 ml/min per 100 g body wt. During infusion of PTH, inulin clearance decreased insignificantly to 1.20 ± 0.57 ml/min per 100 g body wt, while mannitol clearance decreased to 1.08 ± 0.50 ml/min per g body wt. The difference between mannitol clearances during control and PTH infusion was not significant. However, when the individual ratios of mannitol to inulin clearance for each clearance period are compared, the decrease from a ratio of 0.98 ± 0.02 to 0.89 ± 0.04 during infusion of PTH was highly significant (P < 0.001). Inulin clearance also tended to decrease with time. When initial clearance periods are compared to those after reequilibration, average inulin clearance decreased from 1.34 ± 0.49 to 1.17 ± 0.45 ml/min per 100 g body wt. This difference was not significant.

Random measurements of intratubular pressures in

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**FIG. 1.** Mannitol and inulin recoveries after simultaneous early-proximal microinjections during control conditions and during infusion of PTH. Dashed horizontal line represents 100% recovery of injected substances. Solid lines connect reinjection pairs performed at same puncture site.
proximal convolutions were made during control and experimental periods in all animals studied. Average intratubular pressure during control periods was 12.2 ± 2.3 mmHg (n = 116). During experimental periods, average proximal intratubular pressure was 12.4 ± 2.5 mmHg (n = 110). This difference was not significant. To determine if there was a significant renal response to the exogenous PTH, urinary excretion of cAMP and inorganic phosphorus (Pi) was measured at the beginning and end of each control and experimental period during the microinjection studies and measured in each clearance period during the micropuncture and clearance studies. During control conditions, urinary cAMP excretion averaged 72.6 ± 45.1 pmol/liter (n = 76). During infusion of PTH urinary cAMP averaged 171.6 ± 92.3 pmol/liter (n = 76). This difference was highly significant (P < 0.001). Inorganic phosphorus excretion during control conditions averaged 6.6 ± 6.1 µg/m and increased to 20.7 ± 11.6 µg/m (n = 76). This increase was also highly significant (P < 0.001).

Excretion of mannitol and inulin by the contralateral kidney was monitored after each microinjection. After early-proximal microinjections during PTH administration, 54.2 ± 9.7% of the mannitol not recovered from the experimental kidney was excreted by the contralateral kidney. After late-proximal microinjections during PTH administration, mannitol excretion by the contralateral kidney averaged 47.4 ± 11.3% of that not recovered from the experimental kidney.

Mean arterial blood pressure during control conditions averaged 87 ± 11 mmHg (n = 119) and during experimental periods averaged 89 ± 10 mmHg (n = 119). Blood pressure did decrease when initial microinjections and tubular fluid collections are compared to reinjections and recollections. Mean arterial pressure during the initial series was 92 ± 6 mmHg and during reinjections or recollections decreased significantly to 81 ± 7 mmHg (P < 0.001). For purposes of analysis, the mean blood pressure recording immediately after a microinjection or the midpoint of a clearance period was chosen as the value for analysis. Transient decreases in blood pressure after administration of pentobarbital were not considered for analysis.

DISCUSSION

The results of this study demonstrate that the tubular epithelium undergoes a change in its permeability characteristics during the infusion of PTH. The segmental recovery of mannitol after experimental microinjection is shown in Table 2. When mannitol recovery during infusion of PTH after early proximal injec-
has been similar in both control and experimental periods. Any effects of hypothyroidism on tubular permeability should have been evident during the control series of microinjections. No loss of inulin or mannitol was evident after control microinjections.

Previous work has shown that elevated intratubular pressure can lead to increased permeability of the nephron to mannitol and other usually impermeable molecules (3, 17). However, there was no difference in intratubular pressures between control injections and those performed during infusion of PTH. Transit time to the distal tubule was prolonged during reinjections. This was presumably due to lower arterial blood pressure since previous work has shown that aortic constriction above the renal arteries will prolong transit time (17). However, prolongation of transit time does not cause significant mannitol loss from the nephron (17). Since the order of the control injections and those performed during PTH were alternated, any loss of mannitol due to prolongation of transit time alone should have showed in those control injections performed after PTH infusion. The micropuncture studies were in accord with the microinjection studies in that the late-proximal TF/P mannitol ratios were significantly lower than the simultaneously performed TF/P inulin ratios during infusion of PTH. Similarly, the whole-kidney clearance studies demonstrate a net loss of mannitol from the tubule as compared to inulin since the ratio of mannitol clearance to inulin clearance decreased significantly during infusion of PTH. These studies do not rule out the possibility of bidirectional movements of mannitol across the tubular epithelium; however, they all do show that the predominant flux is from the tubular lumen to the peritubular capillary.

These results are similar to those previously reported from this laboratory during infusion of cAMP or dibutyryl cAMP (16). Both studies demonstrate that

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<th>TABLE 1. Effect of PTH on mannitol and inulin clearance</th>
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<tr>
<td>Control</td>
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<td>( C_{\text{ur}} ), ml/min per 100 g body wt</td>
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<td>(n = 21)</td>
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<tr>
<td>( C_{\text{mannitol}} ), ml/min per 100 g body wt</td>
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<tr>
<th>TABLE 2. Segmental mannitol recovery during PTH infusion</th>
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<td>% Mannitol Recovery</td>
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<tr>
<td>Early proximal ((n = 34))</td>
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<td>Late proximal ((n = 33))</td>
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<td>Distal ((n = 34))</td>
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exogenous cAMP or endogenous stimulation of AMP by PTH can alter the passive permeability characteristics of the renal tubular epithelium. However, these studies are not strictly comparable because the animals studied here had previously had a thyroparathyroidectomy, and systemic infusion of cAMP induces alterations in systemic hemodynamics (15).

Although there is no evidence from this study which suggests the possible route of mannitol flux secondary to this alteration of permeability, it would seem reasonable to suggest that there is an alteration in the permeability characteristics of the tight junction with the Necturus nephron to raffinose during conditions of intercellular spaces. Boulpaep (5) has shown that there is an alteration in the permeability characteristics of the Necturus nephron to mannitol during conditions of volume expansion. Additionally, the studies of Bulger et al. (6) have shown changes in the tight junction with expansion of the lateral intercellular spaces of the proximal tubule rat associated with increased intratubular pressure which alters proximal tubular permeability to mannitol. A decrease in net transport then would result by increased backdiffusion from the peritubular capillary into the tubular lumen with no effect on the active unidirectional movement from the tubular lumen to the peritubular capillary. This could explain the decreased net transport of such varied substances as bicarbonate, sodium, and amino acids which have been directly observed or inferred as being secondary to PTH stimulation of the proximal convoluted tubule (1, 9, 20).

Although the micropuncture studies did not show a significant decrease in sodium transport in the proximal tubule, TF/P inulin ratios were lower during PTH infusion. Since the order of PTH infusion was alternated, other variables may have affected sodium transport. Arterial blood pressure was significantly lower during PTH infusion compared to saline loading. Microperfusion studies (18) suggest that phosphate transport at some point beyond the late proximal convoluted tubule. There was no evidence of a change in renal tubular permeability during PTH stimulation in relation to phosphate transport at some point in the nephron beyond the proximal convoluted tubule and perhaps in the distal convoluted tubule. There was no evidence of a change in the permeability of the distal tubular epithelium or points beyond to mannitol during PTH stimulation. Further, microperfusion studies (18) suggest that phosphate transport in the proximal tubule is characterized by primarily a lumen-to-plasma flux with little or no backdiffusion. It therefore seems likely that the permeability change observed may not contribute to the inhibition of phosphate transport during PTH infusion.

The significance of this observed change in renal tubular permeability during PTH stimulation in relation to phosphate transport is questionable. Recent observations (2, 14) suggest that there is indeed active phosphate transport at some point in the nephron beyond the proximal convoluted tubule and perhaps in the distal convoluted tubule. There was no evidence of a change in the permeability of the distal tubular epithelium or points beyond to mannitol during PTH stimulation. Further, microperfusion studies (18) suggest that phosphate transport in the proximal tubule is characterized by primarily a lumen-to-plasma flux with little or no backdiffusion. It therefore seems likely that the permeability change observed may not contribute to the inhibition of phosphate transport during PTH infusion.

This study was supported by National Institutes of Health Grant AM-17855.

Received for publication 10 March 1976.

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