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Thyroid hormone modulates rabbit proximal straight tubule paracellular permeability

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Abstract

Proximal straight tubules (PST) from both neonatal and hypothyroid adult rabbits have a lower rate of passive volume absorption when perfused with a high-chloride solution simulating late proximal tubular fluid than adult rabbit PST. We hypothesized that the maturational increase in serum thyroid hormone levels mediates the developmental changes in PST paracellular permeability. Neonatal tubules had lower chloride permeability, higher transepithelial resistance, but comparable mannitol permeability compared with adult PST. The present in vitro microperfusion study directly examined whether thyroid hormone affects passive solute flux and whether thyroid hormone could explain the developmental changes in PST paracellular permeability. Passive chloride transport was 62.1 ± 4.5 , 23.1 ± 7.7 , and 111.6 ± 5.6 $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ in PST from euthyroid, hypothyroid, and hypothyroid animals that received thyroid treatment, respectively (control different from hypothyroid and thyroid treatment at $P < 0.05$). This was due to a thyroid hormone-mediated change in chloride permeability (P_{Cl}). Mannitol permeability was 3.65 ± 1.03 , -0.19 ± 0.72 , and $3.60 \pm 1.12 \times 10^{-6}$ cm/s in PST from euthyroid animals, hypothyroid animals, and hypothyroid rabbits that received thyroid replacement, respectively ($P < 0.05$ hypothyroid vs. euthyroid and thyroid replacement). We demonstrate that PST from hypothyroid animals have a higher passive $P_{\text{Na}}/P_{\text{Cl}}$ and $P_{\text{HCO}_3}/P_{\text{Cl}}$ than euthyroid controls. Finally, we examined whether these changes in permeability were paralleled by a change in PST paracellular resistance. Resistance was measured by current injection and cable analysis. The resistance in PST from hypothyroid rabbits was $6.3 \pm 0.8 \Omega \cdot \text{cm}^2$, which was not different from control of $4.8 \pm 0.7 \Omega \cdot \text{cm}^2$, or $7.0 \pm 0.7 \Omega \cdot \text{cm}^2$ in hypothyroid animals that received thyroid replacement. Therefore, the maturational increase in thyroid hormone levels does not fully explain the developmental changes in the paracellular pathway.

Keywords

paracellular pathway; resistance; chloride permeability; mannitol permeability

The proximal tubule receives an ultrafiltrate from the glomerulus and reabsorbs two-thirds of this fluid in a nearly isosmotic fashion. The early proximal tubule reabsorbs glucose and amino acids and preferentially reabsorbs bicarbonate over chloride ions (18, 27). This leaves the luminal fluid in the late proximal tubule devoid of organic solutes and with a higher chloride concentration and lower bicarbonate concentration than that in the peritubular plasma (18, 27). The proximal tubule reabsorbs ~60% of the filtered chloride (2, 17).

In proximal tubules perfused with a high chloride-low bicarbonate solution simulating late proximal tubular fluid, approximately one-half of NaCl transport is active and transcellular, and one-half is passive and paracellular (1, 2, 4, 29, 30). Active NaCl transport is mediated by the parallel operation of the Na^+/H^+ exchanger and a Cl^-/base exchanger (2, 29). Passive Cl absorption is mediated, in large part, by diffusion of chloride down its concentration gradient (4, 6, 17). Thus the permeability properties of the paracellular pathway can affect a significant amount of proximal tubule NaCl transport.

We recently demonstrated that adult rabbit proximal straight tubules (PST) perfused with a late proximal tubular fluid had a 50% reduction in the rate of volume absorption when active transport was inhibited by 10^{-5} M bath ouabain (29, 30). In adult rabbits made hypothyroid by administration of water containing 0.1% propylthiouracil (PTU) from 26 days gestation to the mothers and subsequently to the rabbits after weaning until 8 wk of age, the rate of passive volume absorption from a high-chloride solution was not different from zero and increased to control values with administration of thyroid hormone. The reduction in volume absorption under these conditions in hypothyroid rabbits was consistent with an effect on the paracellular pathway affecting passive NaCl transport. We also demonstrated that the rate of volume absorption from a high-chloride solution in neonatal PST in the presence of 10^{-5} M bath ouabain, to inhibit active transport, was not different from zero and increased to adult levels with thyroid hormone treatment (30). The purpose of the present study was to examine directly whether the thyroid hormone status of rabbits alters the permeability properties of the PST.

METHODS

Animals

New Zealand White rabbits were born at our institution and studied between 7 and 9 wk of age. Hypothyroidism was induced by adding 0.1% PTU (Sigma, St. Louis, MO) to the drinking water of pregnant rabbits from *day* 26 of gestation (term gestation ~31 days) until the time of the experiment (8, 30). Untreated age-matched rabbits served as control adults. T4 levels were 5.0 ± 0.3 and 2.5 ± 0.4 $\mu\text{g}/\text{dl}$ in control and hypothyroid animals, respectively ($P < 0.0001$). Thyroid replacement (Sigma) was administered to some hypothyroid animals by daily subcutaneous injection of triiodothyronine (T3; 10 $\mu\text{g}/100$ g body wt) for 3 days and in the morning 2 h before death resulted in levels >800 ng/ml as previously described (30).

In vitro microperfusion flux studies

Isolated segments of superficial PST were perfused as previously described (4, 24, 28, 30, 31). Briefly, tubules were dissected in Hanks' balanced salt solution containing (in mM) 137 NaCl, 5 KCl, 0.8 MgSO₄, 0.33 Na₂HPO₄, 0.44 KH₂PO₄, 1 MgCl₂, 10 Tris·HCl, 0.25 CaCl₂, 2 glutamine, and 2 L-lactate at 4°C. Tubules were transferred to a 1.2-ml temperature-controlled bath. The tubules were perfused using concentric glass pipettes at 38°C. There was no difference in the tubular lengths in the groups perfused in any group as shown in RESULTS. The inner diameter was not significantly different between the three groups $P = 0.08$ ($n = 14$). The inner diameters were 25.6 ± 0.6 , 22.9 ± 1.4 , and 25.7 ± 0.9 μm in the control, hypothyroid, and thyroid replacement groups, respectively. However, the outer diameter was less in the hypothyroid group than the other two ($n = 14$). The outer diameters were 45.2 ± 0.6 , 40.0 ± 1.3 , and 44.6 ± 0.7 μm in the control, hypothyroid, and thyroid replacement groups, respectively ($P < 0.05$).

PST were perfused at ~10 nl/min. In flux studies the bathing solution was a serum-like albumin solution containing (in mM) 115 NaCl, 25 NaHCO₃, 2.3 Na₂HPO₄, 10 Na acetate, 1.8 mM CaCl₂, 1 MgSO₄, 5 KCl, 8.3 glucose, 5 alanine, and 6 g/dl bovine serum albumin. The perfusion solutions are described below. The osmolality of all solutions was adjusted to 295 mosmol/kgH₂O. The pH and osmolality of the bathing solution were maintained constant by continuously changing the bath at a rate of 0.5 ml/min. Net volume absorption (J_V ; in nl·mm⁻¹·min⁻¹) was measured as the difference between the perfusion (V_O) and collection (V_L) rates (nl/min) normalized per millimeter of tubular length (L). Exhaustively dialyzed [methoxy-³H] inulin was added to the perfusate at a concentration of 75 $\mu\text{Ci/ml}$ so that the perfusion rate could be calculated. The collection rate was measured with a 50-nl constant-volume pipette. The length (in mm) and internal diameter (in μm) were measured with an eyepiece micrometer. Tubules were incubated for at least 20 min before initiation of the control period. The transepithelial potential difference (PD; in mV) was measured using the perfusion pipette as the bridge into the tubular lumen.

In the first series of experiments, we examined mannitol permeability (P_{mann}) in euthyroid and hypothyroid rabbits and in rabbits that received thyroid treatment. PST were perfused with a solution containing (in mM) 10 mannitol, 110 NaCl, 30 Na gluconate, 5 NaHCO₃, 5 KCl, 1 Na₂HPO₄, 1.8 CaCl₂, 1 MgSO₄, 1 acetazolamide, and [¹⁴C]mannitol (25 $\mu\text{Ci/ml}$). Tubules were bathed in a serum-like albumin solution. We previously showed that under these conditions the rate of volume absorption was not different from zero (24, 28). P_{mann} was calculated as previously described in our laboratory (24, 28).

In the next series of experiments, we examined chloride permeability and net chloride flux in PST from euthyroid and hypothyroid rabbits and in rabbits that received thyroid replacement. Tubules were perfused at ~10 nl/min with a high-chloride solution simulating late proximal tubular fluid containing (in mM) 140 NaCl, 5 NaHCO₃, 5 KCl, 4 Na₂HPO₄, 1 CaCl₂, and 1 MgSO₄. Ouabain (10⁻⁵ M) was then added to the bathing solution to inhibit active transport. Lumen-to-bath chloride permeability was determined by the addition of ³⁶Cl to the tubular lumen (50 $\mu\text{Ci/ml}$) using the following equation

$$P_{Cl} = \frac{V_O - V_L}{A} \left[\frac{\ln C_O^* / C_L^*}{\ln V_O / V_L} + 1 \right]$$

where A is the area of the luminal area calculated from the internal radius and length, C_O^* and C_L^* are the concentration of ^{36}Cl (in counts·min⁻¹·nl⁻¹) in the perfusate and bath, and V_O and V_L are the perfusion and collection rates.

The chloride concentrations were also measured to determine the net chloride flux using the microtitration method of Ramsay (26). Net passive chloride flux was determined using the following equation

$$J_{Cl} = (V_O C_O - V_L C_L) / L$$

From the passive chloride flux, the chloride permeability was calculated using the following equation

$$P_{Cl} = \frac{[J_{Cl} - (1 - \sigma_{Cl}) \bar{C} J_V + J_{act}]}{DF_{Cl}}$$

where DF_{Cl} , the driving force for Cl, is defined as

$$DF_{Cl} = (\bar{C}_L - C_B) - \left(\bar{C} F \frac{\Delta V}{RT} \right)$$

$$\bar{C}_L = (C_O + C_L) / 2$$

is the mean luminal chloride concentration, and

$$\bar{C} = (\bar{C}_L + C_B) / 2$$

is the mean chloride concentration where C_B is the bath chloride concentration. V is the transepithelial PD, F is the Faraday constant, T is the temperature in Kelvin, and R is the gas constant. J_{act} was zero in these studies because tubules had ouabain in the bathing solution.

There were at least three measurements of each parameter in each period. The mean rate of volume absorption was used as the rate for that tubule. Analysis of variance using the Student-Newman-Kuels method was used to determine statistical significance. There were at least six experiments in each group.

Measurement of transepithelial resistance

The specific resistance (R_m) was measured as described by Berry and by our laboratory (6, 25). Briefly, PST from control, hypothyroid, and hyperthyroid New Zealand White rabbits

were perfused and bathed with Hanks' solution at 37°C. Tubules were perfused with a double-barreled pipette made from theta glass (Hilgenberg Glass, Hilgenberg, Germany). One barrel of the pipette was used to measure the PD at the perfusion end, whereas the other barrel was used to pass current (30–60 nA) from a Grass S44 stimulator (Grass Instruments, Quincy, MA) via silver wire. Details of the methodology have been published by our laboratory (25). Cable analysis was used to calculate the length constant (λ ; μm), input resistance (Ω), transepithelial resistance ($\Omega\cdot\text{cm}$), and the specific resistance ($\Omega\cdot\text{cm}^2$) as previously described (6, 19).

In the final series of experiments, we measured the relative sodium to chloride permeability ($P_{\text{Na}}/P_{\text{Cl}}$) and bicarbonate to chloride permeabilities ($P_{\text{HCO}_3}/P_{\text{Cl}}$) in PST from rabbits in the three groups. $P_{\text{Na}}/P_{\text{Cl}}$ and $P_{\text{HCO}_3}/P_{\text{Cl}}$ were calculated from the passive transepithelial PD due to imposed ion concentration gradients exactly as previously described in our laboratory (25) and using similar methodology as used by others (7, 25, 32). All bathing solutions contained 10^{-5} M ouabain to inhibit any transepithelial PD generated from active transport. Tubules were perfused with an ultrafiltrate-like solution. The composition of the bathing solutions used to measure dilution potentials is shown in Table 1.

RESULTS

In the first series of experiments, we examined P_{mann} in euthyroid, hypothyroid, and rabbits that received thyroid treatment. Mannitol is a sugar that is neither metabolized nor actively transported by the proximal tubule. The mean tubular lengths were 1.8 ± 0.1 , 1.8 ± 0.1 , and 1.5 ± 0.1 mm in the euthyroid, hypothyroid, and thyroid replacement groups, respectively [P = not significant (NS)]. The perfusion solution was designed to produce a rate of volume absorption of zero in tubules bathed in a serum-like albumin solution (28). The rate of volume absorption was 0.01 ± 0.02 , -0.16 ± 0.09 , and -0.03 ± 0.03 $\text{nl}\cdot\text{mm}^{-1}\cdot\text{min}^{-1}$ in the euthyroid, hypothyroid, and thyroid replacement groups, respectively (P = NS). The P_{mann} s in the three groups are shown in Fig. 1. As can be seen, P_{mann} in the hypothyroid group was significantly less than the euthyroid or thyroid replacement groups ($P < 0.05$). Three days of thyroid replacement resulted in an increase in P_{mann} to a value not different than the euthyroid control group.

In the next series of experiments, we examined the effect of thyroid hormone on the rate of passive volume absorption, net passive chloride transport, and the transepithelial PD in PST perfused with a high-chloride solution simulating late proximal tubular fluid and bathed in a serum-like albumin solution. The mean tubular lengths were 1.9 ± 0.1 , 1.6 ± 0.1 , and 1.6 ± 0.1 mm in the euthyroid, hypothyroid, and thyroid replacement groups, respectively (P = NS). The results of these experiments are shown in Fig. 2. As is seen, the rate of volume absorption in the hypothyroid group was not different from zero and was less than that in the euthyroid rabbits and those that received thyroid treatment. The rate of net passive chloride transport was less in the hypothyroid group than in the other two groups. The thyroid treatment group had a higher rate of chloride transport than did the euthyroid controls. The transepithelial PD in the hypothyroid group was not different from zero and was significantly less than the positive PD in the proximal tubules from euthyroid rabbits and rabbits that received thyroid replacement.

We also examined chloride permeability in PST from euthyroid, hypothyroid, and rabbits that received thyroid replacement. The mean tubular lengths were 1.9 ± 0.1 , 1.6 ± 0.1 , and 1.6 ± 0.1 mm in the euthyroid, hypothyroid, and thyroid replacement groups, respectively ($P = \text{NS}$). We assessed chloride permeability measuring lumen-to-bath ^{36}Cl permeability as well as net chloride permeability. The results are shown in Fig. 3. As expected, the $P_{^{36}\text{Cl}}$ was higher than the P_{Cl} , because $P_{^{36}\text{Cl}}$ is unidirectional and P_{Cl} is a measure of net permeability that also accounts for chloride flux from the bath into the lumen. In both groups of experiments, the chloride permeability was less in the PST from hypothyroid rabbits than euthyroid rabbits and rabbits that received thyroid replacement. The rabbits that received 3 days of thyroid hormone had a higher chloride permeability than the euthyroid controls. Thus the thyroid hormone status of the animal affects the chloride permeability of the PST.

We next assessed whether thyroid hormone affected the resistance of the PST. Short tubules were purposefully dissected in these experiments. The mean tubular lengths were 170 ± 10 , 180 ± 10 , and 210 ± 10 μm in the euthyroid, hypothyroid, and thyroid replacement groups, respectively ($P = \text{ns}$). The results are shown in Fig. 4. As can be seen, there was no difference in the transepithelial resistance in proximal tubules from control, hypothyroid animals, and hypothyroid animals that received thyroid replacement. Thus the changes in proximal tubular permeability were not paralleled by measurable changes in resistance.

In the final series of experiments, we measured the PD generated by imposed ionic gradients to determine the $P_{\text{Na}}/P_{\text{Cl}}$ and the $P_{\text{HCO}_3}/P_{\text{Cl}}$ in control, hypothyroid, and rabbits that received thyroid replacement. As shown in Fig. 5, PST from hypothyroid rabbits had a significantly higher $P_{\text{Na}}/P_{\text{Cl}}$ than control rabbits and hypothyroid rabbits that received thyroid replacement. The $P_{\text{HCO}_3}/P_{\text{Cl}}$ was also higher in hypothyroid PST than control but thyroid replacement, while causing a reduction, was not significantly different than the hypothyroid group ($P = 0.10$).

DISCUSSION

This present study directly demonstrates that thyroid hormone alters the permeability properties of the paracellular pathway. Mannitol, a sugar that is not actively transported, had a permeability that was less in PST from hypothyroid rabbits than in control rabbits. P_{mann} returned to control values with thyroid treatment of hypothyroid animals. Hypothyroid animals also had a reduction in chloride permeability, which reduced passive net chloride transport and volume absorption in tubules perfused with a late proximal tubular fluid and bathed in a serum-like albumin solution containing ouabain to inhibit active transport. The fact that the chloride permeability and net chloride flux in hypothyroid animals that received thyroid replacement were greater than controls is likely the result of the fact that these rabbits had elevated levels of thyroid hormone at the time of study (30).

The rates of active and passive volume absorption in neonatal PST perfused with a high-chloride-low-bicarbonate solution simulating late proximal tubular fluid are both less in neonates than in adult PST (29, 30). There is a postnatal increase in thyroid hormone levels (5). We hypothesized that this postnatal increase in thyroid hormone resulted in the

developmental changes in PST paracellular permeability (30). Although the maturational changes in PST chloride permeability are prevented in hypothyroid animals, there were distinct differences in the paracellular maturation of passive transport that cannot be fully explained by the developmental changes in thyroid hormone (25). In this study, thyroid hormone affected the chloride and mannitol permeability but had no effect on the paracellular resistance. This is distinctly different from what we saw in the maturation of PST. We found that there is a maturational decrease in paracellular resistance from $11.3 \pm 1.4 \Omega \cdot \text{cm}^2$ in neonatal PST compared with $6.7 \pm 0.7 \Omega \cdot \text{cm}^2$ in adult tubules; however, there was no developmental change in P_{mann} . Thus our hypothesis that the developmental changes in thyroid hormone can explain all the developmental changes that occur in the paracellular pathway is clearly incorrect. It must be noted that there is an inconsistency in our data in that hypothyroid animals had significant differences in chloride permeability, P_{mann} , $P_{\text{Na}}/P_{\text{Cl}}$, and $P_{\text{HCO}_3}/P_{\text{Cl}}$ compared with euthyroid controls; however, there was no difference in another marker of paracellular properties, resistance. However, it is possible that the difference in resistance was too small with changes in thyroid hormone status to measure a difference in resistance using the technique employed in this study.

The transepithelial PD in PST perfused with a high-chloride solution simulating late proximal tubular fluid and bathed in a serum-like albumin solution is lumen positive. This transepithelial PD is not affected by inhibiting active transport (29, 30) and is mediated by the transepithelial chloride and bicarbonate concentration gradients and the greater permeability of the superficial proximal tubule to chloride ions than bicarbonate ions (4, 6, 13, 32). In hypothyroid animals, the transepithelial PD was not different from zero, consistent with a reduction in P_{Cl} relative to P_{HCO_3} . This was directly demonstrated in studies showing that the $P_{\text{HCO}_3}/P_{\text{Cl}}$ was higher in hypothyroid than control PST. We also demonstrate that there is a significantly higher $P_{\text{Na}}/P_{\text{Cl}}$ in PST from hypothyroid animals than those from euthyroid control animals.

Thyroid hormone has been shown previously to affect several aspects of renal function. Hypothyroid rats have a lower renal blood flow and glomerular filtration rate but a higher fractional excretion of sodium than euthyroid rats (14, 22). In regard to the proximal tubule, hypothyroid rats have a lower rate of volume absorption compared with euthyroid controls (10, 11, 22). Thyroid hormone has been shown to affect proximal tubule Na^+/H^+ exchanger and Na-K-ATPase activities (3, 5, 9, 12, 15, 16, 33). The present study shows that the thyroid hormone status of the animal can affect the permeability properties of the paracellular pathway.

Previous studies were consistent with thyroid hormone altering the properties of the paracellular pathway (20, 21). In isolated toad bladders, addition of thyroid hormone increased the short-circuit current without a change in the transepithelial PD consistent with an increase in paracellular chloride permeability (20). In addition, the lumen-to-bath and bath-to-lumen flux of ^{36}Cl and ^{32}P were increased in toad bladders incubated with thyroid hormone consistent with an increase paracellular permeability (20). Thyroid hormone, however, does not cause an increase in permeability in all epithelia. This group subsequently demonstrated that small intestine passive phosphate and calcium transport were less in rats that received thyroid hormone for 7 days than in control rats (23). It is unclear at present

what is mediating the change in the properties of the paracellular pathway by thyroid hormone.

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Fig. 1. Mannitol permeability (P_{mannitol}) in perfused proximal straight tubules from 8-wk-old euthyroid, hypothyroid, and hypothyroid rabbits that received thyroid replacement. Tubules were perfused with a 0 J_v solution so that all transport would be passive. As can be seen, hypothyroid rabbits had a mannitol permeability not different from 0. The mannitol permeability was normalized with thyroid treatment. There were at least 6 experiments in each group.

Fig. 2.

Volume absorption (J_v), chloride transport (J_{Cl}), and transepithelial potential difference (PD) in proximal straight tubules from euthyroid, hypothyroid, and hypothyroid rabbits that received thyroid replacement. All tubules had 10^{-5} M ouabain in the bathing solution to inhibit active transport. The rates of passive volume absorption, net passive chloride transport, and PD were less in the hypothyroid group compared with control and hypothyroid animals that received thyroid treatment. There were at least 6 experiments in each group.

Fig. 3.

Passive chloride permeability using ^{36}Cl ($P_{36\text{Cl}}$; A) and microtitration of Ramsey (P_{Cl} ; B) in euthyroid, hypothyroid, and hypothyroid rabbits that received thyroid replacement. All tubules had 10^{-5} M ouabain in the bathing solution to inhibit active transport. The lumen-to-bath and net passive chloride permeability were lower in the hypothyroid group and increased with thyroid hormone treatment. There were at least 6 experiments in each group.

Fig. 4.

Transepithelial resistance using current injection and cable analysis in euthyroid, hypothyroid, and hypothyroid rabbits that received thyroid replacement. There was no difference in the transepithelial resistance comparing euthyroid, hypothyroid, and hypothyroid animals that received thyroid replacement. There were at least 6 experiments in each group.

Fig. 5.

P_{Na}/P_{Cl} (A) and P_{HCO_3}/P_{Cl} (B) in euthyroid, hypothyroid, and hypothyroid rabbits that received thyroid replacement. All tubules had 10^{-5} M ouabain in the bathing solution to inhibit active transport. Hypothyroid P_{Na}/P_{Cl} and P_{HCO_3}/P_{Cl} were significantly greater than control. Thyroid replacement to hypothyroid animals normalized P_{Na}/P_{Cl} ($P < 0.05$) and decreased the P_{HCO_3}/P_{Cl} to near control levels ($P = 0.10$).

Table 1Solutions used to measure $P_{\text{Na}}/P_{\text{Cl}}$ and $P_{\text{HCO}_3}/P_{\text{Cl}}$

	Ultrafiltrate-Like Solution, mM	NaCl Dilution, mM	NaHCO ₃ Dilution, mM
NaCl	104	54	104
NaHCO ₃	25	25	5
Na ₂ HPO ₄	4	4	4
NaAc	7.5	7.5	7.5
CaCl ₂	1	1	1
MgSO ₄	1	1	1
KCl	5	5	5
Glucose	5	5	5
Alanine	5	5	5
Urea	5	5	5
Mannitol		*	*

P_{Na} , P_{Cl} , and P_{HCO_3} : sodium, chloride, and bicarbonate permeability, respectively.

* Mannitol was added to increase the osmolality to 295 mosmol/kgH₂O.