Published in final edited form as:
Am J Physiol Renal Physiol. 2000 April ; 278(4): F596–F602.

Maturation of proximal straight tubule NaCl transport: role of thyroid hormone

MEHUL SHAH[1], **RAYMOND QUIGLEY**[1], and **MICHEL BAUM**[1,2]

[1]Departments of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, Texas 75235-9063

[2]Departments of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas 75235-9063

Abstract

We have recently demonstrated that the rates of both active and passive proximal straight tubule (PST) NaCl transport in neonatal rabbits were less than in adults. In this segment NaCl entry across the apical membrane is via parallel Na^+/H^+ and Cl^-/OH^- exchangers, which increases in activity with maturation. The present in vitro microperfusion study examined whether thyroid hormone plays a role in the maturational increase in PST NaCl transport. Neonatal and adult PST were perfused with a high-chloride-low bicarbonate solution without organic solutes, simulating late proximal tubule fluid. Thyroid hormone-treated neonates had a higher rate of PST total and passive NaCl transport. In 8-wk-old animals that were hypothyroid since birth, the maturational increase in total and passive NaCl transport was prevented. Thyroid treatment for 4 days in hypothyroid 8-wk-old rabbits increased the rate of both total and passive NaCl transport. The maturational increases in both Na^+/H^+ and Cl^-/OH^- exchange activities were blunted in 8-wk-old hypothyroid animals and increased to control levels with thyroid treatment. This study demonstrates that thyroid hormone is a factor responsible for the maturational increase in both active and passive PST NaCl transport.

Keywords

sodium/hydrogen antiporter; chloride/base exchanger; kidney development; passive transport

---

The Proximal Tubule Reabsorbs ~60% of the filtered NaCl. The preferential reabsorption of organic solutes and bicarbonate by the early proximal tubule leaves the luminal fluid with a higher chloride and lower bicarbonate concentration than the peritubular plasma (19, 24). In the proximal tubule approximately one-half of NaCl transport is passive and paracellular (4, 25). The parallel operation of the apical membrane Na^+/H^+ antiporter and Cl^-/base exchangers mediate NaCl entry into the proximal tubule cell (1, 18, 25).

Address for reprint requests and other correspondence: M. Baum, Dept. of Pediatrics, Univ. of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9063 (Mbaum@mednet.swmed.edu)..

We have recently examined the postnatal developmental changes in proximal straight tubule (PST) NaCl transport (25). In rabbit PST perfused with a high chloride-low bicarbonate solution simulating late proximal tubular fluid and bathed in a serum-like albumin solution, the rate of volume absorption, a reflection of both active and passive NaCl transport, was over twofold higher in the adult than neonatal segment (25). NaCl transport was inhibited by 50% by bath ouabain in adult tubules and totally inhibited in the neonatal segment. Thus the rates of active and passive NaCl transport were higher in the adult tubule than were those in the neonate (25).

In the rabbit PST there was a fivefold increase in the Na^+/H^+ antiporter activity during postnatal development (25). Cl^-/base exchange in neonatal and adult PST was not affected by cyanide and acetazolamide, CO_2 and bicarbonate, or the addition of formate, consistent with Cl^-/OH^- exchange (18, 25). There was a sixfold maturational increase in apical membrane PST Cl^-/OH^- exchange activity (25).

The factors that produce the profound postnatal changes in active and passive proximal tubular transport remain to be elucidated. Both serum glucocorticoid and thyroid hormone levels are lower in neonates than in adults and increase at about the time of weaning (5, 14, 16, 26, 28). The purpose of the present study was to examine whether thyroid hormone affects neonatal PST active and passive NaCl transport. Our findings in this segment are consistent with an important role for thyroid hormone in the postnatal maturation of both active and passive NaCl transport. Most importantly, this study demonstrates that the postnatal increase in thyroid hormone can affect the permeability properties of the paracellular pathway.

METHODS

Animal preparation

Superficial PST from neonatal (18 ± 1 days) and adult New Zealand White rabbits (55 ± 1 days) were studied. Both male and female rabbits were used in these studies. Hyperthyroidism was induced by daily subcutaneous injection of 10 μg/100 g 3,5,3 -L-triiodothyronine (T_3; Sigma Chemical, St. Louis, MO) for 3 days and in the morning 2 h before death. Control neonates were injected with vehicle.

Hypothyroidism was induced by adding 0.1% propylthiouracil (PTU; Sigma Chemical, St. Louis, MO) to the drinking water of pregnant rabbits from the 26th day of gestation (term gestation ~31 days) until the time of experiment at ~8 wk of age (6). Untreated age-matched rabbits served as control adults. Thyroid treatment was administered to some hypothyroid animals by daily subcutaneous injection of T_3 (10 μg/100 g body wt) for 3 days and in the morning 2 h before death.

In vitro microperfusion

Isolated segments of neonatal and adult superficial PST (S2 segments) were perfused by using concentric glass pipettes as previously described (25). Briefly, tubules were dissected in Hanks' balanced salt solution containing (in mM) 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 10 Tris, 0.25 $CaCl_2$, 2 glutamine, and 2 lactate at 4°C.

Tubules were transferred to a 1.2-ml temperature-controlled bath for flux studies and a 0.2-ml chamber, in which the bathing solution was preheated to 38°C for intracellular pH (pH_i) studies.

In vitro microperfusion flux studies

Tubules were perfused at ~10 nl/min with a high-chloride solution simulating late proximal tubular fluid containing (in mM) 146 NaCl, 5 $NaHCO_3$, 5 KCl, 2.3 Na_2HPO_4, 1 $CaCl_2$, 1 $MgCl_2$, and 0.1 formate. Formate was added because it has been shown to stimulate NaCl transport in this segment (18). The bathing solution was a serum-like albumin solution containing (in mM) 115 NaCl, 25 $NaHCO_3$, 2.3 Na_2HPO_4, 10 Na acetate, 1.8 mM $CaCl_2$, 1 $MgSO_4$, 5 KCl, 8.3 glucose, 5 alanine, 1 butyrate, 1 glutamine, and 6 g/dl bovine serum albumin. The osmolality of these solutions was adjusted to 295 mosmol/kgH_2O. The pH and osmolality of the bathing solution were maintained constant by continuously changing the bath at a rate of 0.5 ml/min in flux studies.

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*-^{3}H]inulin was added to the perfusate at a concentration of 75 μCi/ml so that the perfusion rate could be calculated. The collection rate was measured with a 50-nl constant-volume pipette. The length was measured with an eyepiece micrometer.

The transepithelial potential difference (PD, in mV) 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, via bridges containing perfusion and an ultrafiltrate of the bathing solution, respectively, 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. The recording and reference calomel half-cells were connected to the high- and low-impedance sides, respectively, of an electrometer (model 602; Keithley Instruments, Cleveland, OH).

Tubules were incubated for at least 15 min before initiation of the control period. There were at least three collections in each period for measurement of volume absorption. The mean rate was used as the rate of volume absorption for that tubule. Ouabain (10^{-5} M) was then added to the bathing solution to inhibit active transport, and repeat collections were performed after incubation of 10 min.

Measurement of pH_i

The solutions used in these experiments are shown in Table 1. The fluorescent dye 2 ,7 -bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) was used to measure pH_i as described previously (3, 18, 25). pH_i was measured by using a Nikon inverted epifluorescent microscope attached to a PTI Ratiomaster at a rate of 30 measurements/s. A variable diaphragm was placed over the area to be measured. To calculate pH from the ratio of fluorescence (F_{500}/F_{450}), a nigericin calibration curve was performed as previously

described (3, 25). There was no difference in the calibration curves of adult and neonatal PST.

Tubules were incubated with the initial luminal and bathing solutions for at least 10 min after loading with 5×10^{-6} MBCECF and had a constant pH_i for several minutes before the measurement of the transporter activity. dpH_i/dt was measured from the slope of the change in pH_i immediately after a luminal fluid change. Steady-state pH_i values were reached within 1 min after a luminal fluid exchange, but pH_i was measured for several minutes to ensure a steady-state pH_i was achieved.

Apparent buffer capacity (β) was measured as previously described by using NH_3/NH^+_4 (3, 18, 25). Solutions (*C* and *F*) used for measurement of apparent buffer capacity did not contain Na^+ or Cl^- to inhibit all acidification mechanisms due to Na^+- and Cl^--dependent transporters. In the absence of HCO^-_3, buffer capacity was 28.1 ± 5.0 mM/pH in neonatal PST and 43.0 ± 6.6 mM/pH in adult PST (*P* = not significant) (25). Tubular volume was calculated from the measured inner and outer tubular diameters at ×400 magnification by using an eyepiece reticle.

Proton flux rates[1] (J_H, in $pmol \cdot mm^{-1} \cdot min^{-1}$) resulting from a luminal fluid change were calculated by using the following formula

$$J_H = \frac{dpH_i}{dt} \cdot \frac{V}{mm} \cdot \beta$$

where dpH_i/dt is the rate of initial change in pH_i after a luminal fluid change, V is the tubular volume in liters, and β is the buffer capacity.

Statistics

Data are expressed as means ± SE. Analysis of variance and the Student's *t*-test for paired and unpaired data were used to determine statistical significance.

RESULTS

Effect of thyroid hormone status in neonatal and adult animals

The mean weights of PTU water-treated adult animals and adult control animals were 1.3 ± 0.1 and 1.8 ± 0.1 kg, respectively ($P < 0.05$). Similarly, the mean weight of the kidney in the PTU water-treated adults (6.4 ± 0.3 g) was significantly lower than that in adult control animals (7.8 ± 0.4 g, $P < 0.05$). As shown in Table 2, serum T_3 levels in the PTU water-treated animals were comparable to neonates and both were ~50% that of adult control animals. However, the serum corticosterone level in the PTU water-treated group was similar to that in the adult control group, demonstrating that these animals were not glucocorticoid deficient. Thyroid administration given to neonates and PTU-treated adults resulted in levels >800 ng/dl when assayed at the time of death, 2 h after thyroid hormone administration.

[1]All proton fluxes are presented as absolute values and are expressed as J_H in picomoles per millimeter per minute.

Effect of thyroid hormone status on PST volume

The mean tubular volume of PST in the control and thyroid-treated neonates were 440.0 ± 14.4 × 10^{-12} and 476.9 ± 14.3 × 10^{-12} l/mm.The mean tubular volume of PST from hypothyroid and control adult animals and tubules from hypothyroid animals that received thyroid treatment were 845.1 ± 37.3 × 10^{-12}, 1,075 ± 47.9 × 10^{-12}, and 1,042 ± 31.6 × 10^{-12} l/mm, respectively. The tubular volume of the PST from the hypothyroid group was less than the control and thyroid replacement group ($P < 0.01$).

Effect of thyroid hormone on neonatal PST NaCl transport

In the first series of experiments PST were perfused with a high-chloride low-bicarbonate solution without organic solutes, simulating late proximal tubular fluid. Tubules from control and thyroid-treated neonates (10 μg/100 g body wt for 3 days and in the morning 2 h before death) were compared as shown in Fig. 1. As can be seen, PST from thyroid-treated neonates had a higher rate of volume absorption than that of vehicle-treated control neonates. Ouabain (10^{-5} M) was then added to the bathing solution to inhibit active transport. The rate of passive transport was greater in PST from the thyroid-treated than that from vehicle-treated control neonates. These data are consistent with thyroid hormone causing acceleration of maturation of PST active and passive NaCl transport.

The transepithelial potential difference in adult proximal tubules perfused with a high-chloride solution simulating late proximal tubular fluid is lumen positive due to the chloride and bicarbonate concentration gradients and the greater permeability of the paracellular pathway to chloride ions (4). As is shown in Fig. 1, the transepithelial potential difference was greater in thyroid-treated tubules than control PST ($P < 0.05$). These data are consistent with a greater chloride than bicarbonate permeability in thyroid-treated neonates. Addition of bath ouabain did not cause a significant change in the transepithelial potential difference in either group, indicating that the potential difference was not due to active transport. The rates of active and passive volume absorption and the lumen-positive potential difference in thyroid-treated neonates are all comparable to that previously found in adult PST (25).

Effect of thyroid hormone on neonatal apical Na^+/H^+ and Cl^-/OH^- exchange activity

We first examined the apical Na^+/H^+ antiporter activity in PST perfused with *solution D* and bathed with *solution C*. Both were HEPES-buffered solutions without sodium and chloride. We excluded chloride from all the solutions to prevent Cl^-/base exchange from attenuating any pH_i changes. In the experimental period, luminal sodium was added (*solution E*) and dpH_i/dt and J_H were measured. As shown in Fig. 2, Na^+/H^+ antiporter activity in thyroid hormone-treated neonates was significantly greater than that in the vehicle-treated control neonates.

We next examined the effect of thyroid hormone on Cl^-/base activity. In PST apical Cl^-/base activity is due to Cl^-/OH^- exchange in both adult and neonatal tubules (18, 25). In these studies, tubules were perfused (*solution B*) and bathed (*solution A*) with chloride-containing HEPES-buffered solutions without sodium. We excluded sodium from all the solutions to prevent Na^+-dependent transporters from attenuating any pH_i changes. In the experimental period, luminal chloride was removed (*solution D*) and dpH_i/dt and J_H

resulting from the transporter activity were measured. As is shown in Fig. 3, PST from neonatal rabbits injected with thyroid hormone had an increase in Cl^-/OH^- activity. These data demonstrate that Cl^-/OH^- activity is affected by thyroid hormone.

Effect of hypothyroidism and thyroid treatment on NaCl transport in adult PST

If the postnatal rise in thyroid hormone was responsible for the maturational increase in NaCl transport, then the developmental increase in NaCl transport should be prevented in animals made hypothyroid from birth. The rates of PST volume absorption from a high-chloride perfusate were the same in these 8-wk-old rabbits as we have measured in adult rabbits of this age and older (25). As is shown in Fig. 4, hypothyroid animals had a lower rate of total and passive transport than age-matched controls. The rates of volume absorption in PST of hypothyroid 8-wk-old rabbits were comparable to the rates in neonatal rabbit PST. In hypothyroid rabbits that received thyroid replacement, the rates of total and passive volume absorption were comparable to controls and greater than the hypothyroid group.

In these experiments the transepithelial potential difference in control tubules was not different after the addition of bath ouabain (Fig. 4). This is consistent with the electroneutral active NaCl transport. The potential difference in the hypothyroid PST was -1.4 ± 0.3 mV in the control period and increased to -0.6 ± 0.3 mV after the addition of bath ouabain ($P < 0.01$). The cause for this small increase is unknown. The potential difference in the hypothyroid control and ouabain-treated PST were significantly less than the control groups, consistent with thyroid hormone affecting the maturation of the paracellular pathway. Thyroid replacement resulted in a potential difference not different from the control group and significantly higher than the hypothyroid group.

Effect of hypothyroidism and thyroid treatment on adult PST apical Na^+/H^+ and Cl^-/OH^- exchange activities

To further substantiate the role of thyroid hormone in maturation of apical Na^+/H^+ antiporter activity, we examined PST from hypothyroid animals. As shown in Fig. 5, J_H in PST from hypothyroid adult animals was significantly lower than that from the adult control group. When these hypothyroid adult animals were treated with thyroid hormone, J_H increased to that in the adult control group, confirming an important role of thyroid hormone in the maturation of apical Na^+/H^+ antiporter activity in the neonatal PST.

In the next series of experiments, we examined the effect of hypothyroidism on the apical membrane Cl^-/base activity in the PST. As shown in Fig. 6, J_H in the PST from hypothyroid adult animals was significantly lower than that from control adults. When these hypothyroid animals were treated with T_3, Cl^-/base activity increased significantly to a rate similar to that obtained in adult control PST. These data confirm a role of thyroid hormone in maturation of apical Cl^-/base exchange activity in the PST.

DISCUSSION

The present in vitro microperfusion study examined the effect of thyroid hormone on the maturation of PST NaCl transport. Administration of thyroid hormone to neonatal rabbits resulted in an increase in volume absorption in tubules perfused with a high chloride

solution simulating late proximal tubular fluid and when active transport was inhibited with bath ouabain. Thyroid hormone also increased PST Na^+/H^+ antiporter and Cl^-/OH^- exchange activity. Hypothyroidism blunted the maturational increase in total and passive NaCl transport as well as the developmental increase in Na^+/H^+ antiporter and Cl^-/OH^- exchanger activity in rabbit PST. These were all reversed with thyroid treatment.

Several studies have examined the effect of hypothyroidism on renal function in adult animals. Hypothyroid rats had a lower glomerular filtration rate and renal blood flow (22), and a higher fractional excretion of sodium than euthyroid control rats (15, 22). Lower rates of proximal tubule sodium transport have been implicated to explain the difference in renal sodium handling compared with euthyroid controls (11, 12, 22). In vivo rat micropuncture and shrinking droplet studies demonstrated a lower rate of proximal tubule transport compared with control animals (11, 12, 22). Administration of thyroid hormone to hypothyroid rats restored proximal tubule transport to control levels (11, 12). Hypothyroid rats and rabbits had a reduction in proximal tubule Na^+-K^+-ATPase activity that was restored by administration of thyroid hormone (2, 10, 13). However, administration of thyroid hormone to hypothyroid rats resulted in an increase in the rate of proximal tubule volume absorption before the time when a change in Na^+-K^+-ATPase activity was measured (10-12). These data suggest that the effect of thyroid hormone was not entirely due to its effect on the Na^+-K^+-ATPase, a result in concordance with this and our previous studies (5, 9).

In this study thyroid hormone resulted in an increase in Na^+/H^+ antiporter activity. This is in agreement with previous studies in neonates and adults showing an effect of thyroid hormone on brush-border membrane vesicle Na^+/H^+ antiporter activity (5, 17). The effect of thyroid hormone on Na^+/H^+ antiporter could be indirect and solely mediated by alterations in renal hemodynamics or other neural or hormonal changes. Although a hemodynamic effect of thyroid hormone on proximal tubular transport may be a contributing factor, a direct effect of thyroid hormone on proximal tubular cells to increase Na^+/H^+ antiporter activity has been described (9, 29). Thyroid hormone has been shown to increase the maximum velocity of the Na^+/H^+ antiporter in opossum kidney (OK) cells (28). We have recently found that thyroid hormone increases Na^+/H^+ antiporter by increasing NHE3 mRNA and protein abundance in OK cells in vitro (9). Thyroid hormone had no effect on NHE3 mRNA or protein stability but activated the promotor to increase NHE3 gene transcription (9).

Serum thyroid hormone levels increase during postnatal maturation (5, 26, 28), which may be of importance in renal development. We have recently examined the effect of thyroid hormone on neonatal rat Na^+/H^+ antiporter (5). Neonatal rats made hypothyroid by the addition of PTU to their drinking water had a lower rate of renal cortical brush-border membrane Na^+/H^+ antiporter activity. Renal cortical NHE3 protein abundance was less than one-half of that of euthyroid neonates, however, NHE3 mRNA abundance was comparable. Hyperthyroid neonates had higher rates of brush-border membrane Na^+/H^+ antiporter activity, NHE3 mRNA, and protein abundance than euthyroid control animals. Although these data suggest a possible role for thyroid hormone in the postnatal maturational increase in Na^+/H^+ antiporter in the rat, the effect of thyroid hormone on cortical Na^+/H^+ antiporter

activity was small. There was only a 10% decrease in brush-border membrane Na^+/H^+ antiporter activity in hypothyroid rats and a 10% increase in antiporter activity in rats given thyroid hormone. Thus there may be a difference in the importance of thyroid hormone in promoting the maturation of proximal tubule Na^+/H^+ antiporter in the rabbit and rat.

Perhaps the most important finding in this study is the effect of thyroid hormone on the passive NaCl transport. We had previously found that neonates have lower rates of both active and passive transport when perfused with a high-chloride solution simulating late proximal tubular fluid compared with adult tubules (25). Thyroid-treated neonates had a higher rate of volume absorption from a high-chloride perfusate compared with control tubules in the presence and absence of bath ouabain. In addition, the maturational changes in transport from a high-chloride perfusate in the presence and absence of bath ouabain were prevented in hypothyroid rabbits, which were studied at ~8 wk of age. The maturational increase in the rates of PST Na^+/H^+ antiporter and Cl^-/OH^- exchange activities were also blunted when animals were made hypothyroid.

In addition to the effect on passive and active volume absorption, thyroid hormone affected the transepithelial potential difference. In adult proximal tubules perfused with a late proximal tubular fluid and bathed in a serum-like solution, the transepithelial potential difference is lumen positive due to the anion gradients across the paracellular pathway and the greater permeability of the proximal tubule to chloride ions (4). Inhibition of active transport does not affect the potential difference (4). We found that the potential difference was significantly lower in neonates than adults, consistent with a maturational change in paracellular permeability. Thyroid hormone-treated neonatal PST have a significant increase in the transepithelial potential difference, whereas the maturational increase in potential difference was not seen in hypothyroid adult proximal tubules. Thyroid replacement resulted in an increase in the transepithelial potential difference in 8-wk-old adult rabbits.

Thyroid hormone was shown 35 years ago to affect passive paracellular anion permeability in isolated toad bladders (20, 21). After thyroxine treatment, there was an increase in short-circuit current but no change in electrical potential, consistent with a concomitant increase in paracellular permeability. Addition of thyroxine to toad bladders increased chloride and phosphate permeability. These data are consistent with the present findings, which find a potential role for thyroid hormone in the maturational change in paracellular pathway. Thyroid hormone does not cause a generalized increase in paracellular permeability in epithelia as this group subsequently demonstrated that thyroxine treatment in the rat small intestine decreased passive phosphate and calcium transport (23).

Thyroid hormone has been implicated as a factor in other aspects of renal development. There is evidence that thyroid hormone is a factor in the maturational increase in several proximal tubule mitochondrial oxidative enzymes (27). The growth of the kidney is impaired in hypothyroid rats (8). Importantly, the proximal tubule is shorter in hypothyroid rats (7). Thyroid-treated neonatal PST were of comparable volume per millimeter as that of control. Eight-week-old hypothyroid rabbits had a significant reduction in tubular volume compared with control 8-wk PST, which increased to the control tubular volume with thyroid treatment.

In summary, this study confirms that there is a postnatal rise in both active and passive NaCl transport in the PST. Administration of thyroid hormone to neonates resulted in an increase in volume reabsorption from a high-chloride perfusate. The increase in active NaCl transport was mediated in part by an increase in apical membrane Cl^-/OH^- and Na^+/H^+ exchange activity as well as by changes in passive NaCl transport. The postnatal maturational increases in both active and passive NaCl transport were prevented and the maturational increases in $Cl^-/OH^-$and Na^+/H^+ exchange activity were blunted in hypothyroid rabbits. This study is consistent with an important role for thyroid hormone in postnatal maturation of PST NaCl transport.

Acknowledgments

This work was supported by National Institute of Diabetes and Digestive and Kidney Disease Grant DK-41612 (to M. Baum).

We are grateful for the secretarial assistance of Janell McQuinn.

REFERENCES

1. Aronson PS, Giebisch G. Mechanisms of chloride transport in the proximal tubule. Am J Physiol Renal Physiol. 1997; 273:F179–F192.
2. Barlet C, Doucet A. Kinetics of triiodothyronine action on Na-K-ATPase in single segments of rabbit nephron. Pflügers Arch. 1986; 407:27–32. [PubMed: 3016635]
3. Baum M. Neonatal rabbit juxtamedullary proximal convoluted tubule acidification. J Clin Invest. 1990; 85:499–506. [PubMed: 2153709]
4. Baum M, Berry CA. Evidence for neutral transcellular NaCl transport and neutral basolateral chloride exit in the rabbit proximal tubule. J Clin Invest. 1984; 74:205–211. [PubMed: 6736248]
5. Baum M, Dwarakanath V, Alpern RJ, Moe OW. Effects of thyroid hormone on the neonatal renal cortical Na^+/H^+ antiporter. Kidney Int. 1998; 53:1254–1258. [PubMed: 9573540]
6. Boerth SR, Artman M. Thyroid hormone regulates Na^+-Ca^{++} exchanger expression during postnatal maturation and in adult rabbit ventricular myocardium. Cardiovasc Res. 1996; 31:E145–E152. [PubMed: 8681339]
7. Bradley SE, Bradley GP, Stéphan F. Role of structural imbalance in the pathogenesis of renal dysfunction in the hypothyroid rat. Trans Assoc Am Physicians. 1972; 85:344–352. [PubMed: 4660014]
8. Bradley SE, Stéphan F, Coelho JB, Réville P. The thyroid and the kidney. Kidney Int. 1974; 6:346–365. [PubMed: 4431166]
9. Cano A, Baum M, Moe OW. Thyroid hormone stimulates the renal Na/H exchanger NHE3 by transcriptional activation. Am J Physiol Cell Physiol. 1999; 276:C102–C108.
10. Capasso G, Lin J-T, De Santo NG, Kinne R. Short term effect of low doses of tri-iodothyronine on proximal tubular membrane Na-K-ATPase and potassium permeability in thyroid-ectomized rats. Pflügers Arch. 1985; 403:90–96. [PubMed: 2984639]
11. De Santo NG, Capasso G, Kinne R, Moewes B, Carella C, Anastasio P, Giordano C. Tubular transport processes in proximal tubules of hypothyroid rats. Lack of relationship between thyroidal dependent rise of isotonic fluid reabsorption and Na^+-K^+-ATPase activity. Pflügers Arch. 1982; 394:294–301. [PubMed: 6292823]
12. De Santo NG, Capasso G, Paduano C, Carella C, Giordano C. Tubular transport processes in proximal tubules of hypothyroid rats. Pflügers Arch. 1980; 384:117–122. [PubMed: 6446079]
13. Garg LC, Tisher CC. Effects of thyroid hormone on Na-K-adenosine triphosphatase activity along the rat nephron. J Lab Clin Med. 1985; 106:568–572. [PubMed: 2997354]
14. Henning J. Plasma concentrations of total and free corticosterone during development in the rat. Am J Physiol Endocrinol Metab Gastrointest Physiol. 1978; 235:E451–E456.

15. Holmes EW Jr, DiScala VA. Studies on the exaggerated natriuretic response to a saline infusion in the hypothyroid rat. J Clin Invest. 1970; 49:1224–1236. [PubMed: 5422024]

16. Hummelink R, Ballard PL. Endogenous corticoids and lung development in the fetal rabbit. Endocrinology. 1986; 118:1622–1629. [PubMed: 3948795]

17. Kinsella J, Sacktor B. Thyroid hormones increase Na^+-H^+ exchange activity in renal brush border membranes. Proc Natl Acad Sci USA. 1985; 82:3606–3610. [PubMed: 2987936]

18. Kurtz I, Nagami G, Yanagawa N, Li L, Emmons C, Lee I. Mechanisms of apical and basolateral Na^+-independent Cl^-/base exchange in the rabbit superficial proximal straight tubule. J Clin Invest. 1994; 94:173–183. [PubMed: 8040258]

19. Liu F-Y, Cogan MG. Axial heterogeneity in the rat proximal convoluted tubule. I. Bicarbonate, chloride, and water transport. Am J Physiol Renal Fluid Electrolyte Physiol. 1984; 247:F816–F821.

20. Matty AJ, Green K. Effect of thyroxine on ion movement in isolated toad bladder. Gen Comp Endocrinol. 1964; 4:331–338. [PubMed: 14181481]

21. Matty AJ, Green K. Permeability and respiration effects of thyroidal hormones on the isolated bladder of the toad *Bufo Bufo*. J Endocrinol. 1963; 25:411–425. [PubMed: 13933918]

22. Michael UF, Barenberg RL, Chavez R, Vaamonde CA, Papper S. Renal handling of sodium and water in the hypothyroid rat. J Clin Invest. 1972; 51:1405–1412. [PubMed: 5024038]

23. Noble HM, Matty AJ. The effect of thyroxine on the movement of calcium and inorganic phosphate through the small intestine of the rat. J Endocrinol. 1967; 37:111–117. [PubMed: 6017299]

24. Rector FC Jr. Sodium, bicarbonate, and chloride absorption by the proximal tubule. Am J Physiol Renal Fluid Electrolyte Physiol. 1983; 244:F461–F471.

25. Shah M, Quigley R, Baum M. Maturation of rabbit proximal straight tubule chloride/base exchange. Am J Physiol Renal Physiol. 1998; 274:F883–F888.

26. Walker P, Dubois JD, Dussault JH. Free thyroid hormone concentrations during postnatal development in the rat. Pediatr Res. 1980; 14:247–249. [PubMed: 7383746]

27. Wijkhuisen AF, Djouadi J, Vilar J, Merlet-Benichou C, Bastin J. Thyroid hormones regulate development of energy metabolism enzymes in rat proximal convoluted tubule. Am J Physiol Renal Fluid Electrolyte Physiol. 1995; 268:F634–F642.

28. Wysocki SJ, Segal W. Influence of thyroid hormones on enzyme activities of myelinating rat central-nervous tissues. Eur J Biochem. 1972; 28:183–189. [PubMed: 4341736]

29. Yonemura K, Cheng L, Sacktor B, Kinsella JL. Stimulation by thyroid hormone of Na^+-H^+ exchange activity in cultured opossum kidney cells. Am J Physiol Renal Fluid Electrolyte Physiol. 1990; 258:F333–F338.

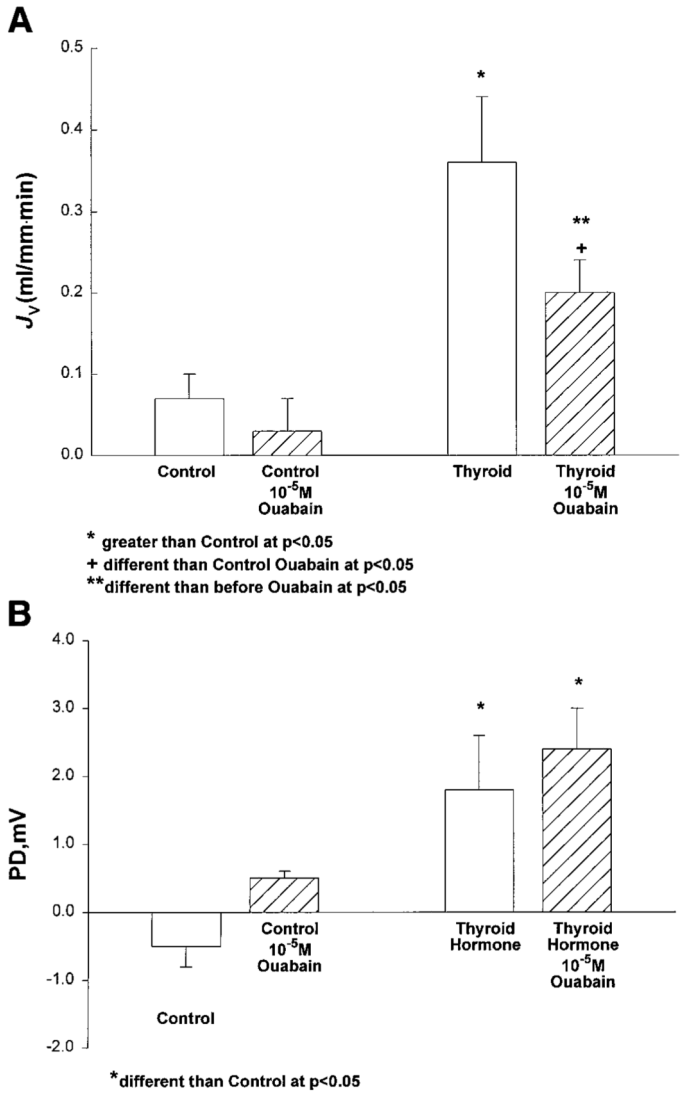

Fig. 1.

Effect of thyroid hormone on neonatal NaCl transport. Neonatal proximal straight tubules (PST) were perfused with a high-chloride solution simulating late proximal tubular fluid and bathed in a serum-like albumin solution in the 1st period, and ouabain was then added to inhibit active transport. *A*: rate of volume absorption (J_V) in PST from neonatal rabbits injected with vehicle and thyroid hormone [10 μg 3,5,3 -L-triiodothyronine (T_3)/100 g body wt for 3 days and in the morning 2 h before death]. *B*: transepithelial potential difference (PD). n = 7 Tubules in control group and 6 in thyroid hormone group.

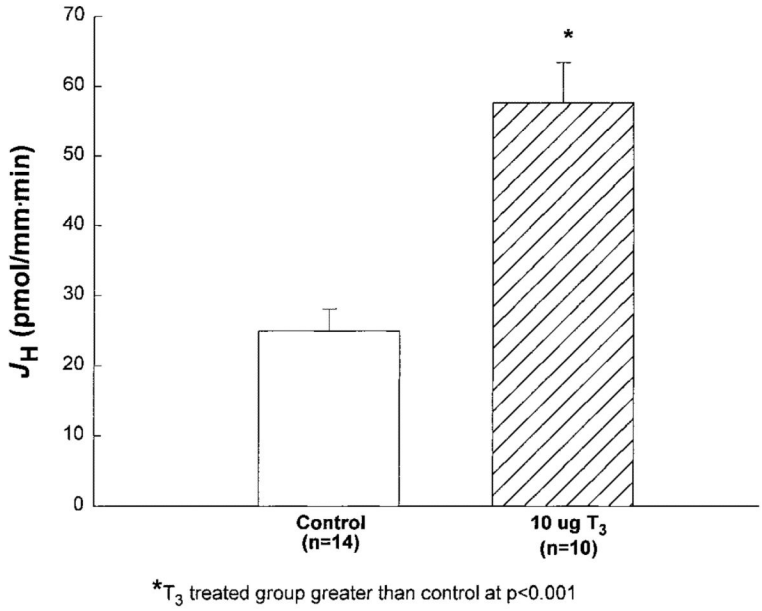

Fig. 2.

Neonatal PST apical Na^+/H^+ antiporter activity (J_H) in control and T_3-injected neonates.

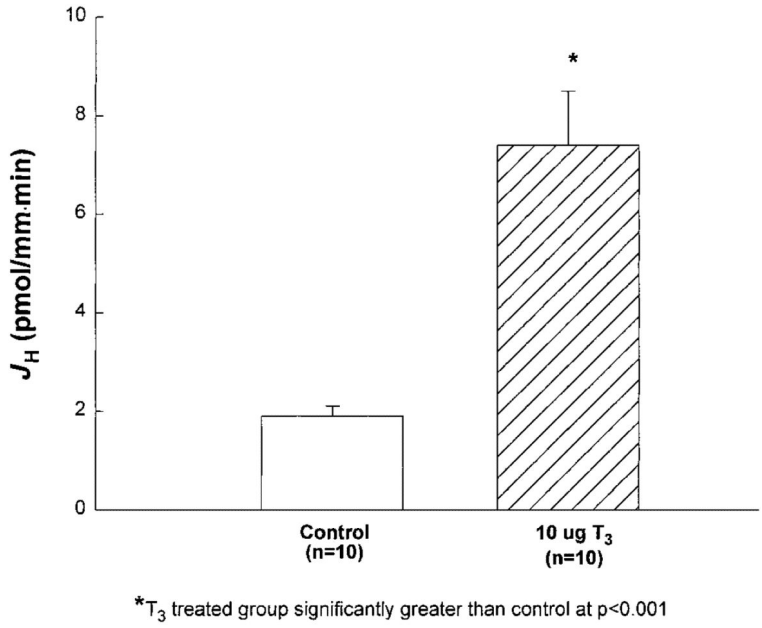

Fig. 3.

Neonatal PST apical Cl^-/OH^- exchanger activity (J_H) in control and T_3-injected neonates.

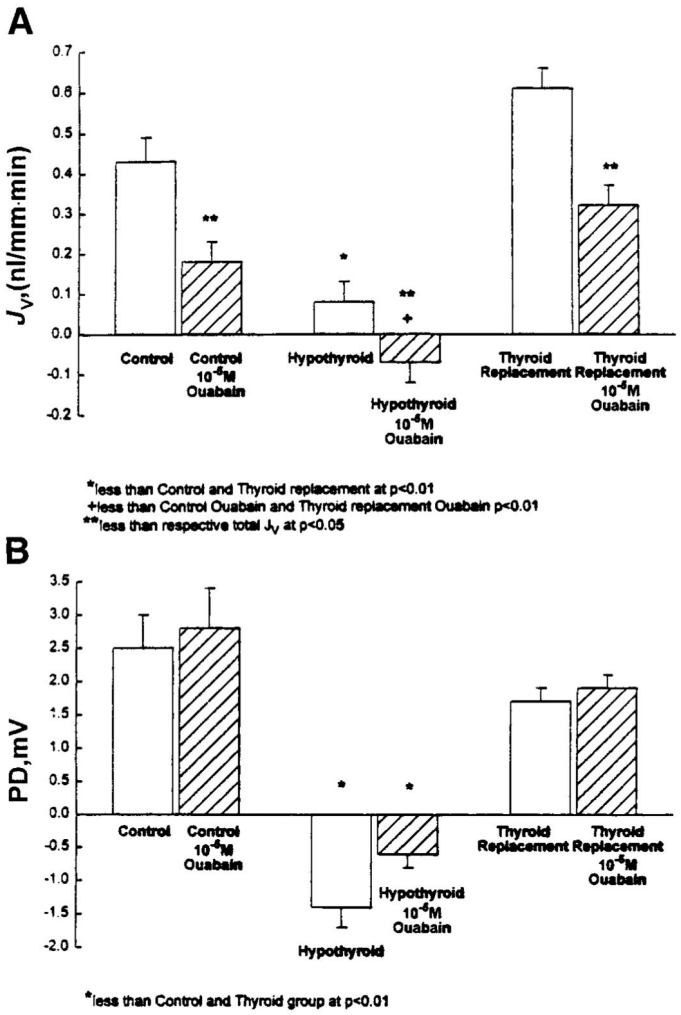

Fig. 4.

Volume absorption (*A*) and PD (*B*) in PST perfused with late proximal tubular fluid in 8-wk-old control ($n = 9$), hypothyroid ($n = 8$), and hypothyroid rabbits that received thyroid treatment ($n = 6$).

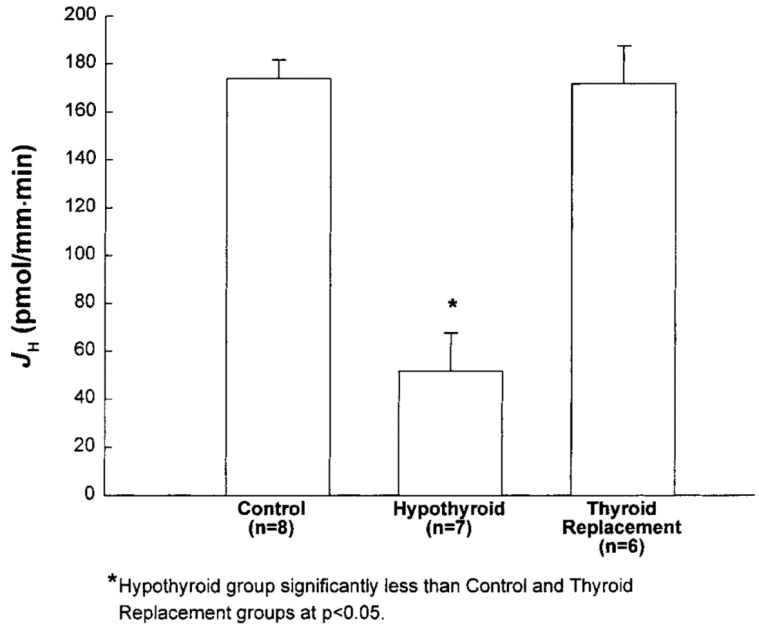

Fig. 5.

Apical Na^+/H^+ antiporter activity (J_H) in 8-wk-old control, hypothyroid, and hypothyroid rabbits that received thyroid treatment.

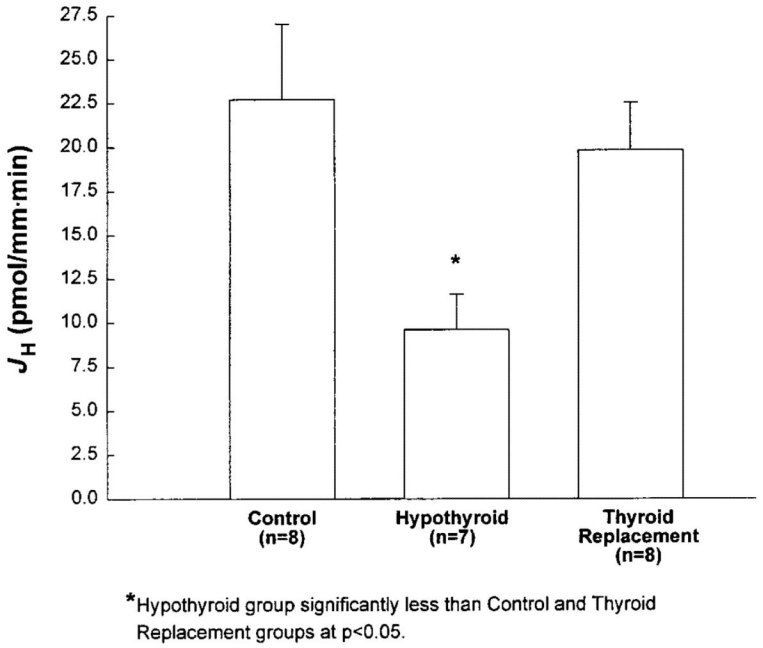

Fig. 6.

Apical Cl^-/OH^- exchanger activity (J_H) in 8-wk-old control, hypothyroid, and hypothyroid rabbits that received thyroid treatment.

Table 1

Solutions used in pH_i studies

	High Cl^- 0 Na^+ (With Organics)	High Cl^- 0 Na^+ (No Organics)	0 Cl^- 0 Na^+ (With Organics)	0 Cl^- 0 Na^+ (No Organics)	0 Cl^- High Na^+ (No Organics)	0 Cl^- 0 Na^+ + 20NH^+_4
	Solution A	*Solution B*	*Solution C*	*Solution D*	*Solution E*	*Solution F*
TMA-Cl	140	145				
TMA-OH			140	145		120
NH_4OH						20
Na gluconate					145	
Gluconic acid lactone			140	145		140
K_2HPO_4	2.5	2.5	2.5	2.5	2.5	2.5
$MgCl_2$	1	1				
Mg gluconate			1	1	1	1
$CaCl_2$	1	1				
Ca gluconate			12.5	12.5	12.5	12.5
Glucose	5		5			5
L-Alanine	5		5			5
HEPES	5	5	5	5	5	5

All constituents are in mM. All solutions were adjusted to an osmolality of 295 mosmol/kgH_2O, bubbled with 100% O_2, and had a pH of 7.4. pH_i, intracellular pH; TMA, tetramethylammonium.

Table 2

Thyroid and corticosterone hormone levels in neonatal and adult rabbits

	Serum T_3, ng/dl	Serum Corticosterone, ng/ml
Neonatal rabbits (n = 8)	172±23*	12.1 ± 2.1*
Adult rabbits (0.1% PTU in drinking water) (n = 5)	159±21*	40.4 ± 5.3
Adult control rabbits (n = 7)	326 ± 8	35.2 ± 8.3

Values are means ± SE; n = no. of experiments. T3, 3,5,3 -L-triiodothyronine; PTU, propylthiouracil.

* Different from corresponding adult control value at $P < 0.01$.