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Kinetic Studies on the Stimulation of Na^+-H^+ Exchange Activity in Renal Brush Border Membranes Isolated from Thyroid Hormone-Treated Rats

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Summary. Na^+-H^+ exchange activity in renal brush border membrane vesicles isolated from hyperthyroid rats was increased. When examined as a function of [Na^+], treatment altered the initial rate of Na^+ uptake by increasing V_m (hyperthyroid, 18.9 ± 1.1 nmol $Na^+ \cdot mg^{-1} \cdot 2\ sec^{-1}$; normal, 8.9 ± 0.3 nmol $Na^+ \cdot mg^{-1} \cdot 2\ sec^{-1}$), and not the apparent affinity K_{Na^+} (hyperthyroid, 7.3 ± 1.7 mM; normal, 6.5 ± 0.9 mM). When examined as a function of [H^+] and at a subsaturating [Na^+] (1 mM), hyperthyroidism resulted in the proportional increase in Na^+ uptake at every intravesicular pH measured. A positive cooperative effect on Na^+ uptake was found with increased intravesicular acidity in vesicles from both normal and hyperthyroid rats. When the data were analyzed by the Hill equation, it was found that hyperthyroidism did not change the n (hyperthyroid, 1.2 ± 0.06; normal, 1.2 ± 0.07) or the $[H^+]_{0.5}$ (hyperthyroid, 0.39 ± 0.08 μM; normal, 0.44 ± 0.07 μM) but increased the apparent V_m (hyperthyroid, 1.68 ± 0.14 nmol $Na^+ \cdot mg^{-1} \cdot 2\ sec^{-1}$; normal 0.96 ± 0.10 nmol $Na^+ \cdot mg^{-1} \cdot 2\ sec^{-1}$). The uptake of Na^+ in exchange for H^+ in membrane vesicles from normal and hyperthyroid animals was not influenced by membrane potential. H^+ translocation or debinding was rate limiting for Na^+-H^+ exchange since Na^+-Na^+ exchange activity was greater than Na^+-H^+ exchange activity. Hyperthyroidism caused a proportional increase and hypothyroidism caused a proportional decrease in Na^+-Na^+ and Na^+-H^+ exchange. We conclude that hyperthyroidism leads to either an increase in the number of functional exchangers in the membrane or exactly proportional increases in the rate-limiting steps for Na^+-Na^+ and Na^+-H^+ exchange activity.

Key Words L-thyroxine · triiodo-L-thyronine · sodium transport · proximal tubule · acid secretion · kidney

Introduction

Thyroid hormones (thyroxine, T_4; triiodothyronine, T_3) have a significant role in controlling kidney growth and function. For example, hyperthyroidism increases renal blood flow, glomerular filtration rate, concentrating and diluting capability, oxygen consumption, and reabsorption of phosphate, Ca^{++} and Na^+ [8, 15]. Hypothyroidism decreases these parameters. Thyroid hormones stimulate (Na^+-K^+)-ATPase by inducing the synthesis of more pumps [13, 25]. Changes in renal (Na^+-K^+)-ATPase activity closely parallel alterations in the net transport of Na^+ [14]. The enhanced Na^+ reabsorption in the proximal tubule of the kidney may be caused by: (i) induction of Na^+ pump elements in the basal-lateral membrane; (ii) an increase in the filtered Na^+ load; (iii) an increase in Na^+ reabsorption across the brush border membrane; and (iv) a concerted action combining the above factors.

Na^+, at physiological concentrations, is largely transported across the luminal brush border membrane of the proximal tubule by exchange for H^+ [7, 33]. A specific protein carrier mediates the electroneutral exchange of Na^+ and H^+, so that under normal conditions Na^+ enters the cell as H^+ exits the cell [16, 18, 27]. Amiloride, at relatively high concentrations (K_i = 0.05 mM in rat brush border membranes), competitively inhibits exchange activity [18]. In contrast, the basal-lateral membrane contains (Na^+-K^+)-ATPase which serves to pump Na^+ from the cell into the interstitium [31].

We have reported that Na^+-H^+ exchange activity is subject to hormonal and pathophysiological regulation. Glucocorticoids and metabolic acidosis increase Na^+-H^+ exchange activity [9, 20]. The exact mechanism for the increased activity is not known, although both treatments increase V_{max} measurements of transport without altering apparent affinities for Na^+ or H^+ [21, 22]. More recently, we have found that chronic hyperthyroidism increases and chronic hypothyroidism decreases Na^+-H^+ exchange activity [23]. The intact cell is necessary to demonstrate an effect on thyroid hormones on the Na^+-H^+ carrier, since preincubation of isolated membranes with T_3 or T_4 does not alter Na^+ uptake [23]. Thyroid hormones may alter exchanger activity by: (i) altering the affinities of Na^+

or H^+; (ii) increasing the incorporation of functional exchangers into the membrane; (iii) increasing the turnover rate of existing exchangers; (iv) changing the properties of the intravesicular H^+ modifier site; or (v) changing the exchange stoichiometry. In the present study, we examine some of these kinetic mechanisms.

Materials and Methods

Animals

Euthyroid Sprague-Dawley male rats (250–300 g) were fed *ad libitum* rat chow (Teklad Test Diet 83006), containing 20% protein, 0.7% calcium, and 0.5% phosphorus. Hyperthyroid rats were given thiouracil added to the chow (3 g/kg) and to the water (0.25 g/liter) and thyroid powder (recrystallized 3×) from ICN Nutrional Biochemicals to the thiouracil-containing diet (1 g/kg chow). Hypothyroid rats received only the thiouracil-containing diet. All rats were kept on the test diets for 3 weeks. At sacrifice, aortic blood was collected. Serum concentrations of T_3 and T_4 were determined with Corning Medical T_3 and T_4 RIA kits. Standards were made by diluting T_3 and T_4 into rat serum collected 4 weeks after thyroparathyroidectomy. Serum T_3 and T_4 were 9.0 ± 1.2 ng/ml and 24.8 ± 2.1 ng/ml (hyperthyroid); 1.9 ± 0.2 ng/ml and 10.1 ± 1.2 ng/ml (euthyroid); and 0.3 ± 0.2 ng/ml and 0.4 ± 0.2 ng/ml (hypothyroid).

Brush Border Membrane Vesicles

Rat renal cortex brush border membrane vesicles were prepared by differential centrifugation in the presence of 4 mM $MnCl_2$, as previously described [6, 9]. After brush border membrane vesicles were prepared in a buffer containing 15 mM HEPES and 300 mM mannitol, adjusted to pH 7.5 with KOH, the membranes were resuspended in the isolation buffers (the compositions of which are described below) and then centrifuged at 15,000 rpm for 20 min in a SW-40 (Sorvall) rotor. The pellet was resuspended in the same buffer and respun. The final pellets were resuspended in their isolation buffers and allowed to equilibrate for 1 hr before transport assays were started. The quality of the membrane preparations, evaluated by enrichment of a brush border marker enzyme maltase, was not altered by thyroid treatments. The enrichments in specific activity compared to crude homogenates ranged from 10- to 15-fold.

Uptakes of ^{22}Na (0.1–0.2 μCi) and D-[^{3}H]glucose (0.1–0.2 μCi) were measured at 20°C by a rapid filtration technique with 0.65-μm filters [9]. The membrane vesicle suspensions (10–25 μl, containing 10–20 mg of protein/ml) were pre-incubated for 1 min at 20°C before dilution to 50 μl with various uptake solutions. Isotope uptakes were terminated by addition of 3 ml of ice-cold solution containing 0.1 mM amiloride, 150 mM KCl, 15 mM HEPES,[1] pH 7.5, with KOH. All incubations were carried out in triplicate with fresh membranes. A metronome was used to time the initial uptakes.

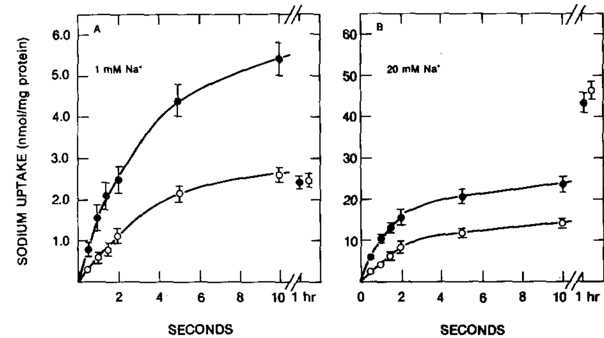

Fig. 1. Effect of thyroid hormones on brush border membrane Na^+-H^+ exchange activity. Na^+ (*A*, 1 mM; *B*, 20 mM) uptakes were measured in membrane vesicles from hyperthyroid (●) and normal (○) rats. The initial pH gradient was $pH_i = 5.5$, $pH_o = 7.5$. The results are presented as the mean ± SE of three experiments

Vesicles were isolated in 25 mM MES, 150 mM KCl, adjusted to pH 5.5 with KOH, for experiments measuring the initial rate and kinetics of Na^+ uptake with an intravesicular > extravesicular pH gradient. In experiments measuring the effect of intravesicular pH on Na^+ uptake, vesicles were isolated in 20 mM MES, 10 mM MOPS, 10 mM HEPES, 150 mM KCl, pH 5.5, 6.2, 6.5, 6.8, 7.2, and 7.5, adjusted with KOH. Uptake solutions for these experiments contained 150 mM (Na + K)Cl, 15 mM HEPES, with appropriate amounts of KOH to adjust the pH of uptake and membrane solution to 7.5. When amiloride/HCl was added, it was substituted for KCl isosmotically. The amiloride-insensitive Na^+ uptake was subtracted from the total Na^+ uptake in all the experiments. We previously demonstrated that amiloride-insensitive Na^+ uptake was not changed by the thyroid status of the animal [23].

The effect of *trans* Na^+ on Na^+ uptake was determined by isolating membrane vesicles in a solution containing 150 mM KCl or 140 mM KCl and 10 mM NaCl buffered by 20 mM MES, 10 mM MOPS, 10 mM HEPES, pH 6.5. Membrane vesicles (10 μl) were diluted 10× into a solution containing 1 mM ^{22}Na, 149 mM KCl, 15 mM HEPES, with KOH to adjust the extravesicular pH to 7.5.

In experiments in which we determined the effect of membrane potential on Na^+-H^+ exchange and Na^+-D-glucose cotransport, the vesicles were suspended in 25 mM MES, 300 mM mannitol, adjusted to pH 5.5 with KOH. The uptake solution contained either 1 mM $^{22}NaCl$, 149 mM tetramethyl ammonium gluconate, 15 mM HEPES, adjusted to pH 7.5 with KOH, or 25 μM [^{3}H]D-glucose, 150 mM Na gluconate, 15 mM HEPES, adjusted to pH 7.5 with KOH. Membrane potentials were generated by the addition of 80 μM FCCP in ethanol. Controls contained ethanol alone (2% solution).

Results

The experiments illustrated in Fig. 1 demonstrate that hyperthyroidism stimulated Na^+ uptake into brush border membrane vesicles whether the Na^+ concentration was 1 mM (Fig. 1*A*) or 20 mM Na^+ (Fig. 1*B*). In addition, the results show that Na^+ uptakes into vesicles from euthyroid rats were lin-

[1] The abbreviations used are: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; MES, 2-(N-morpholino) ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

Table 1. Effect of membrane potential on amiloride-sensitive Na^+-H^+ exchange and Na^+-glucose cotransport in brush border membrane vesicles from euthyroid and hyperthyroid rats

Experiment	Uptake	
	Euthyroid	Hyperthyroid
	(nmol · mg protein^{-1} · 5 sec^{-1})	
Na^+(1 mM) uptake		
−FCCP	2.07 ± 0.15	3.96 ± 0.36
+FCCP	2.14 ± 0.09	3.96 ± 0.31
(−FCCP versus +FCCP)	NS	NS
	(pmol · mg protein^{-1} · 5 sec^{-1})	
D-Glucose (25 μM) uptake		
−FCCP	126 ± 6	120 ± 13
+FCCP	442 ± 36	440 ± 17
(−FCCP versus +FCCP)	$P < 0.01$	$P < 0.01$

ear for 2 sec at both the low and the high Na^+ concentrations. But, Na^+ uptakes at 2 sec into vesicles from hyperthyroid rats were 80–85% of our estimate of true initial rates at both the low and high Na^+ concentration. The kinetic studies described below were based upon 2-sec uptakes, so that estimates of V_{max} in vesicles from hyperthyroid rats were underestimated by 15 to 20%. Apparent affinities, however, were not affected when the underestimation was the same at all substrate concentrations.

In testing the different mechanisms for the increase in Na^+-H^+ exchange activity in hyperthyroidism, we first examined whether after thyroid extract treatment Na^+-H^+ exchange activity was altered by membrane potential. It was demonstrated previously that Na^+-H^+ exchange activity in membranes from normal animals was not affected by changes in membrane potential [16, 27]. If, during hyperthyroidism, the rate-limiting step in Na^+-H^+ exchange activity became sensitive to membrane potential or the stoichiometry changed so that there was a net movement of charge, then altering the membrane potential could lead to a change in the Na^+ uptake mediated by the exchanger. Table 1 shows the results of experiments in which the proton ionophore, FCCP, was used to generate potentials, in the presence of a pH gradient (pH_i = 5.5; pH_o = 7.5). Under these conditions, FCCP would increase intravesicular negativity. To demonstrate that FCCP did indeed increase the membrane potential, we measured its effect on Na^+-glucose cotransport. As shown in Table 1, FCCP enhanced glucose uptake (5 sec) in both normal and hyperthyroid vesicle preparations to the same extent, about 3.5-fold, findings consistent with the known voltage dependence of Na^+-glucose cotransport [5]. However, in the presence of the same pH gradient,

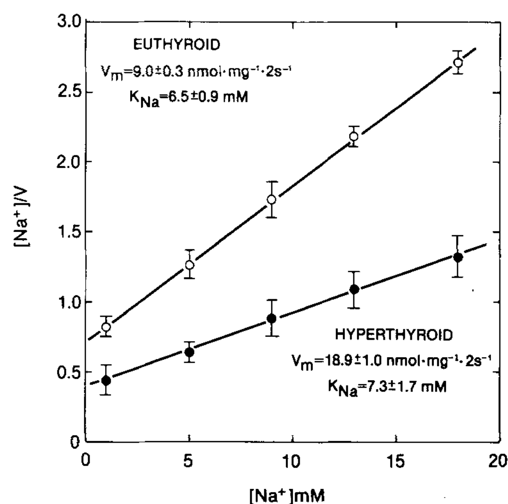

Fig. 2. Effect of hyperthyroidism on brush border membrane Na^+-H^+ exchange kinetics. Uptakes (2 sec) of different Na^+ concentrations were measured in the presence of a pH gradient (pH_i = 5.5; pH_o = 7.5). Membrane vesicles were isolated from hyperthyroid (●) and normal (○) rats. The results are expressed as the mean ± SE of at least five experiments

FCCP had no effect on amiloride-sensitive Na^+ uptake in brush border membranes from normal or hyperthyroid rats. Amiloride-insensitive Na^+ uptake was increased by FCCP in both membrane preparations (*data not shown*), a finding further supporting the view that in these experiments FCCP did generate an inside negative membrane potential. These results suggested that the increased Na^+-H^+ exchange activity found in hyperthyroidism was not due to an alteration in the rate-limiting step for translocation from a membrane potential-insensitive to a sensitive step, or to a change in the stoichiometry of the exchanger.

Next, the kinetic properties of the Na^+-H^+ exchange, with respect to Na^+, were examined to determine how they were influenced by hyperthyroidism. Hyperthyroidism could increase Na^+ uptake by either increasing the apparent K_{Na} or the V_m. The initial rates (2 sec) of Na^+ uptake at various Na^+ concentrations in the presence of a pH gradient (pH_i = 5.5; pH_o = 7.5) are presented in Fig. 2 as a Hanes-Woolf plot. Hyperthyroidism had no effect on the apparent Na^+ affinity (normal, 6.5 ± 0.9 mM; hyperthyroid, 7.3 ± 1.7 mM, $P > 0.05$), but significantly enhanced the maximum velocity (normal, 9.0 ± 0.3 nmol · mg^{-1} · 2 sec^{-1}; hyperthyroid, 18.9 ± 1.1 nmol · mg^{-1} · 2 sec^{-1}, $P < 0.05$). Therefore, the increase in Na^+ uptake into vesicles isolated from hyperthyroid rats shown in Fig. 1 and previously reported [23] was not due to a change in the apparent affinity of the carrier for Na^+ but rather due to an increase in V_{max}. The linearity of these plots (Fig.

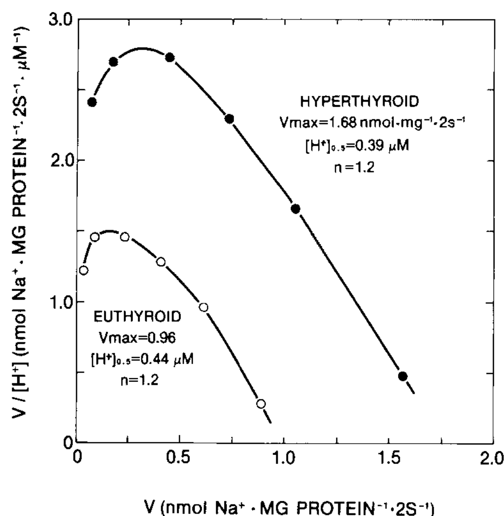

Fig. 3. Effect of hyperthyroidism on brush-border membrane Na^+-H^+ exchange with intravesicular media of different pH. Uptake of 1 mM Na^+ was measured after 2 sec in membrane vesicles from hyperthyroid (●) and normal (○) rats. The extravesicular pH was held constant at pH 7.5, while intravesicular pH was varied between pH 5.5 and 7.5. The data points are expressed as the mean of at least five experiments. The kinetic constants, K'_H, n, and apparent V_{max}, for the individual experiments were determined by the best fit with a MLAB nonlinear least squares curve-fitting routine [24]. The mean of the kinetic constants was used to determine the curve

2) provided further support for the conclusion that only one Na^+ was exchanged for one H^+ in normal and in hyperthyroid vesicle preparations.

Aronson et al. [3] previously reported that the Na^+-H^+ exchanger in rabbit renal brush border vesicles contained a distinct intravesicular H^+ modifier site and an internal H^+ transporting site. Thus, it was possible that hyperthyroidism might increase Na^+-H^+ exchange activity by altering the properties of the H^+ modifier site. In the experiments illustrated in Fig. 3, we measured amiloride-sensitive 1 mM Na^+ uptake (2 sec) into vesicles isolated from euthyroid and hyperthyroid rats as a function of different intravesicular pH values (pH_i = 5.5 to 7.5; pH_o = 7.5). The data are presented in the form of an Eadie-Scatchard plot and fitted to the Hill equation: $V = V_{max} [H^+]n(K'_H + [H^+]n)^{-1}$, with a MLAB nonlinear least squares curve-fitting routine [24]. The nonlinearity demonstrated that intravesicular H^+ influenced Na^+-H^+ exchange by a positive cooperative mechanism. The relationship between K'_H and $[H^+]_{0.5}$ is $K'_H = [H^+]n_{0.5}$. Neither the $[H^+]_{0.5}$ nor the n was affected by hyperthyroidism (normal: $[H^+]_{0.5}$ = 0.44 ± 0.07 μM, n = 1.2 ± 0.07; hyperthyroid: $[H^+]_{0.5}$ = 0.39 ± 0.08 μM, n = 1.2 ± 0.06). Only the apparent V_{max} for 1 mM Na^+ was increased from 0.96 ± 0.10 nmol · mg^{-1} · 2 sec^{-1} to 1.68 ± 0.14 nmol · mg^{-1} · 2 sec^{-1} with hyperthyroidism. Again the findings suggested that hyperthyroidism affected the apparent V_{max} of the exchange, explicable by either the incorporation of more functional exchangers/unit membrane or an alteration in the turnover rate of the carrier. It was reported that Na^+-H^+ exchanger was tightly coupled, i.e., 1 Na^+ was exchanged for 1 H^+ throughout a range of intra- and extravesicular pH [19]. Since the n found in the present experiments was significantly greater than 1 and less than 2 ($P < 0.05$), it was likely that Na^+-H^+ exchange contained one intravesicular H^+ translocating site and one nontransporting H^+ modifier site.

In our use of the Hill equation, since Na^+ concentration was less than saturating, V_{max} equaled the apparent maximum rate of transport, n equaled the apparent number of H^+ binding sites, K'_H equaled the apparent constant comprising the interacting factors and the dissociation constant. Models of cooperative interaction between substrates and activators might be reduced to the Hill equation with an apparent V_{max} substituted for V_{max}, if the affinity for one substrate (Na_o^+) was not altered by the other (H_i^+). The apparent V_{max} would be dependent upon the degree of saturation of the measured substrate (Na_o^+ in this case) [32]. We tested whether changing $[H_i^+]$ altered the V_{max} or the apparent Na^+ affinity to assure validity of our use of the Hill equation. Figure 4*A* and *B* show the effect of intravesicular pH on the apparent affinity of Na^+ and the V_{max} for euthyroid (Fig. 4*A*) and hyperthyroid rats (Fig. 4*B*) at a fixed extravesicular pH (pH_o = 7.5). Increasing the intravesicular H^+ concentration 5 times from pH 6.2 to 5.5 did not significantly change the apparent K_{Na} (pH 6.2: 6.8 ± 0.5 mM; pH = 5.5: 7.2 ± 0.6 mM, $P > 0.05$), whereas the V_{max} was significantly increased (pH 6.2: 7.7 ± 0.9 nmol · mg^{-1} · 2 sec^{-1}; pH 5.5: 11.7 ± 1.2 nmol · mg^{-1} · 2 sec^{-1}, $P < 0.01$) in membranes isolated from euthyroid rats. Very similar results were found when we examined membranes isolated from hyperthyroid rats, the apparent K_{Na} values were not changed (pH 6.2: 7.0 ± 0.7 mM; pH 5.5: 6.5 ± 0.5 mM, $P > 0.05$) and V_{max} increased (pH 6.2: 15.6 ± 1.2 nmol · mg^{-1} · 2 sec^{-1}; pH 5.5: 21.6 ± 2.7 nmol · mg^{-1} · 2 sec^{-1}, $P < 0.01$). The results presented in Fig. 4, demonstrated that (i) V_{max} changed and K_{Na} did not change in response to pH_i regardless of the thyroid status of the animal; and (ii) the use of the Hill equation was justified, although the values represent apparent rather than true kinetic constants.

Renal Na^+-H^+ exchange could function in multiple exchange modes involving Na^+, H^+, Li^+ and NH_4^+ all of which were sensitive to amiloride [2, 3, 16, 17]. Furthermore, there were differences in the

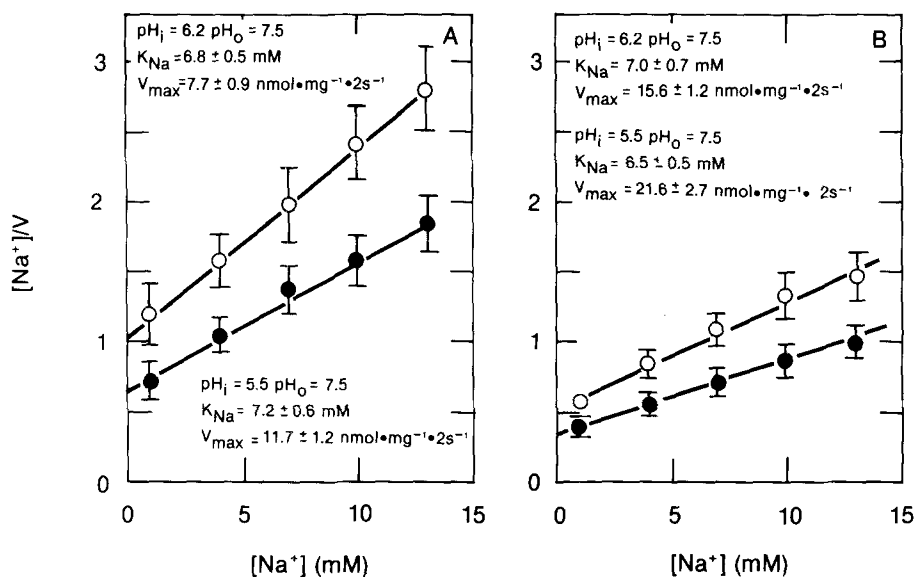

Fig. 4. Effect of intravesicular pH on brush border membrane Na^+-H^+ exchange kinetics in normal and hyperthyroid animals. Uptakes (2 sec) of different Na^+ concentrations were measured in the presence of either $pH_i = 5.5$, $pH_o = 7.5$ (●) or $pH_i = 6.2$, $pH_o = 7.5$ (○). Membrane vesicles were isolated from normal (*A*) or hyperthyroid (*B*) animals. The results are expressed as the mean ± SE of at least five experiments

Table 2. Na^+-H^+ and Na^+-Na^+ exchange activities in brush border membrane vesicles isolated from hypo-, eu-, and hyperthyroid rats

Thyroid status	Na^+-H^+ exchange	Na^+-Na^+ exchange[a]	Na^+-Na^+/Na^+-H^+
	(nmol Na^+ · mg protein^{-1} · 2 sec^{-1})		(% change)
Hypothyroid	0.54 ± 0.05	0.80 ± 0.07	48 ± 7
Euthyroid	0.74 ± 0.06	1.07 ± 0.08	47 ± 9
Hyperthyroid	1.25 ± 0.06	1.87 ± 0.19	51 ± 9

[a] The values for Na^+-Na^+ exchange represent the sum of the Na^+ for Na^+ exchange and the residual Na^+ for H^+ exchange remaining in the presence of 10 mM Na_i^+. If additivity is assumed, the interpretation of the data would not be altered.

relative rates of transport of these substrates; for example, Na^+-Na^+ exchange occurred at a higher rate than Na^+-H^+ exchange [3, 17]. These results suggested, if we assume a consecutive model for the exchange reaction, i.e., Na^+ binds, translocates, and dissociates before a H^+ binds, translocates and dissociates, that the rate-limiting step for Na^+-H^+ exchange involved either a H^+ translocating or H^+ debinding step and not a Na^+ translocating or a debinding step. Our results showed that hyperthyroidism increased V_{max}, which could be interpreted as either more transporter or a change in the activity of the rate-limiting step. If hyperthyroidism increased Na^+-H^+ exchange by increasing the activity of the rate-limiting step (H^+ translocation or debinding), then the ratio of Na^+-Na^+ exchange to Na^+-H^+ exchange should be reduced during hyperthyroidism. The results of experiments comparing Na^+-Na^+ exchange activity to Na^+-H^+ exchange activity are shown in Table 2. Both Na^+-Na^+ and Na^+-H^+ exchange activity increased in hyperthyroidism and decreased in hypothyroidism compared to euthyroid rats. In each case Na^+-Na^+ exchange was increased about 50% compared to Na^+-H^+ exchange. These findings would be consistent with the hypothesis that hyperthyroidism increased Na^+-H^+ exchange activity by increasing the amount of active exchanger in the brush border membrane rather than by selectively altering the activity of the rate-limiting step. Alternatively, however, if we assume a simultaneous model for the exchange reaction in which the exchange of extravesicular Na^+ for intravesicular Na^+ is faster than the exchange of outside Na^+ for inside H^+, then thyroid hormone could have increased the rate-limiting steps in both the Na^+-Na^+ and Na^+-H^+ exchange modes.

Discussion

Previously we reported that renal brush border Na^+-H^+ exchange activity was altered by various hormonal and metabolic treatments [9, 20, 23]. When Na^+-H^+ exchange activity in vesicles from euthyroid, hypothyroid, and hyperthyroid rats were compared, hyperthyroidism increased H^+ and amiloride-sensitive Na^+ flux, increased Na^+ and amiloride-sensitive H^+ flux, and increased passive (Na^+-independent) pH gradient dissipation [23]. Hypothyroidism had opposite effects [23]. The biochemical basis for the change in Na^+-H^+ exchange activity was not examined. In the present paper, we studied several different possible mechanisms for the regulation of transport activity.

Hypothetical mechanisms tested included whether the stoichiometry changed or the rate-limiting step became sensitive to membrane potential. Kinsella and Aronson [19] previously reported that over a range of pH the stoichiometry of the exchange was fixed at one Na^+ for one H^+ and under the same conditions the carrier rate remained insensitive to an alteration in membrane potential. Our results, demonstrating that amiloride-sensitive Na^+ uptake in vesicles from normal and hyperthyroid rats was insensitive to changes in membrane potential (Table 1), suggested that it was unlikely that hyperthyroidism changed the stoichiometry of one Na^+ exchanging for one H^+. If the stoichiometry were two Na^+ translocated in for each H^+, then the inside vesicle negativity produced by the FCCP might be expected to increase the rate of Na^+ uptake by providing a pathway for charge compensation brought about by the excess in Na^+ over H^+ translocated. Alternatively, hyperthyroidism might have modified the rate-limiting step in the cycling of Na^+ and H^+ by the carrier so as to make it sensitive to membrane potential without changing the stoichiometry. Again, the lack of effect of FCCP on amiloride-sensitive Na^+ uptake made this hypothesis unlikely.

In Fig. 1*A*, we measured amiloride-sensitive 1 mM Na^+ uptake, a Na^+ concentration below its apparent K_m. Changes in Na^+ uptake at this concentration could be due to an effect on either the apparent K_{Na} or the V_{max}. Our results shown in Fig. 2 demonstrated that hyperthyroidism increased the V_{max} for Na^+ without affecting the K_m of Na^+. This finding was consistent with the observation (Fig. 1*B*) that hyperthyroidism enhanced amiloride-sensitive Na^+ uptake when the Na^+ concentration was 20 mM, a value approximately three times the apparent K_m. However, whether hyperthyroidism led to an increased incorporation of active exchangers in the brush border membrane or an increased turnover of existing exchangers could not be determined by this experiment.

Previously, Aronson et al. [3] reported that the Na^+-H^+ exchanger in brush border membranes had an internal nontransporting H^+ modifier site. Acidification of the vesicle interior increased amiloride-sensitive unidirectional influx and efflux of Na^+ through the amiloride-sensitive Na^+-H^+ exchange. However, the kinetic properties of the modifier site were not determined, since through the pH range investigated, Na^+ transport did not saturate. Under the experimental conditions used in the present study, we were able to approach maximum 1 mM Na^+ uptakes with respect to internal H^+ concentration and therefore could obtain a good estimate of the apparent V_{max}. We specifically wanted to know if in hyperthyroidism, the increase in V_{max} (Fig. 2) could readily be explained by some change in the properties of the H^+ modifier site. However, by a Hill analysis (Fig. 3), we found that neither the $[H^+]_{0.5}$ nor n were changed, rather, the apparent V_{max} was increased by hyperthyroidism. We interpret these results as suggesting either more active exchangers or an increased turnover of the rate-limiting step. Regardless of which mechanism, an increase in V_{max} during hyperthyroidism would result in equivalent percent increase in exchange activity within the range of intravesicular pH measured.

It should be emphasized that the kinetic values determined by the Hill equation were useful in comparing normal and hyperthyroid animals, but it should be recognized that they represented apparent values derived under the specific conditions of these experiments. For example, V_{max} was underestimated since we used less than saturating amounts of Na^+ and external H^+ compete with Na^+ for transport with an apparent K_m = 35 nM [4]. Thus, under the conditions described in Fig. 3, most of the turnover of the exchanger was likely facilitating undetected H^+-H^+ exchange and not Na^+-H^+ exchange.

Models of cooperative interaction between substrate and activators might be reduced to equations similar to the Hill equation with an apparent V_{max} if less than saturating substrate concentrations were used. As we showed in Fig. 4, the apparent affinities of Na^+ for the Na^+-H^+ exchange were the same for euthyroid and hyperthyroid and unchanged by a variation in the intravesicular proton concentration. Similar findings were also described in preparations of isolated intestinal brush borders [12] and thymocytes [11]. In another study, it was found in isolated rabbit renal brush border membranes that increasing intravesicular proton concentration increased the apparent Na^+ affinity without affecting the V_{max} [28]. The reason for the discrepancy between our study and this earlier study is unknown. Using rabbit brush border membranes in our laboratory, we confirmed our finding with rat brush border membranes that increasing intravesicular proton concentration increased V_{max} without changing the apparent Na^+ affinity[2].

Hyperthyroidism increased the V_{max} for Na^+ uptake at different intravesicular pH (Figs. 2 and 4), and increased the V_{max} for intravesicular H^+ stimulation (Fig. 3). Thyroid hormones did not alter the apparent Na^+ affinity (Figs. 2 and 4), the Hill coeffi-

[2] J. Kinsella and B. Sacktor, *unpublished observations*.

cient (Fig. 3), the $[H^+]_{0.5}$ for activation (Fig. 3), or the potential sensitivity (Table 1). These results might be explained by mechanisms that either increase the number of functional exchangers in the membrane or increase the velocity of the rate-limiting step for exchange. Since in a consecutive model for Na^+-H^+ exchange the rate-limiting step involved H^+ translocation or debinding, an increased velocity for this step during hyperthyroidism would be detected by a decreased ratio of Na^+-Na^+ exexchange to Na^+-H^+ exchange activity compared to controls. Neither hypothyroidism nor hyperthyroidism altered the ratio of activities; i.e. Na^+-Na^+ exchange activity changed in exact proportion to Na^+-H^+ exchange. We concluded from this experiment that the thyroid status of the animal regulated the number of functional exchangers in the membrane. An alternative explanation, albeit less likely but not precluded, was that the rate-limiting step for H^+ translocation or debinding in Na^+-H^+ exchange was changed in exact proportion to the rate-limiting step for Na^+ translocation or debinding in Na^+-Na^+ exchange. Further, if Na^+-H^+ exchange involved the simultaneous exchange of cations, then the present results could not distinguish between an increase in the rate-limiting step and an increase in the concentration of functional exchangers.

Previous studies also examined the hormonal regulation of Na^+-H^+ exchange activity. We reported the stimulation of exchange activity in renal brush border membranes isolated from rats administered glucocorticoids [9, 22]. Similar to the findings reported in the present paper, glucocorticoids were found to increase the V_{max} for Na^+ uptake and the V_{max} for intravesicular H^+ stimulation of 1 mM Na uptake [22]. In addition, the apparent affinity for Na^+, the n value, and the $[H^+]_{0.5}$ were not changed by dexamethasone [22]. In contrast to the actions of thyroid hormone and glucocorticoids on the exchange system in the kidney, other investigations showed that growth factors, including epidermal growth factor, platelet-derived growth factor, insulin and α-thrombin, activated Na^+-H^+ exchange in cultured fibroblasts by a different mechanism [26, 29]. In this system, there was an apparent decrease in the $[H^+{}_{0.5}$ value to a more alkaline pH, without changing the apparent Na^+ affinity or V_{max} [26, 29]. The change in $[H^+]_{0.5}$ might involve protein kinase C because phorbal esters mimicked the effects of the growth factors (10). In other studies of Na^+-H^+ exchange in *Necturus* gallbladder, cAMP inhibited exchange activity by decreasing the V_{max} without changing the apparent Na^+ affinity or the $[H^+]_{0.5}$ [30]. Whether these multiendocrine inputs for regulating exchange activity are present in all cells that possess Na^+-H^+ exchange has not been determined, but such a diversity of effectors would permit exquisite regulation of this crucial ion transport system.

Cell pH is dependent upon a steady state between acid-generating and acid-consuming mechanisms. The proximal tubular cell of the kidney must not only maintain cellular pH but since it is an acid secreting epithelium it must balance the rate of acid secretion across the brush border membrane with base secretion across the basolateral membrane. Not only is the activity of Na^+-H^+ exchange important for regulating tubular fluid acidification, but all the transcellular and paracellular pathways that influence Na^+ and H^+ (HCO_3^-) fluxes will also influence acid secretion. Thus, the rate of H^+ secretion into the tubular fluid will be directly dependent upon acid pump-leak mechanisms in the brush border membrane and indirectly dependent upon cellular metabolism and basolateral base pump-leak mechanisms. Since the H^+ secretory rate is a steady state that reflects the rate of all these processes, the regulation of H^+ secretion in vivo is most likely kinetically and not thermodynamically controlled [1]; i.e., pH gradient across the brush border membrane never exceeds or equals the Na^+ gradient [1]. This concept gains added support from the experiment illustrated in Fig. 3. At an intravesicular pH of 7.5 the rate of Na^+-H^+ exchange was comparatively low. Thus, if the membrane vesicle experiment, in vitro, were applicable to the situation, in situ, then regardless of the energy available in the transmembrane Na^+ gradient, the rate of H^+ secretion would remain low. Obviously, H^+ secretion could change if the rate of any one process involved in the steady-state acid-base metabolism changed its activity, resulting in compensating changes in all the other pump-leak systems until a new steady state would be reached. Intracellular acid shifts would result in a dramatic increase in Na^+-H^+ exchange activity because of the exchanger activation by the H^+ modifier site (positive cooperativity). The consequence of intracellular acidosis with an internal H^+ modifier site would be that a new steady state would be reached, reflecting a less-than-expected acid shift in cellular pH and a greater-than-expected rate of acid secretion. Hyperthyroidism, by proportionally increasing Na^+-H^+ exchange activity at any intracellular pH, would result in a more alkaline cellular pH and a higher H^+ secretory rate. This discussion assumes that thyroid hormones solely determine Na^+-H^+ exchange activity without altering other pathways for Na^+ or H^+. However, we previously found that the rate of pH gradient dissipation across the brush border membrane increased during hyper-

thyroidism [23]. The consequence of increased proton permeability and increased Na^+-H^+ exchange activity would be increased Na^+ reabsorption without a concomitant increase in the H^+ gradient. Indeed, preliminary observations indicated that hyperthyroidism increased Na^+ reabsorption by the kidney without any systemic acid-base changes or changes in urinary acid secretion[2].

In summary, hyperthyroidism induced an increase in Na^+-H^+ exchange activity in renal brush-border membranes. We found no change in the stoichiometry or the sensitivity to membrane potential. Furthermore, the apparent affinity for Na^+, the K'_H and the Hill coefficient, n, were not altered by hyperthyroidism, while the V_{max} for Na^+ uptake and the activation by internal H^+ were both increased. Since both Na^+-Na^+ and Na^+-H^+ exchange activity increased proportionally during hyperthyroidism, it is likely that hyperthyroidism increased the number of functional exchangers if the Na^+-H^+ translocation steps are separate. Nevertheless, at this time, we cannot distinguish between the possibilities of more carriers per unit membrane or a more rapid and proportional change in the rate-limiting steps for Na^+-Na^+ and Na^+-H^+ exchange. Both of these possibilities remain until a method is developed which quantitates the amount of functional exchangers. Regardless of the exact mechanism, these results demonstrate that one of the renal responses to hyperthyroidism was to increase Na^+ reabsorption by increasing Na^+-H^+ exchange activity in the brush border membrane.

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