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ABSENCE OF INCREASED ELECTRONEUTRAL Na^+–H^+ EXCHANGE IN RENAL CORTICAL BRUSH-BORDER MEMBRANES FROM HYPERTHYROID RATS

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Abstract—The fluorescence quenching of acridine orange was used to compare Na^+–H^+ exchange and ion conductances in renal cortical brush-border membrane vesicles (BBMV) isolated from euthyroid and hyperthyroid rats. In BBMV from euthyroid animals, Na^+–H^+ exchange was entirely electroneutral. In BBMV from hyperthyroid rats, the total rates of Na^+–H^+ exchange were about 30% higher than in BBMV from euthyroid animals. However, the electroneutral exchange in these membranes was similar to that in BBMV from euthyroid rats; the observed increase in exchange was due to electrically coupled Na^+ and H^+ movements through conductive pathways in the membranes. Ion conductances in isolated BBMV were tested with outwardly directed K^+ gradients in the presence of carbonyl cyanide *m*-chlorophenylhydrazone (K^+ conductance) or valinomycin (H^+ conductance). The K^+ conductance was negligible and similar in BBMV from both groups of rats. A significant H^+ conductance was present in both kinds of membrane preparations and was by 37% higher in BBMV from hyperthyroid animals. Therefore, our experiments failed to demonstrate an increased electroneutral Na^+–H^+ exchange in BBMV from hyperthyroid rats. Instead, a finding of a significant electrically coupled Na^+–H^+ antiport in the presence of increased H^+ conductance in BBMV from hyperthyroid rats indicates that these membranes may also have increased Na^+ conductance.

Thyroid hormones (thyroxine, T_4; triiodothyronine, T_3) have a potent influence on renal function. Thyroid-deficient animals exhibit a number of renal functional defects such as decreased renal plasma flow and glomerular filtration rate (GFR),† an impaired ability to concentrate and dilute urine, smaller oxygen consumption and impaired tubular reabsorption of PO_4^{3-}, Ca^{2+} and Na^+ [1–3]. The effects of thyroid hormones are largely confined to proximal tubules. Here, the isotonic fluid reabsorption, which is closely linked to transepithelial Na^+ transport, is reduced in thyroidectomized rats and largely normalized by the substitution of T_3 [4, 5]. The mechanism of action of thyroid hormones on Na^+ reabsorption in the proximal tubule is poorly understood and several hypotheses have been proposed. First, thyroid hormones could cause an increase in the GFR thereby increasing the amount of filtered Na^+ [6]. The increased Na^+ load would secondarily stimulate the activity and synthesis on (Na^+ + K^+)-ATPase in the basolateral cell membrane [6, 7] thus resulting in an increased transtubular Na^+ transport. However, the T_3-dependent rise of isotonic fluid reabsorption in proximal tubules increased well before actual increase in filtered Na^+ load and (N^+ + K^+)-ATPase activity [4, 8]. Second, T_3 and T_4 could affect the Na^+ entry step in the proximal tubular cells by altering either the conductance of the cell membrane for Na^+ and/or the activity of Na^+–H^+ exchanger in the luminal membrane. As judged from studies on rat renal brush-border membrane vesicles (BBMV), physiological doses of thyroid hormones failed to induce any change in luminal membrane conductance for Na^+ and the activity of Na^+–H^+ exchanger [9]. On the contrary, other authors [3, 10, 11] demonstrated an enhanced exchanger activity in renal luminal membranes from hyperthyroid rats. Finally, Capasso *et al.* [12] suggested that thyroid hormones may increase the K^+ permeability of the tubular cell membrane. The leak of K^+ out of the cell would indirectly stimulate the activity of (Na^+–K^+)-ATPase in the basolateral membrane thus increasing the driving force for Na^+ entry at the luminal membrane.

In this work, we studied the effects of thyroid hormones upon Na^+–H^+ exchange and ion conductances in rat renal cortical BBMV. The rats were made hyperthyroid by a long-term treatment with pharmacological doses of thyroid hormones. In renal brush-border membranes obtained from such animals no enhanced electroneutral Na^+–H^+ exchange could be demonstrated. Instead, an increment of electrically coupled Na^+ and H^+ movements was observed possibly due to increased intrinsic H^+ and Na^+ conductances.

MATERIALS AND METHODS

Experiments were carried out on Wistar strain male rats at an age of 4–5 months. Throughout the experiments, control (euthyroid) animals were supplied with pelleted food (Sljeme, Zagreb) *ad*

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† Abbreviations used: AO, acridine orange; BBMV, brush-border membrane vesicles; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; GFR, glomerular filtration rate; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; TMA^+, tetramethylammonium ion.

libitum. In order to induce a hyperthyroid state, the rats were fed the same diet as above to which a commercial preparation of dessicated thyroid (Thyral, Galenika, Belgrade, Yugoslavia) was added. In this group, each animal consumed daily 1 pellet containing desiccated thyroid (0.5 mg organically-bound iodine) and thereafter was fed regular thyroid-free pellets *ad libitum* for the rest of the day. The treatment lasted for 4 weeks. All rats were offered tap water *ad libitum*.

The oxygen consumption was determined by the method of MacLagan and Sheahan [13], as modified by Tomich and Woollett [14] and expressed in liters of oxygen per square meter of surface area per hour ($l \cdot m^{-2} \cdot hr^{-1}$). The surface area was calculated using the formula [15]; surface area (in cm^2) = $11 \cdot (\text{body mass in g})^{0.63}$. The measurements of oxygen consumption were made 1–3 days before killing of the animals.

Total serum T_3 was determined by using a radioimmunoassay kit (Biodata Labs., Roma, Italy).

The renal cortical BBMV were isolated by the Mg/EGTA-precipitation method of Biber *et al.* [16], but modified for low gravitational forces [17].

$(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was measured by the coupled optical test as described by Berner and Kinne [18]. Leucine arylamidase (EC 3.4.1.2) and alkaline phosphatase (EC 3.1.3.1) activities were determined by using commercial kits (Merckotest No. 3359 and 3344, respectively). The reactions were continuously recorded at room temperature on a Pye Unicam SP 600 UV spectrophotometer with a recorder. The enzyme activities were calculated from the initial rates of the reactions.

The proteins were determined by the method of Bradford [19] using bovine serum albumin as the standard.

Changes in transmembrane ΔpH in isolated membrane vesicles were followed by the fluorescence quenching of acridine orange (AO) as described in detail previously [17, 20, 21]. Prior to the measurements, vesicles were preloaded with the corresponding buffer by two washings followed by an incubation in the same buffer at room temperature for 2 hr.

Na^+–H^+ exchange was studied in both ΔpH-developing and pH-jump modes [17]. In the ΔpH-developing mode, 10 μl of vesicles (0.1 mg protein), preloaded with Na/K-buffer (50 mM mannitol, 150 mM sodium gluconate, 50 mM potassium gluconate, 5 mM HEPES/Tris, pH 7.0), were added into 2.0 ml TMA/K-buffer (50 mM mannitol, 150 mM tetramethylammonium gluconate, 50 mM potassium gluconate, 5 mM HEPES/Tris, pH 7.0) which, in addition, contained 6 μM AO and either 5 μl ethanol (total Na^+–H^+ exchange) or 2.5 μM valinomycin (electroneutral Na^+–H^+ exchange).

In the pH-jump experiments, vesicles (10 μl, 0.1 mg protein) were preloaded with K-buffer A (150 mM mannitol, 150 mM potassium gluconate, 10 mM HEPES/Tris, pH 7.0) and added into TMA-buffer A (149 mM mannitol, 1 mM potassium gluconate, 150 mM tetramethylammonium gluconate, 10 mM HEPES/Tris, pH 7.0) which also contained 6 μM AO and 2.5 μM valinomycin. This condition led to the formation of ΔpH [17]. After the signal of ΔpH had fully developed, concentrated solutions of either sodium gluconate or tetramethylammonium gluconate (final concentration of cations 9.7 mM) were added into outside buffers and the rates of dissipation of the pH gradients were recorded. When necessary, amiloride was added into outside buffer (final concentration 0.5 mM) 30 sec before addition of cations.

The conductances in isolated BBMV were studied as described in detail previously [20, 21]. Vesicles (10 μl, 0.1 mg protein), preloaded with K-buffer B (150 mM mannitol, 150 mM potassium gluconate, 5 mM HEPES/Tris, pH 7.0) were diluted into 2.0 ml TMA-buffer B (150 mM mannitol, 150 mM tetramethylammonium gluconate, 5 mM HEPES/Tris, pH 7.0) which also contained 6 μM AO and either 2.5 μM valinomycin or 5 μM CCCP, or both ionophores (added from ethanol stocks). Controls contained 5 μl of ethanol.

The measurements of fluorescence were started 5 sec after the addition of vesicles to the cuvette. The fluorescence was continuously monitored at room temperature in a Farrand Ratio fluorometer (excitation filter, 490 nm; emission filter, 511 nm). The initial rates of fluorescence changes were estimated by drawing the tangent to the initial part of an Na^+ gradient-driven fluorescence quenching (ΔpH-developing mode) or dissipation of fluorescence quenching (pH-jump mode) and were expressed as fluorescence change per minute (ΔF/min). In the ΔpH-developing mode, the initial rates in the presence of a cation gradient were subtracted for the rates observed in the absence of the gradient.

The data are shown either as a single experiment representative for several repetitions with different membrane preparations (the recordings) or as means ± SE. The differences between the corresponding data were analysed by Student's *t*-test and were considered to be significant at P values less than 0.05.

Chemicals. Acridine orange was from Eastman Kodak (Rochester, NY). Valinomycin and CCCP were purchased from Boehringer (Mannheim, F.R.G.). Other chemicals used were of analytical grade.

RESULTS

The treatment of rats with thyroid hormones for 4 weeks resulted in a smaller body mass and significant increase of kidney mass, oxygen consumption, serum T_3 concentration, and renal cortical $(Na^+ + K^+)$-ATPase activity in comparison with control animals (Table 1). The observed differences between control and hyperthyroid rats are in complete agreement with previously published data of Katz and Lindheimer [6]. In accord with the data reported by Capasso *et al.* [22], we also found a smaller activity of alkaline phosphatase in renal homogenate and in brush-border membranes obtained from rats treated with thyroid hormones. Therefore, all findings indicate a highly hyperthyroid state in rats treated with desiccated thyroid.

The isolated BBMV were enriched in specific activities of luminal membrane marker enzymes, leucine arylamidase and alkaline phosphatase, about 10-fold and 7-fold, respectively. The enrichment

Table 1. Renal enzymes and other parameters in euthyroid and hyperthyroid rats

Parameter	Euthyroid	Hyperthyroid
Body mass (g)	338 ± 14	304 ± 10
Kidney mass (g)	1.78 ± 0.07	2.14 ± 0.09*
Oxygen consumption ($l \cdot m^{-2} \cdot hr^{-1}$)	6.58 ± 0.20	10.97 ± 0.34*
Serum triiodothyronine (nM)	0.93 ± 0.04	1.80 ± 0.12*
($Na^+ + K^+$)-ATPase activity (nmol min^{-1} mg^{-1})		
Homogenate	9.58 ± 1.86	16.51 ± 1.62*
Brush-border membranes	1.47 ± 0.32	2.29 ± 0.63
Enrichment factor	0.21 ± 0.07	0.22 ± 0.07
Leucine arylamidase activity (μmol min^{-1} mg^{-1})		
Homogenate	30.0 ± 0.75	28.3 ± 2.25
Brush-border membranes	281.1 ± 25.9	310.8 ± 30.9
Enrichment factor	9.4 ± 0.84	11.1 ± 1.11
Alkaline phosphatase activity (μmol min^{-1} mg^{-1})		
Homogenate	0.42 ± 0.04	0.27 ± 0.04*
Brush-border membranes	2.87 ± 0.28	1.51 ± 0.09*
Enrichment factor	7.4 ± 1.13	6.5 ± 0.88

Shown are means ± SE from 5 to 7 experiments. Statistically significant differences are indicated by an asterisk.

factor for basolateral marker, ($Na^+ + K^+$)-ATPase activity, was less than 0.5. As judged from similar enrichment factors of these enzyme activities in control and hyperthyroid animals, the efficiency of isolation of luminal membranes was equal in both groups of rats.

In the ΔpH-developing experiments, an Na^+ gradient ($Na^+_i > Na^+_o$) in vesicles from control animals led to a time-dependent fluorescence quenching (final quenching about 10%) due to a Na^+–H^+ exchange (Fig. 1, Control, −Val). In the vesicles from hyperthyroid rats, we observed much faster development of the quenching and stronger final quenching (about 20%) (Fig. 1, Hyperthyroid, −Val). The initial rates of quenching, which are a reliable measure of Na^+–H^+ exchange in BBMV [17], were by 30% higher in vesicles from hyperthyroid than from euthyroid rats (Table 2, −Valinomycin). The results may indicate that thyroid hormones stimulate the activity of the Na^+–H^+ antiport in renal BBMV, as has recently been described by Kinsella *et al.* [10, 11] and Yusufi *et al.* [3]. However, Na^+–H^+ exchange can result from the action of (a) an electroneutral Na^+–H^+ antiporter, and (b) electrically coupled Na^+ and H^+ movements through coexisting conductive paths in isolated membranes [21, 23]. To test the contribution of the electroneutral and electrically coupled Na^+–H^+ exchange in our membrane vesicles, fluorescence quenching due to a Na^+ gradient was measured in the presence of equal K^+ concentration from both sides of the vesicles membrane and absence of valinomycin (Fig. 1, $Na^+_i > Na^+_o$, −Val; Table 2, −Valinomycin) and compared with the quenching under same conditions, but in presence of valinomycin (Fig. 1, $Na^+_i > Na^+_o$, +Val; Table 2, +Valinomycin). With K^+ plus valinomycin in the test, an electrically coupled intravesicular H^+ uptake

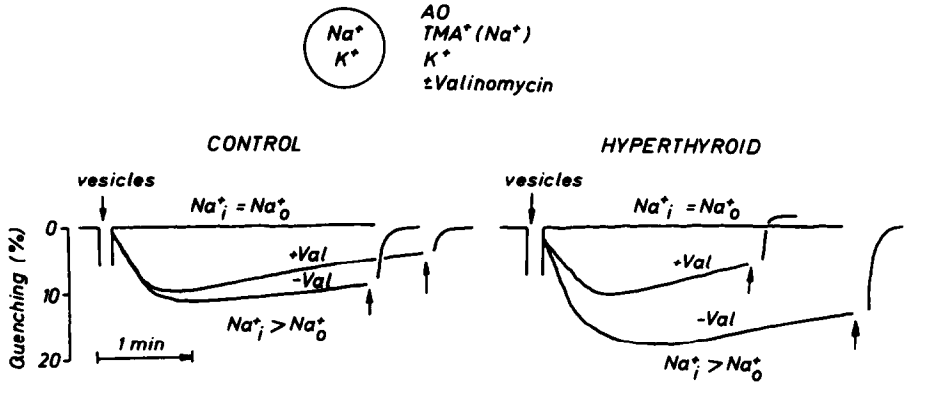

Fig. 1. Na^+–H^+ exchange measured by the ΔpH-developing mode in renal BBMV from euthyroid (Control) and hyperthyroid rats. Vesicles were preloaded with Na/K-buffer and diluted into the same ($Na^+_i = Na^+_o$) or TMA/K buffer ($Na^+_i > Na^+_o$) which contained AO and either ethanol (−Val) or valinomycin (+Val). At the indicated time (arrow), Na^+ was added into the cuvette (final concentration 25 mM) to dissipate the pH gradient.

Table 2. Electroneutral and electrically-coupled Na^+–H^+ exchange in brush-border membrane vesicles from euthyroid and hyperthyroid rats as measured by the ΔpH-developing mode

Animals	Na^+–H^+ exchange (ΔF/min) −Valinomycin	+Valinomycin
Euthyroid	16.5 ± 0.59	15.2 ± 1.33
Hyperthyroid	21.5 ± 1.62*	16.6 ± 1.60**

The experimental conditions are described in the legend of Fig. 1. Shown are initial rates of fluorescence quenching (means ± SE) from 7 independent experiments. Statistically significant differences are indicated by one (vs Euthyroid, −Valinomycin) or two asterisks (vs Hyperthyroid, −Valinomycin).

Table 3. Na^+–H^+ exchange in brush-border membrane vesicles from euthyroid and hyperthyroid rats as measured by the pH-jump mode

Condition	Na^+–H^+ exchange (ΔF/min) Euthyroid (N = 4)	Hyperthyroid (N = 5)
TMA^+	1.13 ± 0.26	1.14 ± 0.11
Na^+	4.50 ± 0.25	4.26 ± 0.22
Na^+ + amiloride	1.13 ± 0.14	1.20 ± 0.19
Amiloride-sensitive portion	3.38 ± 0.26	3.12 ± 0.18

Experimental conditions are described in the legend of Fig. 2. Shown are the initial rates of fluorescence quenching dissipation (means ± SE) with 9.7 mM indicated cations in the absence or presence of 0.5 mM amiloride. N = number of rats.

driven by a Na^+ diffusion potential is minimized and only an electroneutral exchange is recorded [17, 21, 23].

In membrane vesicles from euthyroid rats, the final quenching and the initial rates of quenching were similar in the presence and absence of valinomycin (Fig. 1, and Table 2, +Valinomycin and −Valinomycin, respectively). The experiment indicates a negligible electrically coupled Na^+–H^+ exchange in these membranes which completely agrees with previous findings [17, 23]. On the contrary, in vesicles from hyperthyroid rats, in the presence of valinomycin the final quenching was smaller by 50% (Fig. 1) and the initial rates by 30% (Fig. 1 and Table 2). Moreover, in the presence of valinomycin, the final quenching and the initial rates in vesicles from hyperthyroid animals were practically identical to those in vesicles from control animals (Fig. 1 and Table 2). Therefore, these results indicate that the electroneutral Na^+–H^+ exchange is unaltered whereas the electrically coupled transmembrane movements of Na^+ and H^+ are increased in vesicles from hyperthyroid animals.

Similar activities of electroneutral Na^+–H^+ exchange in vesicles from euthyroid and hyperthyroid rats, as observed by the ΔpH-developing mode, were obtained also in pH-jump experiments. As demonstrated in Fig. 2, addition of Na^+ to the cuvette after ΔpH had been fully developed led to a concentration dependent increase in rates of ΔpH dissipation via Na^+–H^+ exchange. The rates observed at 9.7 mM Na^+ were used to compare Na^+–H^+ exchange in vesicles from euthyroid and hyperthyroid animals and were assumed to be completely electroneutral because (a) K^+ and valinomycin, necessary to diminish electrically coupled Na^+–H^+ exchange, were present in the assay, and (b) 0.5 mM amiloride, an inhibitor of electroneutral Na^+–H^+ exchange, inhibited the Na^+ gradient-driven ΔpH dissipation completely (Fig. 2). The data from this experiment are summarized in Table 3.

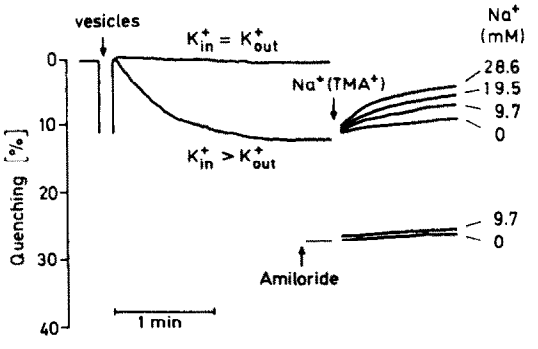

Fig. 2. Na^+–H^+ exchange measured by the pH-jump mode in renal BBMV. Vesicles were preloaded with K-buffer A and diluted into the same ($K^+_{in} = K^+_{out}$) or TMA-buffer A ($K^+_{in} > K^+_{out}$) which contained AO and valinomycin. After the ΔpH had fully developed, either TMA^+ (9.7 mM, 0) or indicated concentrations of Na^+ were added into the cuvette to dissipate pH gradient via Na^+–H^+ exchange. Where indicated, amiloride was added 30 sec prior to cation injection.

The rates of ΔpH dissipation in the absence of Na^+ (TMA^+) were similar in vesicles from both groups of animals. At 9.7 mM Na^+ (Na^+), the rates increased by 4-fold and were also similar in vesicles from euthyroid and hyperthyroid animals. Being completely inhibited by amiloride (Na^+ + amiloride), the Na^+ gradient-dependent rates of ΔpH dissipation can be entirely attributed to the electroneutral Na^+–H^+ exchange. The electroneutral Na^+–H^+ antiport, i.e. amiloride sensitive portion, was similar in vesicles from both groups of animals.

In order to investigate the cause of the increased electrically coupled Na^+–H^+ exchange in membranes from hyperthyroid rats, as detected in ΔpH-developing experiments, we have tested the ion conductances in vesicles from both groups of animals. The AO method is suitable for measurement of the conductances for H^+ and K^+, but not for Na^+ [21]. An H^+ conductance can be visualized by intravesicular acidification after imposing an in-to-out K^+ gradient in the presence of valinomycin [21]. On the other hand, the rate of acidification with outwardly oriented K^+ gradient in the presence of protonophore CCCP is a relative measure for the K^+ conductance [21].

In the presence of a K^+ gradient and absence of any ionophore (Fig. 3, euthyroid and hyperthyroid, EtOH, and Table 4, ethanol) only a small increase in quenching was observed in comparison with that

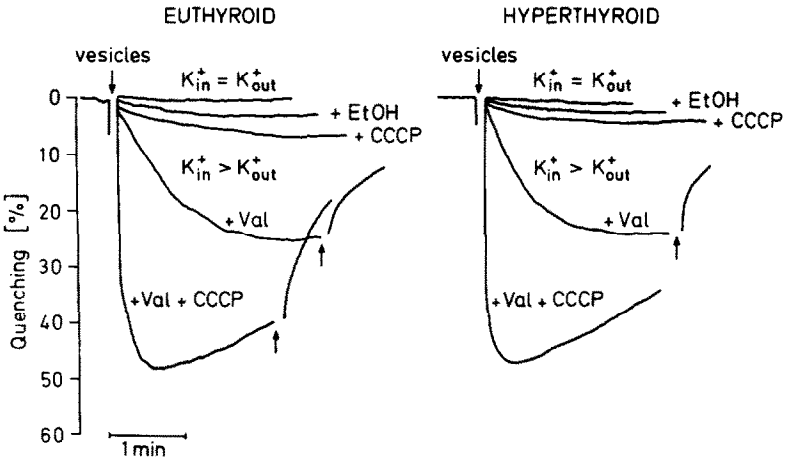

Fig. 3. K^+ gradient-driven acidifications in renal BBMV from euthyroid and hyperthyroid rats. Vesicles were preloaded with K-buffer B and diluted into the same ($K^+_{in} = K^+_{out}$) or TMA-buffer B ($K^+_{in} > K^+_{out}$) which contained AO and either ethanol (+EtOH) or CCCP (+CCCP) or valinomycin (+Val), or both ionophores (+Val +CCCP). At the indicated time (arrow), Na^+ was injected into the cuvette (final concentration 25 mM) to dissipate the pH gradient.

in absence of a K^+ gradient ($K^+_{in} = K^+_{out}$) indicating that both kinds of membrane vesicles possess the small but similar intrinsic K^+ and H^+ conductances. By increasing the H^+ conductance (Fig. 2 and Table 4, CCCP), no additional decrease in fluorescence was observed in both vesicle preparations, suggesting that the intrinsic permeability of the vesicle membranes to K^+ is indeed negligible. On the contrary, in the presence of valinomycin (Fig. 2, Val), the quenchings were much stronger in both kinds of vesicles indicating a significant intrinsic H^+ permeability in membranes from both groups of animals. As indicated in Table 4, the permeability for H^+ in vesicles from hyperthyroid rats was by 37% higher than in euthyroid animals.

The differences in fluorescence quenching observed in this experiment may be an artefact caused by differences in (a) quality of membrane preparations, (b) preloading of the vesicles with K^+, (c) buffering capacity, and/or (d) degree of vesiculation in two membrane preparations. The effect of different preparations can be excluded as the enrichment factors for various marker enzymes were similar in both membrane preparations (Table 1). The other three possibilities were tested in an experiment with in-to-out K^+ gradient and the presence of ionophores for both, K^+ and H^+ (Fig. 2, Val + CCCP). With both ionophores a strong fluorescence quenching was induced which depends on all three parameters: the number of vesicles, intravesicular buffering capacity and loading of vesicles with K^+. The final quenchings in these experiments were similar for vesicles from euthyroid (54.2 ± 1.81%, N = 7) and hyperthyroid rats (46.1 ± 0.97%, N = 7, N.S.) indicating that neither of the above-mentioned possibilities can account for the observed differences in H^+ permeability between both membrane preparations.

Table 4. K^+ gradient-driven acidifications in renal brush-border membrane vesicles from euthyroid and hyperthyroid rats

Additions	Rate of acidification (ΔF/min) Euthyroid	Hyperthyroid
Ethanol	1.39 ± 0.50	0.89 ± 0.25
CCCP	1.27 ± 0.22	1.41 ± 0.16
Valinomycin	12.57 ± 1.13	17.17 ± 1.25*

Experimental conditions are described in the legend of Fig. 3. Shown are the initial rates of fluorescence quenching (means ± SE) from 7 independent experiments. Statistically significant differences, vs corresponding euthyroid data, are indicated by an asterisk.

DISCUSSION

By using the fluorescence quenching of AO we studied Na^+–H^+ exchange and K^+ and H^+ conductances in renal BBMV isolated from euthyroid and hyperthyroid rats. Whereas the electroneutral Na^+–H^+ exchange and K^+ conductances were similar in vesicles from both groups of animals, the conductance for H^+ was higher in vesicles from hyperthyroid rats.

The mechanisms by which thyroid hormones stimulate Na^+ and fluid reabsorption in the mammalian proximal tubule are a matter of dispute [1–12]. The proposal that the thyroid hormone-induced increase in activity of basolateral ($Na^+ + K^+$)-ATPase activity would be a primary driving force for the transepithelial electrolyte and fluid reabsorption, has been challenged. The activity of this enzyme ([2, 4, 6, 12] and this study) and, on a longer run, the synthesis of enzyme units [7] is indeed increased in proximal tubular cells following *in vivo* treatment with thyroid hormones. However, changes in Na^+ and fluid reabsorption and in ($Na^+ + K^+$)-ATPase do not coincide [8, 9]. The second possibility, that thyroid hormones affect the proximal tubular luminal membrane by increasing its permeability for cations has been studied previously [3, 9, 10, 12] and was also under investigation in this paper. Recently pub-

lished data indicated that thyroid hormones may induce the activity of amiloride-sensitive Na^+–H^+ exchange in renal cortical BBMV. The Na^+–H^+ exchange seems to have a major role in Na^+ reabsorption and H^+ secretion in the proximal tubule [24]. The increased activity of Na^+–H^+ exchanger would increase the acidification of the proximal tubular fluid and Na^+ and HCO_3^- reabsorption. However, experiments in which absorption of glycodiazine, a buffer which in the proximal tubule behaves similarly to bicarbonate [25], was studied, failed to demonstrate a significant thyroid-stimulated HCO_3^- reabsorption in the perfused proximal tubule [5]. Compared to glycodiazine absorption in euthyroid rats, the absorption in hypothyroid animals was smaller by only 4% and was restored to normal after T_3-treatment [5]. This is at odds with a large, 45% [3] to 75% [10] increase in Na^+–H^+ exchange observed by rapid filtration techniques in renal luminal membranes from T_3-treated animals. On the contrary, Capasso *et al.* [9] used the same techniques to study Na^+–H^+ exchange as in the above mentioned papers but they did not find an effect of thyroid hormones on amiloride-sensitive antiport activity in rat renal luminal membranes. As shown in this paper, we also found no differences in electroneutral Na^+–H^+ exchange in renal BBMV isolated from euthyroid and hyperthyroid rats.

Next, we considered the effect of thyroid hormones on the K^+ conductance in vesicle membranes. Haber and Loeb [26] showed that thyroid hormones increase the passive permeability to K^+ in rat diaphragm. As suggested by Schultz [27], the K^+ permeability of basolateral membrane could regulate the transepithelial Na^+ transport by increasing indirectly $(Na^+ + K^+)$-ATPase activity thereby increasing the driving force for Na^+ entry at the luminal membrane. Experiments made by Capasso *et al.* [12] indeed indicated an increased K^+ conductance in the proximal tubular cell membrane in T_3-treated rats. However, as to K^+ conductance in luminal membrane, we could not confirm those results; the apparent K^+ conductance in BBMV from both euthyroid and hyperthyroid rats was similar. Our results do not preclude possible changes in basolateral K^+ conductance which, in accordance to the proposed mechanism [27] should be more important; K^+ conductance in basolateral membranes was not investigated in this study.

In experiments with K^+ gradients we found a significant H^+ conductance in brush-border membranes from both groups of rats, in hyperthyroid animals being by 37% higher than in euthyroid. The presence of a H^+ conductance in isolated luminal membranes has been described previously [20, 21, 23]. Meanwhile, this is the second report on increased passive H^+ permeability in renal brush-border membranes due to an action of thyroid hormones. Recently, Kinsella and Sacktor [10] described that the rate of dissipation of preformed pH gradients was much faster in BBMV from hyperthyroid than hypothyroid rats, probably due to higher H^+ conductance in membrane vesicles from hyperthyroid animals. The significance of these findings is not clear.

The electrically coupled Na^+–H^+ exchange in BBMV from hyperthyroid animals, which is not present in euthyroid rats, indicates that besides a significant H^+ conductance, the membranes from hyperthyroid rats may also have an increased conductance for Na^+. The thyroid hormone-dependent high Na^+ conductance of luminal membranes has been previously considered as a reason for increased electrolyte and fluid reabsorption, but not proven [3, 9, 11]. On the other side, the data in this paper indicate that thyroid hormones may affect an Na^+ conductance in renal BBMV, but these indications are indirect. Specific tracer studies should be performed to confirm our findings. However, it is difficult to compare the data on thyroid hormone-dependent changes in plasma membrane properties found by us and by others as BBMV isolated by us (Mg/EGTA-precipitation method) and by others (Ca- or Mn-precipitation method) [3, 9–11] differ significantly in transport characteristics [16, 23] and ion conductances [23]. Also, it has to be pointed out that previous studies on the effect of thyroid hormones upon renal Na^+–H^+ exchange [3, 10, 11] were not performed under conditions with K^+ plus valinomycin. Furthermore, to correlate the data obtained in vesicle studies to those obtained on animals *in vivo* or microperfused proximal tubules *in vitro* is also complicated by the fact that different strains of rats, experimental approaches, durations and intensities of the treatment with thyroid hormones, and different techniques for transport studies have been used. For example, in previous studies of Na^+–H^+ exchange vesicles were isolated from rats which were made hypothyroid by either surgical thyroidectomy or thiouracil treatment and were thereafter made hyperthyroid by the thyroid hormone treatment [3, 10, 11]. Such maneuvers may influence the level of other hormones in the body that may affect the activity of renal Na^+–H^+ exchanger [24]. The experiments described in this paper were performed on intact euthyroid and hyperthyroid animals and thus may be more relevant for *in vivo* situations.

The increased H^+ and Na^+ conductances in luminal membranes from rats treated with pharmacological doses of thyroid hormones for longer time, as indicated in our studies, are not incompatible with the increased Na^+ and fluid reabsorption in hyperthyroid proximal tubules. The Na^+ leakage down its electrochemical gradient across the luminal membrane could facilitate the uptake of fluid and, on a longer run, stimulate the activity and synthesis of $(Na^+ + K^+)$-ATPase in the basolateral membrane. A high H^+ conductance could dissipate the pH gradient and thus would tend to inhibit HCO_3^- reabsorption. However, the backleak of H^+ would increase intracellular concentration of H^+ which in turn have a modifier role in activating the Na^+–H^+ exchanger at the internal site of luminal membrane [28]. Such an accelerated activity of the Na^+–H^+ exchanger, which is not manifested in isolated luminal membranes, can easily keep reabsorption of HCO_3^- normal. In accord with these assumptions are observations reported by Kinsella *et al.* [11] that hyperthyroid rats *in vivo* exhibit no systemic acid-base disturbance or changes in urinary acid secretion.

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