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EFFECTS OF THYROID HORMONES ON SODIUM DIFFUSION ACROSS PROXIMAL TUBULAR BRUSH BORDER MEMBRANES

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Summary

The influence of thyroid hormones on antiporter-mediated sodium uptake was studied in vitro on brush border vesicles. Measurements of kinetic parameters was performed to characterize sodium–hydrogen exchange. Vesicles pre-equilibrated at pH 6 in the presence of the fluorescent probe acridine orange were tested with different sodium concentrations and the change in fluorescence continuously recorded. The results obtained in control conditions were compared to those measured when thyroxine (T_3, 1μM) was present. T_3 raised the K_m from 1.51 to 5.13 ($p<0.01$), while V_{max} was unchanged. Additional studies showed that the brush border contained the monodeiodinase enzyme, capable of converting T_4 to T_3.

These data demonstrate that physiological concentrations of T_3 are capable of influencing the affinity of either sodium or hydrogen or both for the antiporter. Thyroid hormones may be part of a physiological system that controls sodium permeability of the luminal membrane in vivo.

Introduction

Among organic cations, which are important in proximal transport, catecholamines are receiving increasing attention both in vitro [1] and in vivo [2]. Their known positive interaction with thyroid hormones suggests a possible mechanism for control of sodium entry across the brush border. Recently a pathway for sodium entry into the tubular cell from the lumen has been elucidated: it involves an antiporter-mediated diffusional step in exchange with hydrogen, a mechanism where organic cations could act as modulators [3]. Since free thyroid hormones are filtered, the brush border in vivo is in contact with physiological concentrations of T_3 and T_4.

The importance of the luminal side in the control of proximal reabsorption and in the phenomenon of glomerulo-tubular balance has been established [4,5].

The present experiments were undertaken to assess the influence of thyroid hormones on sodium diffusion by antiporter in the isolated perfused brush borders in vitro.

Materials and methods

The experiments were performed on male Wistar rats, weighing 200–300g. Tubular brush border was isolated and purified in the form of vesicles by the technique of Evers et al [6]. Each experiment of in vitro perfused brush border vesicles was performed with the material obtained from a batch of 15–20 rats. Altogether 45 experiments were performed, mostly to improve and modify the technique described by others [7], illustrated in Figure 1. The incubation medium contained sucrose 250mM, Hepes Tris buffer 10mM, pH 8.2 at 25°C. Upon addition of acridine orange 6μM, the fluorescence signal of the probe was continuously recorded. When it became stable after spontaneous drifting, $NaSO_4$ was added, and, after a stable signal was obtained, 10μl of brush border vesicles

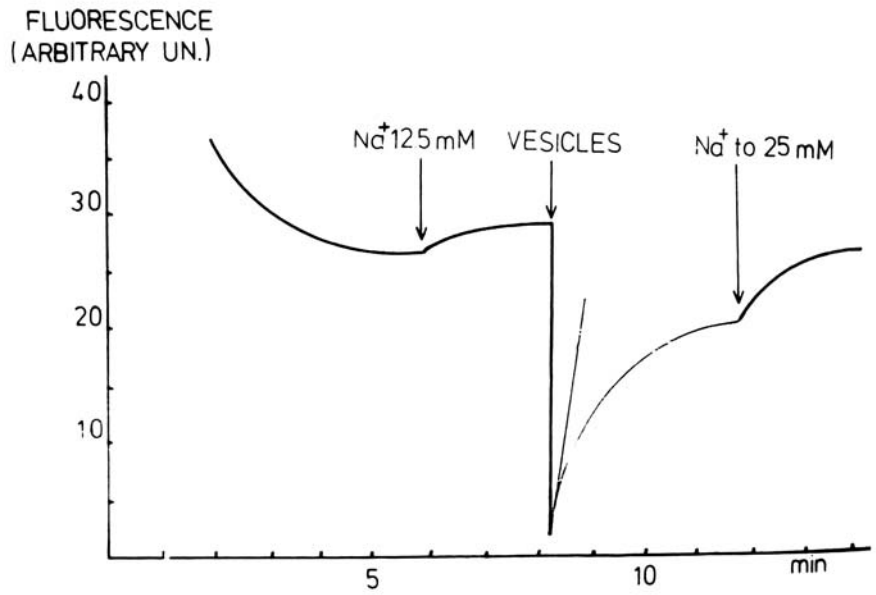

Figure 1. The ordinate is the fluorescence signal generated by acridine orange, in arbitrary units, the abscissa time in minutes. The initial part of the curve indicates a significant spontaneous sequencing and a non-specific rise in the signal after Na addition. The vesicles are added only after stabilization of the fluorescence. They cause an immediate fall in fluorescence, due to a shift of acridine into the vesicles determined by the pH gradient. The slow dissipation of this gradient by H^+ exit causes the subsequent rise in fluorescence. The determination of the kinetic constants is calculated from this part of the experimental recording (tangent at time of the recovery phase). The figure shows the enhancement of the fluorescence determined by a pulse additon of Na^+, which facilitates more H^+ (and, therefore, more acridine orange) exit.

were added, corresponding to 0.2mg of protein. The fluorescence signal fell rapidly, due to the entry of the dye into the vesicles, because of the pH gradient imposed between the incubation medium and the inside of the vesicles (pH 6.00, sucrose 250mM, Hepes Tris 10mM). Subsequently, exit of hydrogen from the vesicles in exchange for the sodium present in the medium slowly dissipated the pH gradient and allowed the acridine orange to diffuse into the outside medium, restoring the fluorescence signal. Fluorescence recovery was accomplished by an antiporter-mediated sodium for hydrogen exchange. It was formed by two exponential functions according to the equation $F=A(1)\cdot(1-e^{-K(1)t}) + A(2)\cdot(1-e^{-K(2)t})$, where F is fluorescence in arbitrary units, K is the rate constant of fluorescence change, t the time.

The derivative of each exponential component was computed from the computer-fitted experimental curve. The initial rates of change in fluorescence (and, hence, in sodium-dependent hydrogen efflux) were used to construct the Lineweaver-Burke plots by least square methods. These were used to calculate the K_m and V_{max} of the antiporter, and the influence of thyroid hormones. The experiments were carried out with either placebo or T_3 1μM in the medium. Under each circumstance experiments were performed at sodium concentrations of 3.12, 6.25, 12.5 and 25.0mM.

The final solution was made isotonic to plasma by appropriate concentrations of sucrose up a final osmolarity of 250mOsm/kg.

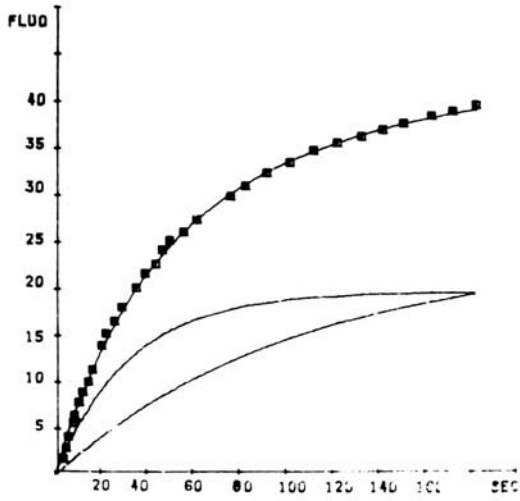

Figure 2. The recovery phase of the fluorescence signal shown in Figure 1. The squares are the experimental recordings. The top line is fitted by computer, the curves below are exponential functions (slow and fast) calculated by the computer to yield the experimental curve. For further explanations see text

Results

Figure 2 shows the results of a typical experiment. A single solution for the computer-derived best fit curve (by using exponential functions only) is obtained by adding the slow (below) and fast (middle) components shown. Table I reports the average values obtained for K_m and V_{max}.

TABLE I

Control				T_3 1μM			
K_m(a)	1.51	V_{max}(a)	0.870	K_m(a)	5.13	V_{max}(a)	0.650
K_m(b)	0.251	V_{max}(b)	0.835	K_m(b)	3.54	V_{max}(b)	0.776
K_m(c)	9.28	V_{max}(c)	0.165	K_m(c)	1.12	V_{max}(c)	0.490
K_m(d)	0.210	V_{max}(d)	0.776	K_m(d)	5.30	V_{max}(d)	0.161

The data were calculated from the experimental curve, either manually (a) or by the computer (b), (c) and (d) refer to data calculated by the computer from the 'slow' and 'fast' components of the experimental curve respectively. K_m is the Michaelis constant in mM/L, V_{max} the maximal velocity of reaction in mM/min

T_3 decreases the overall affinity of the antiporter for sodium, while leaving the maximal transport capacity practically unchanged. This effect is obtained by increasing the affinity (the K_m falls from 9.28 to 1.12mM) of the slow component, the V_{max} of which increases sizeably. Simultaneously the K_m of the

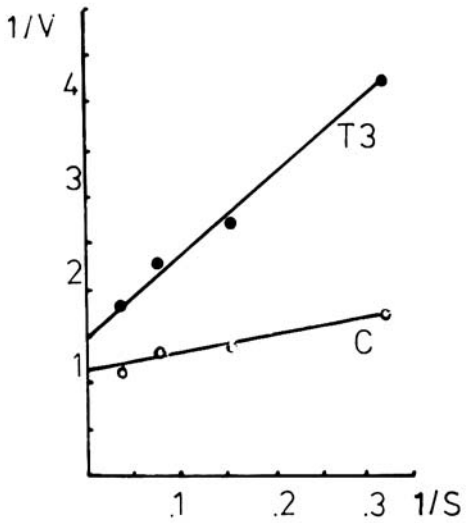

Figure 3. The ordinate is the reciprocal of the initial rate of fluorescence recovery (the tangent drawn manually at time zero in Figure 1) plotted against the reciprocal of the Na concentration in mM/L (abscissa). The reciprocal of the intercept on the Y axis is the V_{max}, on the X axis is the K_m. The lines drawn are the least square fittings for control data (closed circles) and measurements performed in the presence of T_3 (open circles)

fast component increases and the V_{max} falls. Figure 3 shows the Lineweaver-Burke plots obtained in one experiment, and demonstrates the effect of T_3 in reducing the affinity while not affecting V_{max}.

Discussion

Proximal tubule brush border is a highly permeable structure to ions and water, containing a system capable of facilitated diffusion. Among these the antiporter exchanges the reabsorbed sodium for a secreted hydrogen. Since a unique protein seems necessary to operate the mechanism, which can be blocked by Amiloride [3], there may be substances capable of acting as potential modulators by competing with substrates on either side of the brush border membrane, or by changing the affinity of the substrate for the antiporter. We have shown that catecholamines can influence sodium uptake [8], and therefore decided to evaluate the effects of thyroid hormones, known to influence sodium reabsorption and potassium permeability in the proximal tubule [9], and to act in concert with catecholamines in peripheral tissues. Previous work by others [7] established the reliability of the acridine orange method in measuring the antiporter-mediated sodium uptake. This method is reliable and accurate by the modified technique we used. The present data demonstrate that there are two channels available for the antiporter mediated sodium–hydrogen exchange in the brush border membrane.

Normally the slow component contributes little transport since it displays a low V_{max}, notwithstanding the high affinity. Although its affinity is reduced by T_3, its transport capacity is enhanced. The reverse happens for the fast component under the action of T_3, the overall result being a fall in affinity with no significant change in transport. This system, which has a great flexibility, is in apparent contrast with our results with ^{22}Na, which showed increased net sodium flux in brush borders with T_3 [10]. However, if T_3 decreased the K_m for hydrogen, it could favour hydrogen binding and secretion and therefore drive an increased sodium flux in exchange. The concomitant change in the K_m for sodium is uninfluential, since it remains well below the sodium concentration present in the proximal tubule. Thus, we favour the interpretation that thyroid hormones may compete with sodium for binding, decreasing its affinity for the antiporter. Probably sodium and hydrogen are transported in a mutually competitive way, and T_3 may depress the K_m for hydrogen, a value expected in the nanomolar concentration range. If T_3 can change the affinity of the antiporter for its substrates in a reciprocal way, it could exert an important and subtle counter-regulatory function.

For instance, it could help maintain proximal reabsorption in the face of a vanishing pH gradient, as in metabolic acidosis. This regulatory capability could be enhanced by similar effects of thyroid hormones on the permeability of the basolateral membrane [9], where they could act by shifting intracellular sodium and hydrogen concentrations above and near their K_m for the antiporter, thus magnifying their intraluminal and brush border-mediated mechanisms. Finally, both physiologically acting hormones could be important since the brush border membrane can easily convert T_4 to T_3 [10].

In conclusion, the data obtained by the present experiments demonstrate that T_3 and T_4 reduce the affinity of the antiporter for sodium. Therefore, they could influence and act as physiological regulators of proximal sodium transport and acidification.

Acknowledgments

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