Role of Sodium in Thyroid Hormone Uptake by Rat Skeletal Muscle

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Abstract

Whether Na^+ movement through the plasma membrane plays a role in thyroid hormone uptake was investigated in intact rat soleus muscles. After preincubation for 120 min at 37°C in modified Krebs–Ringer bicarbonate containing 140 or 5 mM Na^+ plus choline or lithium to maintain osmolarity, muscles were incubated with 50 pM [^{125}I]triiodo-L-thyronine (T_3) or [^{125}I]L-thyroxine (T_4) for 60 min. T_3 uptake was decreased when extracellular Na^+ was replaced by either choline or lithium, the amount of decrease corresponding to the specific (or saturable) uptake component. Monensin, an ionophore that stimulates Na^+ entry, increased T_3 uptake at 140 mM Na^+ but not at 5 mM Na^+. Amiloride, a Na^+/H^+ exchange inhibitor, had no effect on T_3 uptake under basal conditions or when Na^+ was replaced by choline, but reversed the action of lithium. Ouabain, an inhibitor of Na^+/K^+ ATPase, reduced specific T_3 uptake. T_4 uptake was unaffected by low extracellular Na^+. These results are consistent with a major role of Na^+ movement in T_3 uptake by skeletal muscle, but not in T_4 uptake, and suggest an involvement of membrane pumps in this process.

Introduction

The possible role of the plasma membrane in regulating peripheral thyroid hormone metabolism has been extensively studied (1–9). A specific hormone uptake has been described in hepatocytes (6), erythrocytes (5), lymphocytes (4), pituicytes (8), and fibroblasts (9). Previous studies in our laboratory demonstrated that part of the 3,5,3'-triiodo-L-thyronine (T_3)[1] entering skeletal muscle cells does so by a stereo-specific and energy- and temperature-dependent process (10). In this tissue the nuclear-associated T_3 derives almost exclusively from circulating T_3 (11) without contribution from the intracellular 5'-deoidination of thyroxine (T_4), thus indicating that the plasma membrane might modulate the availability of active thyroid hormone. However, the exact mechanism of thyroid hormone entry into cells is still poorly defined.

A role of sodium in thyroid hormone uptake was suggested in hepatocytes by Krenning et al. (6) and in erythrocytes by

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1. *Abbreviations used in this paper:* KRB, Krebs-Ringer bicarbonate; pH$_i$, internal pH; T_3, 3,5,3'-triiodo-L-thyronine; T_4, thyroxine.

Holm et al. (5) based on ouabain sensitivity of the initial rate of uptake by these tissues. Sodium plays a major role in amino acid transport across the cell membrane, and the "A" system, mediating the concentrative uptake of neutral amino acids, is strictly sodium dependent (12, 13). Thyroid hormone is an amino acid derivative, although apparently not sharing the A system (unpublished data). The aim of the present study was to investigate the relation between sodium movement across the plasma membrane and thyroid hormone entry into rat soleus muscle.

Methods

L-T_3, L-T_4, amiloride, and monensin were purchased from Sigma Chemical Co. (St. Louis, MO). Ouabain was obtained from Calbiochem-Behring Corp. (La Jolla, CA). [^{125}I]L-T_3 (3,300 μCi/μg) and [^{125}I]L-T_4 (4,400 μCi/μg) were purchased from New England Nuclear (Boston, MA). The purity of labeled and unlabeled hormones was assayed by thin-layer chromatography on silica gel K1F (Whatman, Inc., Clifton, NJ) using the solvent system formic acid/methanol/chloroform (1:3:16), according to Sato and Cahnmann (14).

Sprague–Dawley male rats weighing 70–130 g were purchased from Taconic Farms, Inc. (Germantown, NY). The animals were kept for at least 3 d before the experiment, had free access to commercial food pellets (Ziegler Brothers, Inc., Gardners, PA) and water, and were maintained at constant temperature with a light–dark cycle of 12 h. Rats were killed by carbon dioxide inhalation and soleus muscles were excised as previously described (15) and placed in ice-cold buffer for ~ 30 min. Modified Krebs–Ringer bicarbonate (KRB) buffer, containing 5 mM sodium pyruvate and half the usual $CaCl_2$ concentration (i.e., 2.5 mM), was used throughout the study; in low sodium experiments, Na^+ was replaced by equimolar amounts of choline; when lithium was used, it replaced sodium chloride up to a final concentration of 120 mM lithium and choline HCO_3^- was substituted for $NaHCO_3$. In both cases the buffer contained 5 mM sodium due to the presence of Na^+ pyruvate as an energy source.

To perform uptake studies the isolated muscles were preincubated for 120 min at 37°C, pH 7.38, in capped borosilicate vials containing KRB under an atmosphere of O_2/CO_2 (95:5%) in a shaking water bath (40 oscillations/min). Incubation was carried out after addition of 50 pM [^{125}I]T_3 or [^{125}I]T_4 for 60 min unless otherwise specified. Unlabeled thyroid hormone was added, where appropriate, only during incubation; all the other substances were present during both preincubation and incubation. The final pH did not exceed 7.55; experiments with larger pH variations were discarded. The incubation was ended by rinsing the muscles in ice-cold buffer; the muscles were then blotted on filter paper, and tendons were accurately removed. The total radioactivity in each vial was measured before the incubation, and the radioactivity incorporated during incubation was measured in an auto gamma scintillation spectrometer (Hewlett-Packard Co., Palo Alto, CA). The muscles were then dried at 60°C for at least 6 h and weighed; the dry weight was preferred to wet because preliminary experiments showed that, despite a reasonably close correlation, the former eliminates considerable individual variability. Where possible, the experiment was performed using the paired technique, in which the two muscles from the same animal were compared. The uptake was expressed as percent of total [^{125}I]T_3 of T_4 per milligram dry weight. The nonspecific uptake

was determined by adding 10 µg unlabeled T_3 to the incubation buffer, as previously described (10). The specific uptake component was evaluated as the difference between the total uptake and the nonspecific uptake. All experiments were in triplicate or quadruplicate, and the results are expressed as mean±SD of at least three separate experiments. Statistical analysis was performed, where appropriate, using Student's t test for paired data; otherwise, the unpaired t test was used.

Results

The time course of T_3 uptake in rat soleus muscle under basal conditions was compared with the uptake from medium in which sodium was replaced by lithium. The results presented in Fig. 1 show that the curves diverge after 15 min, the effect of sodium depletion being to decrease the total uptake by $\sim 30\%$ at 60 min. Note that the muscles were preincubated for 120 min before the addition of labeled T_3 (see Discussion). It was shown previously that T_3 uptake due to the specific or saturable component of uptake approaches equilibrium after 60 min incubation (10). Furthermore, the amount of decrease in total T_3 uptake observed after incubation with a saturating level of T_3 (10) was the same as that seen in present experiments when sodium was replaced by lithium. The results in Table I show that sodium replacement by either lithium or choline significantly decreased T_3 uptake ($P < 0.001$) and the effect of the two cations did not differ statistically from each other. The addition of excess T_3 to the Na^+-depleted buffer containing lithium produced no further decrease in T_3 uptake, indicating that sodium deprivation affected only the specific T_3 uptake component.

Table I also presents data on the uptake of labeled T_4 by soleus muscle in basal and sodium-depleted buffer. In contrast with the results obtained with T_3, T_4 uptake was not affected by sodium replacement with either lithium or choline, in keeping with the previously recognized absence of a specific T_4 uptake component in this tissue (10).

The decrease in T_3 uptake induced by sodium depletion was proportional to the concentration of sodium in the buffer as shown in Fig. 2. The role of sodium entry on T_3 uptake in the muscle was investigated further by the use of 10 µM monensin, an ionophore that greatly increases the entry of the cation in skeletal muscle (16) through the Na^+ channel or the Na^+/H^+ exchanger (17, 18) (see below). In both lithium- and

Table I. Effect of Sodium Deprivation on Thyroid Hormone Uptake

	Uptake per milligram dry weight*	Change	P‡
	%	%	
T_3 uptake			
Control	1.63±0.5	—	—
Na^+ replaced by Li^+	1.19±.09	−27	<0.001
Control	1.55±.1	—	—
Na^+ replaced by choline	1.20±.14	−22	<0.001
Control	1.67±.09	—	—
Excess T_3	1.23±.15	−26	<0.001
Excess T_3 + Na^+ replaced by Li^+	1.29±.15	−23	<0.001
T_4 uptake			
Control	0.57±.04	—	—
Na^+ replaced by Li^+	0.66±.1	—	NS
Na^+ replaced by choline	0.62±.11	—	NS

* Total uptake by rat soleus muscles at 60 min. Mean±SD of three separate experiments in triplicate.
‡ Compared with control in each group.

choline-containing media, monensin had no effect (Fig. 3), showing that the monensin effect requires the presence of sodium to be expressed.

The possible involvement of the Na^+/H^+ exchanger in T_3 uptake was investigated by using amiloride in conjunction with different cationic buffers. Lithium ion, unlike choline, is able to substitute for sodium in the exchange with intracellular H^+ (17, 19). Addition of 1 mM amiloride to the normal incubation buffer containing 140 mM Na^+ had no effect on T_3 uptake either in the presence of monensin (Table II) or its absence (data not shown). This was not unexpected since in
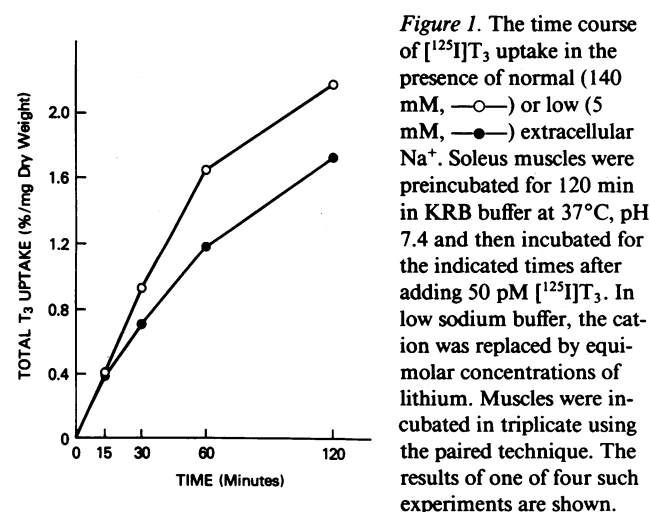

Figure 1. The time course of $[^{125}I]T_3$ uptake in the presence of normal (140 mM, —○—) or low (5 mM, —●—) extracellular Na^+. Soleus muscles were preincubated for 120 min in KRB buffer at 37°C, pH 7.4 and then incubated for the indicated times after adding 50 pM $[^{125}I]T_3$. In low sodium buffer, the cation was replaced by equimolar concentrations of lithium. Muscles were incubated in triplicate using the paired technique. The results of one of four such experiments are shown.
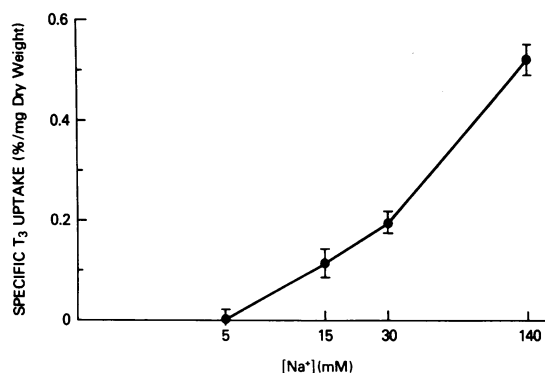

Figure 2. Specific T_3 uptake as a function of extracellular sodium. Soleus muscles were preincubated for 120 min at 37°C, pH 7.4, in KRB buffer containing different amounts of sodium and then incubated 60 min with 50 pM $[^{125}I]T_3$. Lithium replaced sodium to maintain osmolarity. The specific uptake was obtained by subtracting the nonspecific uptake (the T_3 uptake in presence of 10 µM unlabeled T_3) from the total uptake. The values are the mean±SD of triplicate determinations in three different experiments using the paired technique.
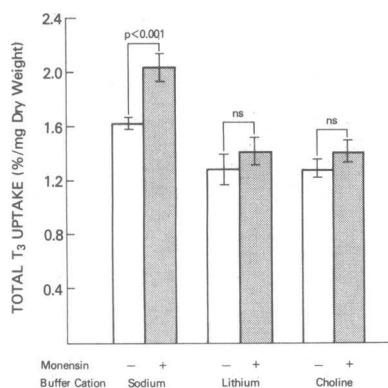
Figure 3. The effect of monensin on T_3 uptake in the presence of normal or low extracellular sodium. Soleus muscles were preincubated for 120 min at 37°C, pH 7.4, and then incubated 60 min with 50 pM [^{125}I]T_3 in KRB buffer containing normal or low extracellular sodium, with or without 10 μM monensin. In low Na^+ experiments the cation was replaced by equimolar lithium or choline. The values are the mean±SD of triplicate determinations in three different experiments.
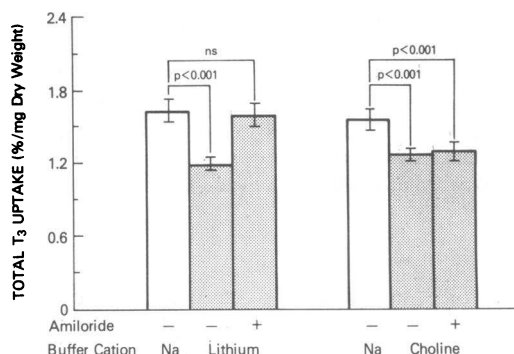

Figure 4. The effect of amiloride on T_3 uptake in the presence of low sodium buffer. Soleus muscles were preincubated for 120 min at 37°C, pH 7.4, and then incubated 60 min with [^{125}I]T_3 in KRB buffer in which lithium or choline was substituted for sodium. Amiloride, 1 mM, was present, where appropriate, during both preincubation and incubation. The values are the mean±SD of triplicate determinations in three different experiments using the paired technique.

many systems a sodium concentration as low as 50 mM inhibits the binding of this drug to the Na^+/H^+ exchanger (20, 21). When amiloride was added to the choline-containing buffer, it also did not alter the depressed T_3 uptake (Fig. 4). When sodium was replaced by lithium, however, amiloride restored T_3 uptake to normal. This suggests an involvement of the Na^+/H^+ exchange system in T_3 uptake since both lithium and amiloride, unlike choline, are known to interact with the Na^+/H^+ exchanger (17, 21) and, further, amiloride antagonizes, in skeletal muscle, the lithium effect on intracellular H^+ extrusion (17).

Since increased T_3 entry seemed to be associated with increased Na^+ entry, and since an increased intracellular Na^+ would be expected to activate the Na^+/K^+ ATPase, the possible role of this pump in T_3 uptake was investigated. The specific inhibitor ouabain, 1 mM, which was shown to block the Na^+/K^+ ATPase activity in myocytes (16, 22), was used and the results are shown in Table III. The mean total T_3 uptake was not reduced significantly, but the mean specific T_3 uptake was reduced 58% ($P < 0.01$), indicating an involvement of the sodium pump in the process of specific T_3 uptake in rat skeletal muscle.

Discussion

In the present study we investigated the relation between sodium movement across the plasma membrane and thyroid

Table II. Effect of Monensin on T_3 Uptake

Additions	n^*	T_3 uptake[‡]	Change	P
		%	%	
None	5	1.37±0.2	—	—
Monensin		1.73±0.2	+27	<0.001[§]
Monensin + amiloride	3	1.69±0.4	+23	NS[‖]

The concentration of monensin and amiloride were 10 μM and 1 mM, respectively.
* No. of experiments.
‡ Percent of total T_3 per milligram dry weight.
§ Vs. no addition.
‖ Vs. monensin alone.

hormone uptake in rat skeletal muscle, a tissue in which our previous studies demonstrated a specific component of T_3 uptake that was saturable and energy as well as temperature dependent (10). In contrast, T_4 uptake was shown to be insensitive to these factors and there was no evidence for a specific process involved in its entry into muscle (10). Holm et al. (5) and Krenning et al. (6) already pointed out that thyroid hormone uptake in erythrocytes and hepatocytes was sensitive to pretreatment with ouabain. Based on this finding they suggested the importance of a sodium gradient across the plasma membrane; Krenning suggested also a transport of thyroid hormones analogous to the transport of other amino acids.

The present study shows that T_3 uptake into muscle is partly dependent on extracellular sodium, whereas sodium concentration does not appear to influence T_4 uptake, confirming a major difference between T_3 and T_4 entry. The sodium-sensitive component of T_3 uptake corresponds to the specific portion of the uptake since the effect of Na^+ deprivation and of a saturating amount of T_3 were not additive (Table I). A direct effect of low extracellular sodium on T_3 uptake is supported by the close correlation between the decrease in specific uptake and the decreasing amount of sodium in the medium (Fig. 2).

The effect of sodium deprivation on T_3 uptake requires time to become evident; despite a 120-min preincubation, the effect of sodium replacement by lithium on T_3 uptake was seen only after 15–30 min of incubation. This lag time is similar to that required to see the effect of excess unlabeled T_3 (10) and is

Table III. Effect of Ouabain on T_3 Uptake

	Minus ouabain	Plus ouabain (1 mM)	Change	P
			%	
Total uptake*	1.88±0.33	1.63±0.29	−13	NS
Specific uptake*	0.41±0.14	0.17±0.08	−58	<0.01

* Percent of total radioactivity per milligram dry weight. Mean±SD of eight separate experiments in triplicate.

most likely explained by the time required for diffusion of T_3 into the intact muscle, as well as the low uptake and the variability between muscles.

Whereas the requirement of extracellular sodium for specific T_3 uptake seems evident, the mechanism involved is less clear. A role of sodium entry and accumulation in muscle cells in the uptake of T_3 is indicated by the effect of monensin. This ionophore is an electroneutral, fully reversible, and symmetric Na^+/H^+ exchanger (21) that is able to increase sodium entry into muscle (16). In the present study monensin increased T_3 uptake by 27% at a concentration similar to that used by Rosic et al. (16) to increase by 100% the sodium uptake into muscle cells. Moreover, when sodium in the medium was replaced by either lithium or choline, the effect of monensin on T_3 uptake disappeared, showing that this effect requires sodium to become evident and does not represent a nonspecific change in membrane permeability.

In mouse soleus muscle, under almost the same experimental conditions used in the present study, Aickin et al. (23) showed that the steady state internal pH (pH_i) is maintained mainly by the Na^+/H^+ exchange system. They also pointed out that a progressive reduction of external sodium causes a significant slowing of pH_i recovery after acidification, and complete removal of sodium almost stops pH_i recovery. These findings were confirmed by Vigne et al. (17) in chick skeletal muscle cells, and it was shown that an inward Na^+ gradient causes intracellular alkalinization and an outward Na^+ gradient, acidification. These observations raise the question whether the monensin effect on T_3 uptake, and T_3 uptake itself, are related to sodium entry through the Na^+/H^+ exchange system or to a change in pH_i subsequent to a change in Na^+ gradient.

A direct measurement of pH_i variations is a subject for another study using a muscle cell system more suitable than intact soleus muscle; however, we investigated the possible involvement of the Na^+/H^+ exchanger by using amiloride. This diuretic drug is the classical inhibitor of the Na^+/H^+ exchange system: its action is to block the Na^+-dependent H^+ efflux from cells in a large number of tissues, including skeletal muscle (16–21). Unfortunately, Na^+ and amiloride compete for the external transport site of the Na^+/H^+ pump and the Michaelis constant for Na^+ is quite low (< 50 mM). Therefore, in the presence of a physiological Na^+ concentration (140 mM), amiloride is not effective as an inhibitor (20). As we expected, amiloride had no effect on T_3 uptake in the presence of a normal sodium concentration, nor was it able to block the monensin effect, but it was useful as a negative control for nonspecific effects (for a review see reference 20).

In the presence of low extracellular sodium plus lithium, amiloride was able to restore T_3 uptake to normal. This effect confirms the possibility of an involvement of the Na^+/H^+ exchange system since both lithium and amiloride interact with this exchanger in soleus muscle (23) and amiloride is known to reverse lithium effects at that level (17, 21). That the amiloride effect on T_3 uptake may involve the Na^+/H^+ pump is also indirectly confirmed by the lack of an effect of the drug when sodium was replaced by choline, a cation unable to interact with the pump (17). From this last experiment it becomes evident also that the amiloride effect is not additive to Na^+ deprivation in decreasing T_3 uptake. It is of interest to note that amiloride also interferes with pH_i recovery after acidification in soleus muscle (23). However, the exact mechanism of the amiloride effect on T_3 uptake in the presence of lithium is

not completely clarified in terms of ion movement, due to the limitations of amiloride use as inhibitor and the complexity of the Na^+/H^+ system (19).

Whatever mechanism of sodium entry may be involved, an increasing intracellular Na^+ concentration activates the Na^+/K^+ ATPase that, therefore, could participate in T_3 uptake. Our results suggest that activation of the sodium pump is in some way related to T_3 entry into muscle since ouabain reduced the specific uptake by ~ 60%. However, this inhibitory effect was not complete, suggesting that activation of Na^+/K^+ ATPase may play a secondary role when compared with sodium entry.

In conclusion, this study showed that T_3 uptake in rat skeletal muscle is partly sodium dependent and that the sodium related T_3 uptake closely corresponds to the specific component of this uptake. T_4 uptake was shown not to be affected by sodium in accord with previous results indicating that passive diffusion is the major pathway of T_4 entry into muscle cells (10). The presence of a sodium-dependent process closely connected to specific T_3 uptake is of particular interest in skeletal muscle since in this tissue the T_3 associated with the nucleus is exclusively derived from the plasma (11), emphasizing the possible role of the cell membrane in regulating intracellular availability of the hormone.

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