Thyroid-Induced Alterations in Myocardial Sodium- and Potassium-Activated Adenosine Triphosphatase, Monovalent Cation Active Transport, and Cardiac Glycoside Binding

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ABSTRACT The effects of thyroid hormone on guinea pig myocardial NaK-ATPase activity, transmembrane monovalent cation active transport, and cardiac glycoside binding were examined. NaK-ATPase activities of left atrial and left ventricular homogenates of control and triiodothyronine (T_3)-treated animals were determined, and compared to activities of skeletal muscle and liver. T_3 administration was associated with a significant increase of 18% in left atrial and left ventricular NaK-ATPase specific activities. This increment was less than that noted in skeletal muscle (+42%) and liver (+30%). To determine if enhanced NaK-ATPase activity was accompanied by increased monovalent cation active transport, in vitro $^{86}Rb^+$ uptake by left atrial strips and hemidiaphragms was measured. Transition from the euthyroid to the hyperthyroid state resulted in a 68% increase in active $^{86}Rb^+$ uptake by left atrium, and a 62% increase in active uptake by diaphragm. Passive $^{86}Rb^+$ uptake was not affected in either tissue.

Ouabain binding by atrial and ventricular homogenates of T_3-treated animals was increased by 19 and 17%, respectively, compared to controls, in close agreement with thyroid-induced increments in NaK-ATPase activity. Taken together, these results are consistent with enhanced myocardial NaK-ATPase activity and monovalent cation active transport due to an increase in the number of functional enzyme complexes.

INTRODUCTION

Recent studies indicate that thyroid state has an important influence on membrane monovalent cation active transport systems of thyroid-responsive tissues (1). Administration of thyroid hormone to euthyroid and thyroidectomized rats results in significant increases in sodium- and potassium-activated adenosine triphosphatase (NaK-ATPase)[1] activity of liver, kidney, and skeletal muscle (1, 2). The enhanced enzymatic activities of liver and skeletal muscle are accompanied by diminished intracellular Na^+ and increased intracellular K^+, while serum electrolyte concentrations are unchanged, suggesting that transmembrane monovalent cation active transport is augmented in these tissues (3). Additional data suggest that the increased energy required for this increment in active cation transport may account for a substantial portion of the increase in tissue oxygen consumption associated with hyperthyroidism (1, 2).

Although the heart is generally recognized as one of the major target organs of thyroid hormone (4), the extent to which thyroid state may influence the myocardial monovalent cation active transport system has not been investigated systematically. Present understanding of the myocardial NaK-ATPase enzyme complex ascribes to it two important functions. Convincing evidence is now available that NaK-ATPase represents the enzymatic equivalent of the

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[1] *Abbreviations used in this paper*: NaK-ATPase, sodium- and potassium-activated adenosine triphosphatase; T_3, 3,3',5-triiodo-L-thyronine.

membrane transport mechanism responsible for the maintenance of normal sodium and potassium gradients (5). Additionally, it binds cardiac glycosides with high affinity and specificity, and may serve as the pharmacologic receptor for digitalis (6). We have therefore evaluated the effects of thyroid hormone on myocardial NaK-ATPase activity, monovalent cation transport, and cardiac glycoside binding. Because of possible differences in thyroid responsiveness of atrial and ventricular myocardium, these tissues were analyzed independently and compared to alterations in liver and skeletal muscle.

METHODS

Experimental model. Male Hartley albino guinea pigs (400–500 g) maintained on Charles River Guinea Pig Formula (Country Foods, Syracuse, N. Y.) and water ad lib. were employed for all experiments. Hyperthyroidism was induced in one group of animals by administering three 1.3 mg/kg i.p. doses of 3,3',5-triiodo-L-thyronine (T_3) (Sigma Chemical Co., St. Louis, Mo.) over a 5-day period. The hyperthyroid state produced was characterized by a 17% increase in resting oxygen consumption of isolated left atrial strips, measured with a Clark oxygen electrode. This increment in oxygen consumption is somewhat less than the 29% increase noted in rat diaphragm by Asano et al., who employed a comparable T_3 dosage schedule (2). During the T_3 treatment period heart rate increased by 34% compared to pretreatment control values, and body weight declined by 15%. Littermate controls received diluent injections on the same schedule. Heart rate in control animals did not change during the injection period, and body weight increased by 10%.

Preparation of tissue homogenates. 24 h after the final injection animals were sacrificed by cervical dislocation, and the heart, liver, and diaphragm rapidly removed and placed on ice. The entire left atrium was dissected from the left ventricle, and 100-mg samples of the anterolateral left ventricular wall, liver, and diaphragm were freed of connective tissue and fat. The tissues were transferred to a 2.0-ml ground-glass hand-operated homogenizer, and were homogenized with 20 strokes in 1.5–2.0 ml of buffer containing sucrose (0.25 M), EGTA (1.25 mM), and Tris (10 mM), pH 7.0. All of these procedures were carried out at 0–4°C.

Protein concentrations of the homogenates were determined by the method of Lowry et al. (7). All assays were performed in triplicate, with bovine serum albumin as standard.

NaK-ATPase assay. Assays were performed in a total vol of 1.0 ml containing 100 mM NaCl, 10 mM KCl, 50 mM Tris, 1 mM Na_2EDTA, 5 mM $MgCl_2$, 2.5 mM Na_2ATP, pH 7.4, and 125–500 μg enzyme protein. The quantity of homogenate used was adjusted so that less than 15% of the total ATP present was hydrolyzed during the incubation period. Sodium azide (5 mM) was also present to inhibit mitochondrial regeneration of ATP (1), and to reduce background activity by inhibiting other nonouabain inhibitable ATPases such as mitochondrial ATPase (8). Each homogenate was assayed in triplicate with matched tubes containing 1 mM ouabain. After a 3-min preincubation period, reactions were initiated by the addition of ATP and allowed to proceed for 10 min at 37°C. They were terminated by addition of 1.0 ml ice-cold 10% trichloroacetic acid and

centrifugation at 2,600 g for 20 min. Aliquots of the supernatant phase were assayed for inorganic phosphate by the method of Fiske and Subbarow (9). NaK-ATPase activity was calculated as the difference between micromoles Pi released per milligram protein per hour for reactions proceeding in the presence and absence of ouabain.

Rubidium transport studies. Monovalent cation active transport in left atrial myocardium and diaphragm was assessed by measuring Rb^+ uptake in vitro as previously described (10). Paired hemiatria and hemidiaphragms (without ribs) from control and T_3-treated animals were incubated for 15 min at 30°C in Krebs-Ringer bicarbonate buffer (preoxygenated with 95% O_2—5% CO_2), with or without 0.1 mM ouabain. RbCl (0.1 mM) containing 10^6 dpm/ml $^{86}Rb^+$ was present in all vials. Tissue radioactivity was determined by counting of Cerenkov radiation for 1 min in a liquid scintillation counter at 25°C. The tissues were immediately returned to the incubation vials for an additional 30 min, recounted, blotted, and weighed.

Active Rb^+ uptake was calculated at 15 and 45 min of incubation by subtracting uptake in the presence of 0.1 mM ouabain (passive uptake) from uptake in the absence of ouabain. Uptake was expressed as nanomoles Rb^+ per milligram wet weight. Pilot experiments demonstrated that active Rb^+ uptake was linear throughout an incubation period of at least 60 min.

[^3H]Ouabain binding studies. Myocardial ouabain binding was assayed by the method of Schwartz et al. (11) with slight modification. Triplicate assays were performed on left atrial and left ventricular homogenates with 0.6–0.8 mg enzyme protein. [^3H]Ouabain (0.2 μM, 11.9 Ci/mmol) was present with or without an excess of unlabeled ouabain (0.2 mM) to correct for nonspecific binding. Preliminary experiments confirmed that this method of correcting for nonspecific binding gave results indistinguishable from those obtained by omitting ATP from the reaction mixture. In some experiments the [^3H]ouabain concentration was increased to 1 μM. Reactions proceeded for 15 min at 37°C, and were terminated by cooling the tubes to 0°C followed by centrifugation at 100,000 g for 30 min. Preliminary studies demonstrated that the binding reaction had reached equilibrium within 10 min. After careful removal of the supernate, precipitates were solubilized in 0.5 ml 2% sodium dodecyl sulfate and ^3H activity measured by liquid scintillation counting (Packard Instrument Co., Inc., Downer's Grove, Ill.) with toluene-detergent based liquid scintillation fluid (Instagel, Packard Instrument Co., Inc.). Specific [^3H]ouabain binding was calculated as the difference between total binding and nonspecific binding and was expressed as picomoles ouabain bound per milligram homogenate protein. Nonspecific binding was 32% of total counts bound in the case of ventricular homogenates and 55% for atrial homogenates.

RESULTS

Effect of T_3 on NaK-ATPase activity. Determinations of NaK-ATPase activity of left atrial and left ventricular homogenates are summarized in Table I. T_3 administration was associated with an 18% mean increase in NaK-ATPase specific activity of both atrial and ventricular myocardium. The differences between control and hyperthyroid groups were statistically significant for both tissues ($P < 0.005$). There was no significant difference in protein content of atrial or ventricular myocardium (milligram/milli-

gram wet weight) between control and T_3-treated groups (Table I).

To compare the effect of T_3 on liver and skeletal muscle NaK-ATPase in the guinea pig to previous results in the rat (2, 3), NaK-ATPase activities of liver and diaphragm were determined. These data, shown in Table I, indicate a 30% increase in hepatic enzyme activity of hyperthyroid animals compared to controls ($P < 0.001$), and a 42% increase in skeletal muscle activity ($P < 0.025$).

Effect of T_3 on myocardial monovalent cation transport. Active and passive Rb^+ uptake by left atria and diaphragms of control and hyperthyroid animals are shown in Table I and Figs. 1A and B. Rb^+ active transport was increased 68% above control in left atria, and 62% above control in diaphragms of T_3-treated animals ($P < 0.005$ for both tissues). However, there was no significant difference in passive Rb^+ uptake between control and T_3-treated groups.

Effect of T_3 on myocardial ouabain binding in vitro. To obtain information bearing on the mechanism of thyroid-induced enhancement of NaK-ATPase activity and monovalent cation active transport, [^{3}H]ouabain binding studies were performed on myocardial homog-

enates of euthyroid and thyrotoxic animals. The results of these studies are summarized in Table I. With T_3 administration, a 19% increase in ouabain binding by atrial homogenates and a 17% increase in binding by ventricular homogenates were noted. Both differences were statistically significant ($P < 0.025$ for atrial and $P < 0.005$ for ventricular binding).

Increased ouabain binding could be due to an increase in the number of specific binding sites or, if subsaturating ouabain concentrations were used, to an increase in binding affinity. To distinguish between these possibilities a series of binding studies were performed at a fivefold higher [^{3}H]ouabain concentration (1.0 μM). Specific ouabain binding for the control group was 2.42 ± 0.30 pmol/mg protein and for the T_3-treated group 3.56 ± 0.25 pmol/mg protein (mean $\pm$SEM, $n = 4$ for each group). Neither value is statistically different from binding values obtained with 0.2 μM [^{3}H]ouabain ($P > 0.05$). These studies confirm that binding sites were saturated in the presence of 0.2 μM ouabain under the conditions used, and that the number of binding sites has increased in response to thyroid hormone administration.

Turnover numbers calculated from NaK-ATPase ac-

TABLE I
Effect of T_3 on Protein Concentration, NaK-ATPase Activity, Ouabain Binding, and Rb^+ Uptake of Various Tissues in the Guinea Pig

	Atrium			Ventricle			Skeletal muscle			Liver		
	$-T_3$	$+T_3$	$\Delta\%$*	$-T_3$	$+T_3$	$\Delta\%$	$-T_3$	$+T_3$	$\Delta\%$	$-T_3$	$+T_3$	$\Delta\%$
Protein concentration, *mg/mg wet wt*	0.143 $\pm$0.004 ($n = 9$)	0.164 $\pm$0.003 ($n = 9$)	+14	0.150 $\pm$0.004 ($n = 7$)	0.163 $\pm$0.004 ($n = 6$)	+9						
NaK-ATPase, *μmol Pi/mg/h*	1.08 $\pm$0.04 ($n = 15$)	1.27 $\pm$0.05 ($n = 14$)	+18‡	1.53 $\pm$0.06 ($n = 20$)	1.80 $\pm$0.05 ($n = 17$)	+18‡	0.65 $\pm$0.07 ($n = 5$)	0.92 $\pm$0.06 ($n = 4$)	+42§	0.89 $\pm$0.04 ($n = 10$)	1.16 $\pm$0.05 ($n = 8$)	+30‖
Ouabain binding, *nmol/mg*	1.57 $\pm$0.08 ($n = 10$)	1.87 $\pm$0.08 ($n = 10$)	+19§	2.64 $\pm$0.08 ($n = 9$)	3.08 $\pm$0.10 ($n = 8$)	+17‡						
Active Rb^+ uptake, *nmol/mg/45 min*	0.264 $\pm$0.022 ($n = 8$)	0.445 $\pm$0.037 ($n = 10$)	+68‡				0.222 $\pm$0.019 ($n = 5$)	0.360 $\pm$0.041 ($n = 6$)	+62‡			
Passive Rb^+ uptake, *nmol/mg/45 min*	0.175 $\pm$0.019 ($n = 8$)	0.156 $\pm$0.008 ($n = 10$)	−11				0.107 $\pm$0.007 ($n = 5$)	0.112 $\pm$0.012 ($n = 6$)	+5			

Guinea pigs were treated with three 1.3 mg/kg i.p. doses of T_3 over a period of 5 days ($+T_3$). Controls ($-T_3$) received diluent injections on the same schedule. Assays were performed as outlined in the Methods section, and the results are given as the mean $\pm$1SE.
* $\Delta\%$ represents percent change in T_3-treated groups compared to controls.
Significant differences by Student's t test are indicated by symbols.
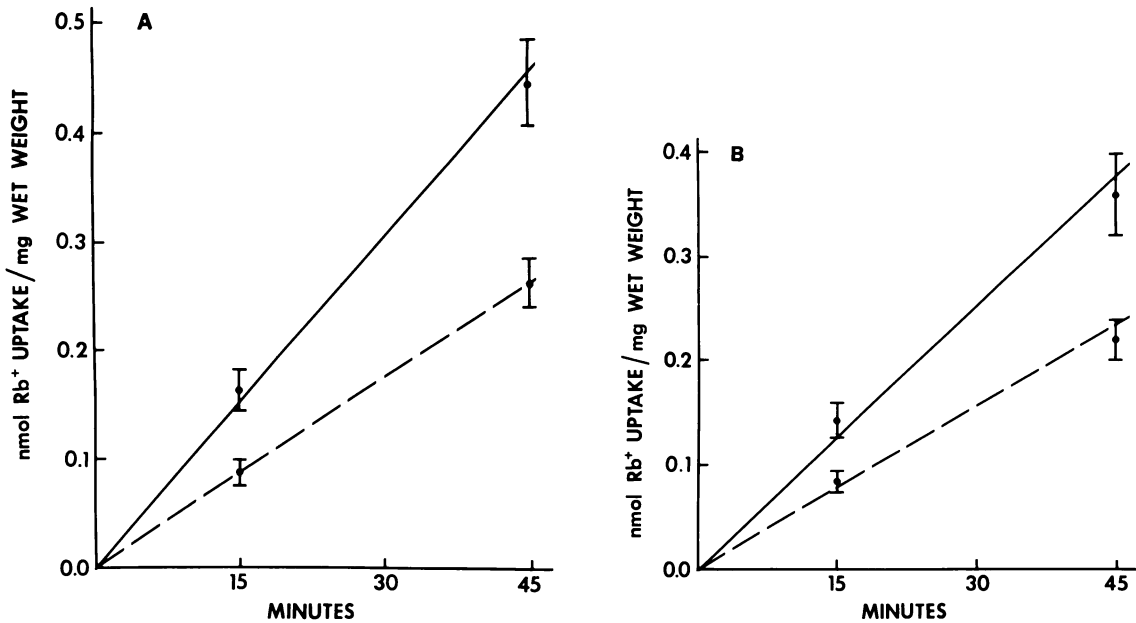‡ $P < 0.005$.
§ $P < 0.025$.
‖ $P < 0.001$.

FIGURE 1 Active Rb^+ uptake as a function of incubation time by left atrial strips (A) and hemidiaphragms (B) from euthyroid (broken lines) and T_3-treated (solid lines) guinea pigs. Each point represents the mean of 6–10 tissues. Brackets show 1 SE above and below the mean. See Methods section for details.

tivities and ouabain binding data, assuming one enzymatic site per ouabain molecule bound, were: control atria 11,465 min^{-1}, ventricles 9,659 min^{-1}; T_3-treated atria 11,320 min^{-1}, ventricles 9,740 min^{-1}. These data indicate that enzyme specific activity and ouabain binding increased in parallel in response to T_3, and that turnover number is not appreciably changed by hormone treatment.

DISCUSSION

Since thyroid state is known to influence cardiac response to digitalis (4), the present investigation was undertaken to examine the effects of thyroid hormone on myocardial NaK-ATPase activity, transmembrane monovalent cation active transport, and cardiac glycoside binding. Previous experiments of Ismail-Beigi and Edelman and Asano et al. (1–3) have shown that T_3 administration to euthyroid and hypothyroid rats is associated with enhanced NaK-ATPase activity and altered intracellular Na^+ and K^+ in skeletal muscle and liver. The results of the present studies demonstrate that experimental hyperthyroidism in the guinea pig is associated with a significant increase in myocardial NaK-ATPase activity. The percentage increases in enzymatic activity of left atrial and left ventricular homogenates were identical, but were less marked than those noted in liver and skeletal muscle. The 30% mean increase in hepatic

enzyme activity and 42% mean increase in skeletal muscle activity of T_3-treated animals are directionally similar to, but differ quantitatively from, the 81 and 25% increases noted in the comparable rat model (1, 2), presumably due to species variability in the response of the NaK-ATPase system to thyroid hormone in the doses used.

To determine if enhanced NaK-ATPase activity was accompanied by increased monovalent cation active transport, Rb^+ uptake by left atria and hemidiaphragms was measured. These tissues are sufficiently thin to avoid a substantial barrier to diffusion during incubation in vitro. The results of these studies provide direct evidence that unidirectional monovalent cation (Rb^+) active transport is significantly enhanced by exogenously administered thryoid hormone, while passive Rb^+ uptake is unchanged. These data support and extend a previous report of Ismail-Beigi and Edelman (3) that T_3 administration in the rat results in a significant increase in intracellular K^+ in heart and diaphragm, and increased Na^+ efflux in liver slices.

Enhancement of NaK-ATPase activity and monovalent cation active transport theoretically could occur by at least two different mechanisms. Increased activity might result from enhanced function of some or all of the pre-existing enzyme complexes. Alternatively, an increased number of normally functioning enzyme units within the cell membrane could produce a similar effect. Kinetic analysis of skeletal muscle

Effect of Thyroid on Myocardial Monovalent Cation Transport   589

NaK-ATPase of euthyroid and hyperthyroid rats has provided support for the latter hypothesis (2). In the studies reported here this question was approached by measuring ouabain binding of atrial and ventricular homogenates in the presence of optimal substrate and cation concentrations. NaK-ATPase binds cardiac glycosides with high affinity and specificity, and previous investigations indicate that ouabain binding is a sensitive method of quantitating numbers of enzyme complexes (6). The findings of a 17% mean increase in ouabain binding by ventricular homogenates and a 19% mean increase in binding by atrial homogenates suggest that experimental hyperthyroidism is associated with augmentation of the number of functional NaK-ATPase complexes in myocardial tissue. These percentage increases in ouabain binding by thyrotoxic atrial and ventricular myocardium agree closely with the 18% increase in NaK-ATPase activity noted in these tissues.

We conclude that T_3 administration in this animal model is accompanied by enhanced myocardial NaK-ATPase activity and monovalent cation active transport. The parallel increases in NaK-ATPase activity and ouabain binding suggest that these effects are mediated by an increase in the number of functional enzyme complexes.

ACKNOWLEDGMENTS

REFERENCES

1. Ismail-Beigi, F., and I. S. Edelman. 1971. The mechanism of the calorigenic action of thyroid hormone. Stimulation of $Na^+ + K^+$-activated adenosinetriphosphatase activity. *J. Gen. Physiol.* **57:** 710–722.
2. Asano, Y., U. A. Liberman, and I. S. Edelman. 1976. Thyroid thermogenesis. Relationships between Na^+-dependent respiration and $Na^+ + K^+$-adenosine triphosphatase activity in rat skeletal muscle. *J. Clin. Invest.* **57:** 368–379.
3. Ismail-Beigi, F., and I. S. Edelman. 1973. Effects of thyroid status on electrolyte distribution in rat tissues. *Am. J. Physiol.* **225:** 1172–1177.
4. Sobel, B. E., and E. Braunwald. 1971. Cardiovascular system. *In* The Thyroid. S. C. Werner, and S. H. Ingbar, editors. Harper & Row Publishers, New York. 551–560.
5. Skou, J. C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* **45:** 596–617.
6. Schwartz, A., G. E. Lindenmayer, and J. C. Allen. 1975. The sodium-potassium adenosine triphosphatase: pharmacological, physiological, and biochemical aspects. *Pharmacol. Rev.* **27:** 3–134.
7. Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
8. Pullman, M. E., H. S. Penefsky, A. Datta, and E. Racker. 1960. Partial resolution of the enzymes catalyzing oxidative phosphorylation. I. Purification and properties of soluble, dinitrophenol-stimulated adenosine triphosphatase. *J. Biol. Chem.* **235:** 3322–3329.
9. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 375–400.
10. Smith, T. W., and H. Wagner, Jr. 1975. Effects of $(Na^+ + K^+)$-ATPase-specific antibodies on enzymatic activity and monovalent cation transport. *J. Membr. Biol.* **25:** 341–360.
11. Schwartz, A., K. Nagano, M. Nakao, G. E. Lindenmayer, and J. C. Allen. 1971. The Na^+ and K^+ activated ATPase system. *In* Methods in Pharmacology. A. Schwartz, editor. Appleton-Century-Crofts, New York. 8th edition. **1:** 361–388.