Renal Sodium- and Potassium-Activated Adenosine Triphosphatase and Sodium Reabsorption in the Hypothyroid Rat

ADRIAN I. KATZ and MARSHALL D. LINDHEIMER

From the Departments of Medicine and Obstetrics and Gynecology, University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637

ABSTRACT The relationship between net tubular reabsorption of sodium and renal microsomal sodium- and potassium-activated adenosine triphosphatase (Na-K-ATPase) was evaluated in hypothyroid and hyperthyroid rats and in age-matched euthyroid controls. Tubular sodium reabsorption per gram of kidney was lower in thyroidectomized rats than in controls (186±14 vs. 246±12 μeq/min; $P < 0.005$) and was accompanied by a quantitatively similar reduction in Na-K-ATPase specific activity (49.4±2.4 vs. 65.8±2.3 μmol inorganic phosphate (P_i)/mg protein per h; $P < 0.001$). This decrement was present in both cortex and outer medulla, and was limited to Na-K-ATPase since other representative enzymes not involved in sodium transport (magnesium-dependent adenosine triphosphatase [Mg-ATPase], glucose-6-phosphatase, 5′-nucleotidase) remained unchanged or increased in the hypothyroid animals. Conversely, Na-K-ATPase rose when sodium reabsorption increased in euthyroid rats treated with triiodothyronine.

Subsequent experiments were performed to determine to what extent the decrease in Na-K-ATPase is due to lack of thyroid hormone per se or to an adaptive response to decreased reabsorptive sodium load. Triiodothyronine in concentrations of 10^{-12} to 10^{-5} M had no effect in vitro on microsomal Na-K-ATPase of either thyroidectomized or euthyroid rats. When hypothyroid rats were uninephrectomized or treated with methylprednisolone, sodium reabsorption per gram kidney increased markedly and was similar to that of intact controls. Despite persistence of the hypothyroid state, Na-K-ATPase specific activity also increased to levels not significantly different from euthyroid animals.

These data suggest that decreased tubular sodium transport is a major determinant of the reduction in renal Na-K-ATPase in thyroid deficiency since the latter can be reversed by increasing sodium reabsorption during continuing hypothyroidism. Furthermore, the modest sodium leak of hypothyroid animals does not appear to be due to decreased Na-K-ATPase since it was not corrected by uninephrectomy despite restoration of both cortical and medullary Na-K-ATPase activity to normal by this maneuver. The close correlation between net sodium reabsorption and Na-K-ATPase in all the experimental situations described here demonstrates that renal Na-K-ATPase changes adaptively in hyper- or hypothyroidism as it does in numerous situations in the normal animal, in accord with its postulated role in the active transport of sodium across the renal tubule.

INTRODUCTION

Thyroid-deficient animals and man exhibit numerous renal functional defects, including decreased renal plasma flow and glomerular filtration rate and impaired tubular reabsorption of sodium (1–4). The mechanisms underlying these defects are incompletely understood.

Recently, Ismail-Beigi and Edelman (5) demonstrated a decrease in sodium- and potassium-activated adenosine triphosphatase (Na-K-ATPase) [1] activity of crude kid-

This work was presented in part at the 64th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., 30 April 1972, and at the 5th International Congress of Nephrology, Mexico City, 9 October 1972.

Received for publication 9 August 1972 and in revised form 13 November 1972.

[1] *Abbreviations used in this paper:* ERPF, effective renal plasma flow; G-6-Pase, glucose-6-phosphatase; Mg-ATPase, magnesium-dependent adenosine triphosphatase; Na-K-ATPase, sodium- and potassium-activated adenosine triphosphatase; PAH, *p*-aminohippurate; Pi, inorganic phosphate; T_3, triiodothyronine.

ney homogenates from hypothyroid rats, accompanied by reduced oxygen consumption in tissue slices. Furthermore, transmembrane sodium and potassium concentration differences in liver and skeletal muscle from euthyroid animals were increased by administration of triiodothyronine (6). The authors postulated that thyroid hormones stimulate Na-K-ATPase differentially, and that this stimulation of energy utilization by the sodium pump may account for their calorigenic effects (5).

The decreased Na-K-ATPase in kidneys of hypothyroid animals cannot be explained solely as a direct effect of thyroid hormone deficiency until other factors known to influence the activity of this enzyme in renal tissues are explored. Na-K-ATPase is involved in the active transport of sodium across epithelial membranes (7, 8) and renal Na-K-ATPase specific activity parallels net sodium reabsorption by the kidney in a variety of physiological and experimental situations (9–11). Thyroid deficiency is known to reduce filtered and reabsorbed sodium (1, 2) and could therefore affect enzymatic activity in this manner.

The present studies were designed to evaluate the effect of hypothyroidism on renal Na-K-ATPase in relation to the alterations in sodium handling found in this condition. Specifically, we sought to determine to what extent the reported decrease in enzymatic activity in hypothyroid animals is due to thyroid hormone deficiency per se, or to an adaptive response to decreased reabsorptive sodium load. The relationship between net tubular reabsorption of sodium and renal Na-K-ATPase was evaluated in rats made hypothyroid by either surgical thyroidectomy or radioactive iodine, and in animals in which hyperthyroidism was produced by repeat triiodothyronine (T_3) administration.

METHODS

Female Sprague-Dawley rats were used in all experiments. Hypothyroidism was produced by surgical thyroidectomy (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), or by the intraperitoneal injection of 1 mCi of [^{131}I]sodium radioiodide (New England Nuclear, Boston, Mass.). Rats subjected to chemical thyroidectomy were given a low iodine diet (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) for 10–14 days before injection. With this exception, all animals were fed a standard rat-chow diet and had free access to tap water. Hypothyroid rats were studied at least 3, and usually 4–8 wk after surgical or chemical thyroidectomy. Plasma total thyroxine, free thyroxine index, calcium, and phosphorus were measured in several groups of randomly selected animals.[2]

Hyperthyroidism was produced by administration of T_3 (Sigma Chemical Co., St. Louis, Mo.). Three doses of 50 μg/100 g body wt T_3 dissolved in dilute NaOH were injected intraperitoneally at 48-h intervals. Hyperthyroid animals and controls injected with equal amounts of diluent were studied on the day after the last injection.

In each experiment, age-matched controls were identically handled and studied simultaneously. Renal function and enzyme activities were measured in three series of experiments performed on (*a*) Surgically and chemically hypothyroid rats and euthyroid controls; (*b*) Hyperthyroid and control animals; (*c*) Hypothyroid rats subjected to maneuvers designed to increase glomerular filtration and reabsorptive sodium load were compared with hypothyroid and euthyroid controls.

In the latter series of experiments, filtered sodium per kidney was increased by contralateral nephrectomy or by the administration of methylprednisolone. The left kidney of hypothyroid animals was removed under ether anesthesia while control hypothyroid and intact animals underwent a sham operation. 3 wk later, renal function and enzymatic activities in the right kidney were measured in uninephrectomized and sham-operated hypothyroid animals and in sham-operated controls. In additional studies, Na-K-ATPase specific activity was determined separately in the cortex and the outer medulla in the same three experimental groups.

In other experiments in this series, hypothyroid rats received daily intramuscular injections of 2.5 mg methylprednisolone in its long-acting form for 4 days. Renal function studies and enzyme assays were performed in this group and in untreated hypothyroid and intact animals on the day after the last methylprednisolone injection.

Enzyme studies

Animals were anesthetized with Pentothal Sodium and killed by rapid exsanguination from the aorta. The kidneys were immediately chilled in an ice-cold buffered sucrose solution used subsequently for tissue homogenization. The renal capsule and papilla were dissected free, the remaining tissue was blotted, weighed, and homogenized in a 10/1 (vol/wt) solution containing 0.25 mol sucrose, 5 mmol Na_2EDTA, 30 mmol histidine buffer per liter and 0.2% sodium deoxycholate at pH 6.8. A portion of the homogenate obtained was frozen for future use, and the remainder centrifuged at 10,800 *g* for 30 min in a refrigerated Spinco model L preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to sediment cell debris, nuclei, and mitochondria. The supernate was carefully removed to avoid contamination and centrifuged at 108,000 *g* for 90 min. The resulting sediment ("microsomal fraction") was gently resuspended in 1.5–2 ml of the original homogenizing solution without deoxycholate, and frozen overnight at −20°C. In certain experiments, kidneys were cut longitudinally, placed in Petri dishes on filter paper moistened with cold saline, and the cortex and outer medulla dissected with iris scissors. The entire outer medulla from each animal and, separately, an equal weight of cortex (approximately 150 mg) were homogenized as described above. In the latter studies, homogenates were spun in 2-ml cellulose tubes using special adapters (Beckman Instruments, Inc., Fullerton, Calif.), and the microsomal pellet (108,000 *g*) was resuspended in 0.5 ml homogenizing solution. Enzyme activities of cortex and medulla from control and hypothyroid rats were assayed simultaneously.

The specific activities of Na-K-ATPase, magnesium dependent adenosine triphosphatase (Mg-ATPase), and glucose-6-phosphatase (G-6-Pase) were determined the following morning, and are expressed as micromoles inorganic phosphate (P_i) liberated/milligram of protein per hour.

[2] We are indebted to Dr. Samuel Refetoff and Dr. Edward Paloyan for performing these studies.

The microsomal fraction was chosen because it contains the highest specific activity of Na-K-ATPase and G-6-Pase of all fractions tested (9). 5′-Nucleotidase, a marker enzyme for plasma membranes, was assayed in the whole tissue homogenate, also within 24 h of preparation.

In vitro studies. ATPase specific activity of microsomes from hypothyroid and control rats was assayed as described below in incubation flasks to which 0.5 ml of serially-diluted T_3 solutions were added to produce final T_3 concentrations ranging between 10^{-5} and 10^{-12} M.

ATPase activity. 0.1 ml of tissue suspension (final protein concentration 80–200 μg/ml) was used in all assays. ATPase activity was determined in 5 ml of a reaction mixture prewarmed at 37°C containing 100 mmol NaCl, 20 mmol KCl, 10 mmol imidazole buffer, 5 mmol $MgCl_2$, and 5 mmol disodium adenosine triphosphate (ATP)/liter at pH 7.8, and in an identical solution containing in addition, 2 mmol ouabain/liter. The reaction was carried out for 5 min at 37°C in a shaking water bath, and terminated by the addition of 1 ml ice-cold 35% (wt/vol) trichloroacetic acid (TCA). The precipitated protein was discarded after centrifugation and the inorganic phosphate in the supernate determined by the method of Fiske and SubbaRow (12). The Na-K-ATPase was defined as the difference between the inorganic phosphate liberated in the absence and presence of 2 mM ouabain in the incubation mixture, and the residual activity was designated Mg-dependent ATPase. Correction was made for the spontaneous, nonenzymatic breakdown of ATP, measured as the inorganic phosphate liberated under the same experimental conditions in the absence of enzyme, which usually accounted for less than 10% of the total inorganic phosphate present.

Glucose-6-phosphatase. 0.1 ml microsomal suspension was incubated for 10 min with 0.1 ml of 80 mM glucose-6-phosphate solution at 37°C and pH 6.5 in a shaking water bath, and the reaction was stopped with 2 ml of 10% TCA (13). The inorganic phosphate liberated was determined in the supernate of this mixture by the method of Fiske and SubbaRow.

5′-Nucleotidase specific activity in the whole homogenate was determined by measuring the inorganic phosphate liberated from adenosine-5′-monophosphoric acid in the presence of 0.1 ml tissue suspension after 45 min of incubation at 37°C (14).

The protein content of tissue suspensions was determined by the method of Lowry, Rosebrough, Farr, and Randall (15), using crystalline bovine albumin as standard. ATP, glucose-6-phosphate, and adenosine-5′-monophosphoric acid were purchased from the Sigma Chemical Co.

Renal function

Euthyroid and hyperthyroid animals were anesthetized with Inactin (Promonta, Hamburg, Germany), 120 mg/kg body wt intraperitoneally. In view of their sensitivity to anesthesia, hypothyroid rats received smaller amounts of the anesthetic (approximately 80–100 mg/kg) initially, with occasional small supplements during the experiment. A tracheostomy was performed and the bladder and a jugular vein were cannulated with polyethylene PE 50 tubing. Animals were placed on a heated board and their rectal temperature, monitored by a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, Ohio), was maintained between 37° and 38°C. During surgery, isotonic saline equal to 0.5–1% of the body wt was infused through the jugular vein catheter to replace estimated fluid losses.

After the completion of surgery, priming doses of 20 mg inulin and 3 mg *p*-aminohippurate (PAH) in isotonic saline were given rapidly, followed by a sustaining solution calculated to maintain plasma inulin and PAH levels at approximately 50 and 3 mg/100 ml, respectively. Fluid was delivered with constant infusion pumps (model 975, Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 20 μl/min. After an equilibration period of 60 min, urine was collected under mineral oil and volumes measured with glass micropipettes. In each experiment three to four consecutive collection periods of 30 min each were obtained. Free-flowing tail blood was collected in heparinized capillary tubes before and after each collection period, and plasma concentrations calculated as the average of the two determinations. At the end of 3 h of infusion, the renal capsule and hilar fat were removed, the kidneys were cut in half along their longitudinal axis, and weighed after light blotting on filter paper (wet weight). Dry kidney weight was measured after desiccation to constant weight at 105°C.

Glomerular filtration rate (GFR) was calculated from the clearance of inulin and effective renal plasma flow (ERPF) from the clearance of PAH. Total thyroxine was determined by the competitive binding assay described by Murphy (16). The free thyroxine index (17), an indirect assessment of the free thyroxine content of plasma, was calculated from the total thyroxine and the resin thyroxine uptake test which measures the availability of unsaturated thyroxine-binding sites. Plasma calcium was determined by the method of Kingsley and Robnett (18) and plasma phosphorus by the method of Fiske and SubbaRow (12). Inulin and PAH were measured by semimicromodifications of the anthrone (19) and diazotization (20) methods. Sodium was measured by flame photometry using lithium as internal standard. Inulin and PAH clearances, filtered load of sodium, fractional and absolute sodium excretion, and net tubular sodium reabsorption were calculated from standard equations.

Results were presented as mean ±1 SEM. The statistical significance of the differences between group means was assessed by Student's "*t*" test, *P* values less than 0.05 were considered significant.

RESULTS

Thyroid function was markedly decreased in both surgically thyroidectomized and ^{131}I-treated rats, whose mean free thyronine index was less than 20% of that measured in intact animals (Table I). Although both hypothyroid and hyperthyroid rats were lighter than controls, the kidneys were smaller in the former but larger in the latter group when compared with euthyroid animals (Table II.)

Enzymes studies. Results of enzyme studies performed on animals in experimental series 1 and 2 are summarized in Table II. Na-K-ATPase specific activity was significantly lower in thyroidectomized animals than in controls (49.4 vs. 65.8 μmol P_i/mg protein per h; Table IIA), while other representative enzymes remained unchanged or increased slightly. These included 5′-nucleotidase, a marker enzyme for plasma membranes, G-6-Pase, a microsomal enzyme and also a phosphatase, and Mg-ATPase, another microsomal ATPase.

TABLE I

Thyroid Function and Serum Calcium and Phosphorus in Thyroidectomized and ^{131}I-Treated Rats

Group	n	Total thyroxine	Free thyroxine index	Ca	P
		μg/100 ml		*mg/100 ml*	*mg/100 ml*
Controls		4.6±0.3	7.3±0.6	10.3±0.2	7.3±0.7
Thyroidectomized	6	1.3±0.1	1.4±0.1	7.6±0.1	10.1±0.4
P		<0.001	<0.001	<0.001	<0.02
Controls		3.4±0.2	5.1±0.4	10.2±0.4	5.2±0.3
^{131}I treated	6	0.6±0.1	0.6±0.1	9.4±0.1	5.6±0.2
P		<0.001	<0.001	NS*	NS*

* = not significant.

During surgical thyroidectomy in the rat the parathyroid glands are usually removed as well. To rule out possible effects of hypocalcemia or hyperphosphatemia (Table I) on the enzyme changes described, the same studies were repeated in rats made hypothyroid with ^{131}I. Thyroid function was similarly depressed in these animals, but serum calcium and phosphorus remained unchanged. As in the surgically thyroidectomized rats, only Na-K-ATPase specific activity was significantly lower than control values (41.5 vs. 65.4 μmol P_i/mg protein per h; Table IIB). Mg-ATPase and 5′-nucleotidase did not change, while G-6-Pase increased compared with controls. The reason for this increment is not clear, although it may reflect an increase in renal gluconeogenesis in the hypothyroid animals.

In additional experiments, Na-K-ATPase was determined separately in the outer medulla and in the cortex of hypothyroid rats and compared with the activity measured simultaneously in control animals. Na-K-ATPase specific activity was lower in both regions of the hypothyroid rat kidney, the relative decrease being similar in the outer medulla and in the cortex (Fig. 1).

In the counterpart of the above studies, rats were made hyperthyroid by repeat T_3 administration. In these animals Na-K-ATPase specific activity was significantly increased, while all other enzymes measured were not different from control values (Table IIC).

Table III summarizes the enzyme studies in experimental series 3, designed to evaluate the effect of chronic increments in filtered sodium load on renal enzymes in hypothyroid rats. Despite persistence of the hypothyroid state, Na-K-ATPase was restored to control levels when the net reabsorption of sodium was increased (see below) by contralateral nephrectomy or by the administration of methylprednisolone. Increasing the filtered and reabsorbed sodium had no predictable effect on other renal enzymes, which remained unchanged or decreased in response to uninephrectomy and

TABLE II

Renal Enzymes in Hypothyroid and Hyperthyroid Rats

	Group	n	Body wt	Kidney wt	Na-K-ATPase	Mg-ATPase	G-6-Pase	5′-Nucleotidase
						Specific activity		
						-μmol P_i/mg protein per h		
A)	Control	6	229±4	1.38±0.03	65.8±2.3	60.3±1.8	8.0±0.9	0.60±0.03
	Thyroidectomized	6	208±6	1.05±0.02	49.4±2.4	62.6±3.2	10.8±0.9	0.54±0.02
	P		<0.025	<0.001	<0.001	NS	⩽0.05	NS
B)	Control	13	281±8	1.52±0.04	65.4±2.0	53.6±1.2	8.7±1.0	0.60±0.02
	^{131}I treated	11	246±3	1.02±0.01	41.5±1.5	50.2±2.2	14.4±1.8	0.59±0.02
	P		<0.001	<0.001	<0.001	NS	<0.01	NS
C)	Control	12	253±4	1.49±0.03	71.0±1.9	51.8±1.1	7.7±0.6	0.69±0.02
	T_3 treated	12	233±3	1.84±0.05	81.9±1.5	50.4±0.8	8.3±0.7	0.67±0.03
	P		⩽0.001	<0.001	<0.001	NS	NS	NS

Na-K-ATPase, sodium-potassium-activated adenosine triphosphatase; Mg-ATPase, magnesium-dependent adenosine triphosphatase; G-6-Pase, glucose-6-phosphatase; T_3, triiodothyronine.

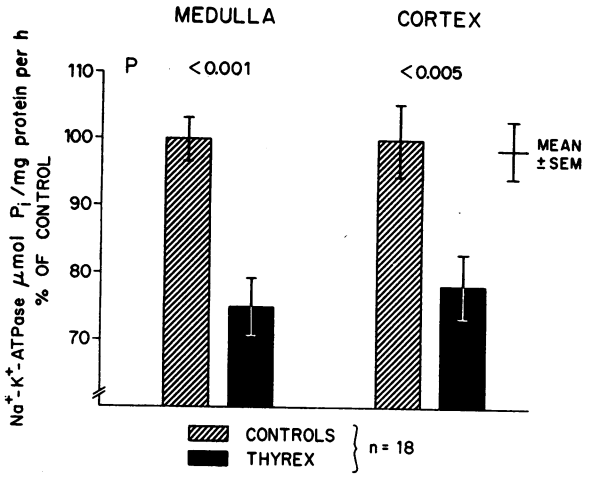

FIGURE 1 Renal Na-K-ATPase specific activity in thyroidectomized and control rats. The relative decrease in enzymatic activity was similar in the cortex and the outer medulla.

treatment with methylprednisolone. After uninephrectomy, Na-K-ATPase specific activity increased in both the cortex and the outer medulla in identical fashion (Table IV and Fig. 2).

Renal function. Renal hemodynamics and sodium handling were measured during hydropenia in thyroidectomized and T_3-treated rats, and results of each group compared with those of its own simultaneously studied controls (Table V). GFR and ERPF were significantly lower, whereas sodium excretion was slightly higher in hypothyroid animals. Net sodium reabsorption per gram of kidney in this group was reduced to 186 μeq/min, compared with 246 μeq/min in controls. The difference in sodium reabsorption between hypothyroid and control animals, whether expressed as absolute reabsorption per minute or related to body or kidney weight, was statistically significant and quantitatively similar to the difference in microsomal Na-K-ATPase specific activity described above.

Administration of T_3 produced the opposite changes in renal hemodynamics and sodium reabsorption and excretion. GFR and ERPF increased significantly, as did the net reabsorption of sodium, whereas the absolute and fractional sodium excretion decreased in treated animals. Net sodium reabsorption calculated per unit body weight was significantly increased in T_3-treated rats. Although net sodium reabsorption per unit kidney mass was also higher in hyperthyroid rats, the difference did not reach statistical significance because of the striking increase in kidney weight which occurred in these animals.

The effect of contralateral nephrectomy and administration of methylprednisolone on renal function is shown in Table VI. Absolute sodium reabsorption was significantly increased in hypothyroid rats by both maneuvers, due primarily to marked increments in filtered sodium. When calculated per unit kidney mass, net sodium reabsorption was increased to levels similar to those measured in intact controls. However, the modest sodium

TABLE III

Effect of Uninephrectomy and Methylprednisolone on Renal Enzymes of Hypothyroid Rats

Group	n	Body wt	Kidney wt	Na-K-ATPase	Mg-ATPase	G-6-Pase	5′-Nucleotidase
					Specific activity		
					μmol P_i/mg protein per h		
1) Control	8	264±4	0.89±0.02*	69.9±1.8	70.1±1.6		
P(1 vs. 2)‡		<0.005	<0.001	<0.001	NS		
2) Hypothyroid	8	234±6	0.69±0.02*	54.7±2.8	72.9±3.4		
P(2 vs. 3)		NS	<0.001	<0.005	<0.05		
3) Hypothyroid + uninephrectomy	8	223±5	0.84±0.04	68.1±2.1	65.1±1.2		
P(3 vs. 1)		<0.001	NS	NS	<0.05		
1) Control	9	224±4	1.38±0.06	59.0±1.7	46.8±1.3	9.7±0.6	0.52±0.04
P(1 vs. 2)		<0.005	<0.001	<0.005	<0.05	<0.005	NS
2) Hypothyroid	8	195±5	0.98±0.03	46.3±3.5	51.8±1.0	16.5±1.1	0.57±0.05
P(2 vs. 3)		NS	<0.05	<0.01	NS	NS	<0.025
3) Hypothyroid + methylprednisolone	9	189±7	1.18±0.06	64.4±3.4	48.2±6.6	20.5±1.9	0.44±0.02
P(3 vs. 1)		<0.005	<0.02	NS	NS	<0.005	NS

Abbreviations as in Table II.

* Right kidney only.

‡ Lines compared for statistical significance.

TABLE IV

Effect of Uninephrectomy on Renal Microsomal Na-K-ATPase from Cortex and Medulla of Hypothyroid Rats

Group	n	Medulla Na-K-ATPase	Cortex Na-K-ATPase
		μmol P_i/mg protein per h	
Control	8	73.6±3.8	46.0±3.4
		$P < 0.005$	$P < 0.02$
Hypothyroid	8	53.4±3.1	34.9±2.0
		$P < 0.005$	$P < 0.02$
Hypothyroid + uninephrectomy	8	70.9±3.4	44.4±2.6

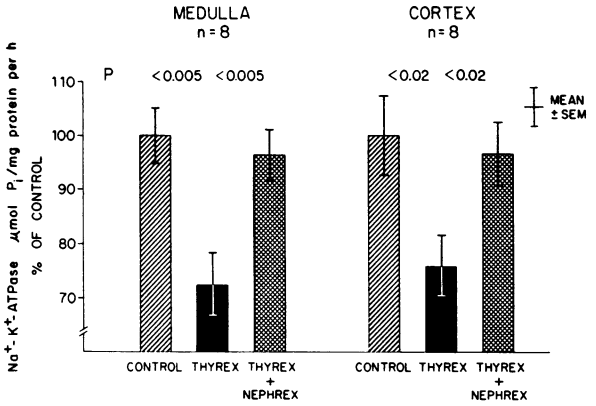

FIGURE 2 Effect of uninephrectomy on renal microsomal Na-K-ATPase in the cortex and the outer medulla of the remaining kidney. The relative increment in enzyme activity was identical in the two regions.

leak present in hypothyroid animals persisted after both uninephrectomy and methylprednisolone treatment. Of interest is the significant increase in both dry and wet kidney weight of the remaining kidney after contralateral nephrectomy. This observation indicates that compensatory kidney growth can occur in the face of markedly reduced thyroid function although normal kidney growth is severely limited in these circumstances.

DISCUSSION

Hypothyroidism is accompanied by widespread alterations in renal hemodynamics and tubular handling of sodium and water. RPF and GFR are markedly reduced in both thyroid-deficient man and rat (1–3). Myxedematous patients have a decreased ability to excrete water loads and frequently manifest fluid retention and dilutional hyponatremia (3, 4). Hypothyroid rats exhibit a limited urinary concentrating ability (21) and an impairment in the renal conservation of sodium, the latter manifested by subtle salt wasting (22) and an exaggerated natriuretic response to saline or water loading (1, 23, 24). Although alterations in urinary concentration and dilution suggest that the sodium leak in hypothyroidism originates in the ascending limb of Henle's loops, the defect in sodium reabsorption may be more generalized and possibly involve both the proximal and the distal nephron (1, 2). The mechanisms responsible for the defect in sodium transport at the cellular level are not known.

Recently Ismail-Beigi and Edelman suggested an important effect of thyroid hormones on transmembrane-active sodium transport. These investigators found decreased oxygen consumption in liver slices and diaphragm of hypothyroid rats which could be increased by thyroid hormone. This increment was largely due to increased energy utilization by the sodium pump, since it could be abolished to a significant extent by ouabain (6). In a subsequent report (5), the same authors described reduced oxygen consumption by liver and kidney slices of hypothyroid rats, and decreased Na-K-ATPase in kidney homogenates. They concluded that thyroid hormones increase energy utilization in active sodium transport by stimulating Na-K-ATPase, and that this effect may account for their calorigenic action.

The above studies were directed at evaluating a general phenomenon and did not deal specifically with the relation of thyroid deficiency to renal physiology and

TABLE V

Renal Hemodynamics and Sodium Handling in Hypothyroid and Hyperthyroid Rats

Group	n	Body wt	Kidney wt	UV	C_{IN}	C_{PAH}	$U_{Na}V$	FE_{Na}	Tubular sodium reabsorption		
		g	*g*	*μl/min*	*ml/min*	*ml/min*	*μeq/min*	%	*μeq/min*	*μeq/min per 100 g*	*μeq/min per g kidney*
Control	16	314±12	1.75±0.10	7.9±0.7	3.26±0.21	7.13±0.39	0.49±0.17	0.11+0.04	471.5±30.2	149.7±7.5	246.2±12.3
Thyroidectomized	16	319±12	1.39±0.10	6.5±0.5	2.38±0.24	5.32±0.37	0.87±0.26	0.29±0.07	342.1±34.0	105.2±8.8	186.4±14.2
P		NS	<0.02	NS	<0.01	<0.01	NS	<0.05	<0.01	<0.001	<0.005
Control	9	251±5	1.34±0.03	8.4±0.5	2.63±0.08	6.42±0.43	0.31±0.07	0.08±0.02	372.8±11.2	149.1±4.5	280.6±11.8
T_3 treated	7	240±7	1.67±0.07	8.6±0.4	3.65±0.22	9.13±0.46	0.09±0.02	0.02±0.005	513.7±32.3	213.2±7.7	307.5±12.6
P		NS	<0.001	NS	<0.001	<0.005	⩽0.02	<0.02	<0.001	<0.001	NS

UV, urine volume; C_{IN}, inulin clearance; C_{PAH}, PAH clearance; $U_{Na}V$, sodium excretion; FE_{Na}, fractional sodium excretion; T_3, triiodothyronine.

TABLE VI

*Effect of Uninephrectomy and Methylprednisolone on Renal Function of Hypothyroid Rats**

Group	n	Body wt	Kidney wt		UV	C_{IN}	C_{PAH}	$U_{Na}V$	FE_{Na}	Tubular sodium reabsorption		
			wet	dry								
		g	*g*	*mg*	*μl/min*	*ml/min*	*ml/min*	*μeq/min*	%	*μeq/min*	*μeq/min per 100 g body wt*	*μeq/min per g kidney*
1. Control	12	249±5	1.55±0.03	344±8	8.9±1.2	2.76±0.10	6.50±0.29	0.57±0.21	0.14±0.05	396.7±16.1	159.3±5.6	259.3±9.9
P(1 vs. 2)‡		<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.02	<0.001	<0.001	<0.001	<0.005
2. Hypothyroid	12	203±4	1.06±0.02	232±5	9.1±1.1	1.48±0.08	3.35±0.31	1.28±0.18	0.60±0.08	211.2±11.0	104.2±5.0	201.2±12.6
P(2 vs. 3)		NS	<0.001	<0.001	<0.001	<0.001		<0.025	NS	<0.001	<0.001	<0.05
3. Hypothyroid + uninephrectomy	11	201±4	0.64±0.01	145±3	8.4±0.8	1.20±0.07	—	1.29±0.26	0.71±0.13	171.6±10.7	85.5±5.1	244.6±16.7
P(3 vs. 1)		<0.001	<0.001	<0.001	<0.001	NS		<0.005	<0.001	NS	NS	NS
1. Control	7	263±11	1.51±0.05	—	10.7±1.8	3.22±0.25	6.71±0.53	0.69±0.24	0.15±0.05	453.8±36.1	171.6±9.5	299.6±21.5
P(1 vs. 2)		NS	<0.001		NS	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001
2. Hypothyroid	10	242±7	1.11±0.04	—	11.1±1.4	1.57±0.06	3.60±0.22	1.61±0.28	0.69±0.10	222.5±8.3	93.1±5.0	201.4±8.5
P(2 vs. 3)		<0.02	NS		NS	<0.001	<0.001	NS	NS	<0.005	<0.001	<0.005
3. Hypothyroid + methylprednisolone	6	213±6	1.13±0.05	—	8.2±0.8	2.28±0.16	7.05±0.95	1.59±0.36	0.49±0.11	318.0±22.4	153.4±13.1	294.4±32.9
P(3 vs. 1)		<0.005	<0.001		NS	<0.02	NS	NS	<0.02	<0.02	NS	NS

Abbreviations as in Table V.

* In the nephrectomy experiments, results are compared with the values calculated for one kidney in the intact control and hypothyroid animals.

‡ Lines compared for statistical significance.

pathophysiology. Nevertheless, they raise a number of interesting questions in this regard. For example, if the decreased Na-K-ATPase in kidney homogenates of hypothyroid rats is a direct consequence of lack of thyroid hormone in the tubular cell, it might explain the defect in sodium reabsorption found in this condition. Another possibility, however, is that the observed decrement in enzymatic activity represents an adaptive change secondary to the reduced reabsorptive sodium load in the hypothyroid rat, analogous to that reported in other conditions (9, 25). The latter hypothesis would suggest that variations in Na-K-ATPase may be the result rather than the cause of changes in sodium transport and, while not ruling out a causal role of this enzyme in the pathogenesis of the sodium reabsorptive defect in hypothyroidism, would make such an explanation less probable.

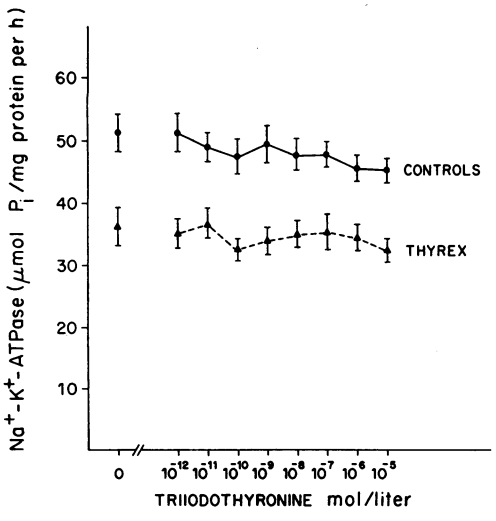

FIGURE 3 Effect of T_3 on microsomal Na-K-ATPase in vitro. T_3 in the concentration range used had no effect on Na-K-ATPase from either hypothyroid or euthyroid rats.

In the present experiments, microsomal Na-K-ATPase specific activity of kidneys from thyroidectomized rats was 25% lower than in euthyroid controls, closely approximating the decrement in net sodium reabsorption per unit kidney mass in these animals. Conversely, the specific activity of Na-K-ATPase increased when net sodium reabsorption was raised by T_3 administration in euthyroid rats. These changes in renal Na-K-ATPase are probably specific, since other enzymes with presumed identical cellular localization but not involved in sodium transport remained unchanged or varied in the opposite direction. This observation, together with the close quantitative correlation with the changes in absolute sodium reabsorption, raised the possibility that the decreased Na-K-ATPase in hypothyroid rats represents mainly an adaptive change in response to decreased substrate (sodium), rather than a direct effect of hormone deficiency.

To test these alternative hypotheses, we attempted initially to demonstrate an effect of T_3 on renal microsomal Na-K-ATPase in vitro. Stimulation of Na-K-ATPase

by T_3 independent of changes in sodium transport would have suggested that thyroid hormone deficit per se is responsible for the decreased renal enzymatic activity in hypothyroidism. T_3 in concentrations of 10^{-12} to 10^{-5} M had no effect on renal Na-K-ATPase of either thyroidectomized or euthyroid animals (Fig. 3), thus failing to support this postulate.

Since failure to demonstrate an effect of thyroid hormone in subcellular particles of disrupted cells may not reflect the situation in the intact kidney, subsequent studies were performed in vivo. We postulated that if reduced Na-K-ATPase is the direct result of lack of thyroid hormone, increasing the reabsorptive sodium load should have little effect on the enzyme since this maneuver does not alter hormone levels.

To increase filtered and reabsorbed sodium per kidney, hypothyroid animals were uninephrectomized, and renal function and Na-K-ATPase were measured after 3 wk, the time required for completion of compensatory kidney growth in the normal rat (26). Net sodium reabsorption in the remaining kidney increased markedly and, when expressed per unit kidney mass, was not significantly different from that of normal controls (Table VI). Despite persistence of the hypothyroid state, Na-K-ATPase also increased significantly in these animals and was similar to controls levels. Na-K-ATPase rose both in the cortex and the outer medulla of the remaining kidney (Table IV), the relative increment being identical in the two regions (Fig. 2). Similar results were obtained in another group of experiments in which hypothyroid rats were treated with methylprednisolone. Again, net sodium reabsorption per gram kidney weight increased in treated hypothyroid rats to levels similar to those measured in euthyroid animals. As in the nephrectomy experiments, Na-K-ATPase specific activity was also increased by this maneuver, to values not significantly different from controls. These results, while not excluding a possible direct influence of thyroid hormone on enzyme activity, suggest that decreased tubular sodium transport is a major determinant of the decrement in renal Na-K-ATPase since the latter can be reversed by restoring sodium reabsorption to normal during continuing hypothyroidism. In addition, they demonstrate that considerable compensatory kidney growth and functional adaptation can take place in the virtual absence of thyroid hormone.

The present studies confirmed the increased sodium excretion in hypothyroid rats previously reported by others (2, 22). Although renal microsomal Na-K-ATPase was decreased, a causal relationship between these observations can not be established for several reasons. First, the reduction in net sodium reabsorption necessary to account for the sodium leak observed was obviously quite small in comparison to that resulting from decreased glomerular filtration, and the decrease in enzymatic activity paralleled the latter. More important, the small sodium leak persisted even when both cortical and medullary Na-K-ATPase specific activity was restored to normal.

In all the experimental situations described above there was a quantitative correlation between net sodium reabsorption per unit kidney mass and Na-K-ATPase specific activity (Fig. 4). These results demonstrate that renal Na-K-ATPase changes adaptively in hyper- or hypo-

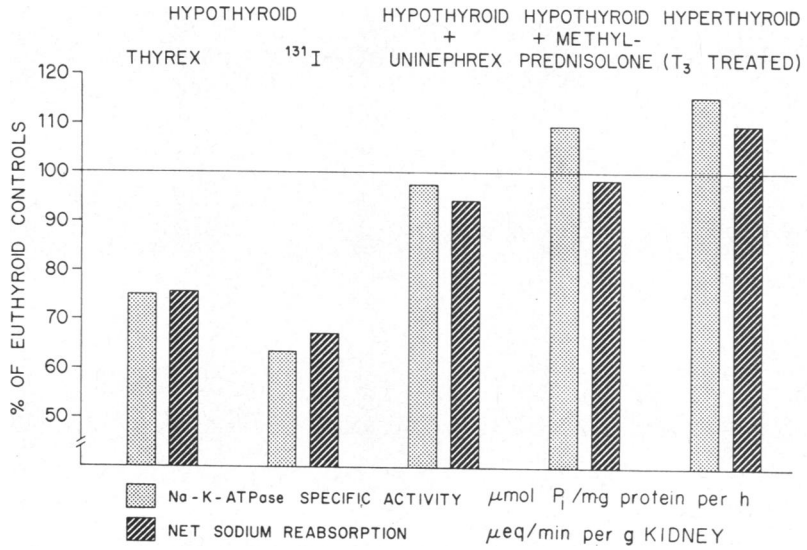

FIGURE 4 Net sodium reabsorption and Na-K-ATPase specific activity in hypo- and hyperthyroid rats. The two functions closely paralleled each other in all the experimental situations studied.

thyroidism as it does in numerous situations in the normal animal, in accord with its postulated role in the active transport of sodium across the renal tubule.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Leslie J. De Groot for helpful advice, and to Mrs. Barbara Corsaw-Hausler and Miss Margaret Lorincz for excellent technical assistance.

This work was supported in part by the Louis Block Fund, by grants AM13601 and HD05572 from the National Institutes of Health, U. S. Public Health Service, and by the Chicago and Illinois Heart Associations.

REFERENCES

1. Holmes, E. W., Jr., and V. A. Di Scala. 1970. Studies on the exaggerated natriuretic response to a saline infusion in the hypothyroid rat. *J. Clin. Invest.* **49**: 1224.
2. Michael, U. F., R. L. Barenberg, R. Chavez, C. A. Vaamonde, and S. Papper. 1972. Renal handling of sodium and water in the hypothyroid rat. *J. Clin. Invest.* **51**: 1405.
3. Di Scala, V. A., and M. J. Kinney. 1971. Effects of myxedema on the renal diluting and concentrating mechanism. *Am. J. Med.* **50**: 325.
4. DeRubertis, F. R., Jr., M. F. Michelis, M. E. Bloom, D. H. Mintz, J. B. Field, and B. B. Davis. 1971. Impaired water excretion in myxedema. *Am. J. Med.* **51**: 41.
5. 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.
6. Ismail-Beigi, F., and I. S. Edelman. 1970. Mechanism of thyroid calorigenesis. Role of active sodium transport. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 1071.
7. Skou, J. C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* **45**: 596.
8. Katz, A. I., and F. H. Epstein. 1968. The physiological role of sodium- and potassium-activated adenosine triphosphatase in the transport of cations across biological membranes. *N. Engl. J. Med.* **278**: 253.
9. Katz, A. I., and F. H. Epstein. 1967. The role of sodium-potassium-activated adenosine triphosphatase in the reabsorption of sodium by the kidney. *J. Clin. Invest.* **46**: 1999.
10. Epstein, F. H., A. Manitius, E. Weinstein, A. I. Katz, and G. E. Pickford. 1969. Sodium- and potassium-activated adenosine triphosphatase in kidneys of Fundulus heteroclitus adapted to fresh and salt water. *Yale J. Biol. Med.* **41**: 388.
11. Lindheimer, M. D., and A. I. Katz. 1971. Kidney function in the pregnant rat. *J. Lab. Clin. Med.* **78**: 633.
12. Fiske, C. H., and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375 .
13. Harper, A. E. 1965. Glucose-6-phosphatase. *In* Methods of Enzymatic Analysis. H. V. Bergmeyer, editor. Academic Press, Inc., New York. 788.
14. Michell, R. H., and J. N. Hawthorne. 1965. The site of diphosphoinositide synthesis in rat liver. *Biochem. Biophys. Res. Commun.* **21**: 333.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
16. Murphy, B. P. 1965. The determination of thyroxine by competitive protein-binding analysis employing an anion-exchange resin and radiothyroxine. *J. Lab. Clin. Med.* **66**: 161.
17. Robin, N. I., S. R. Hagen, F. Collaco, S. Refetoff, and H. A. Selenkow. 1971. Serum tests for measurement of thyroid function. *Hormones (Basel).* **2**: 266.
18. Kingsley, G. R., and O. Robnett. 1958. Further studies on a new dye method for the direct photometric determination of calcium. *Am. J. Clin. Pathol.* **29**: 171.
19. Davidson, W. D., and M. A. Sackner. 1963. Simplification of the anthrone method for determination of inulin in clearance studies. *J. Lab. Clin. Med.* **62**: 351.
20. Smith, H. W., N. Finkelstein, L. Aliminosa, B. Crawford, and M. Graeber. 1945. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* **24**: 388.
21. Réville, P., F. Stephan, and H. Jahn. 1965. Étude comparative des effets de l'hyperthyroïdisme et de l'hypothyroïdisme sur le débit et la concentration des urines du rat. *Arch. Int. Physiol. Biochem.* **73**: 97.
22. Fregly, M. J., R. L. Brimhall, and O. J. Galindo. 1962. Effect of the antithyroid drug propylthiouracil on the sodium balance of rats. *Endocrinology.* **71**: 693.
23. Stephan, F., H. Jahn, and B. Metz. 1959. Action de l'insuffisance thyroïdienne sur l'élimination urinaire de l'eau, du sodium et du potassium chez le rat. *C. R. Seances Soc. Biol. Fil.* **153**: 332.
24. Taylor, R. E., Jr., and M. J. Fregly. 1964. Renal response of propylthiouracil-treated rats to injected mineralocorticoids. *Endocrinology.* **75**: 33.
25. Jørgensen, P. L. 1972. The role of aldosterone in the regulation of $(Na^+ + K^+)$-ATPase in rat kidney. *J. Steroid Biochem.* **3**: 181.
26. Katz, A. I., and F. H. Epstein. 1967. Relation of glomerular filtration rate and sodium reabsorption to kidney size in compensatory renal hypertrophy. *Yale J. Biol. Med.* **40**: 222.