Mechanism of Impaired Water Excretion in the Hypothyroid Rat

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ABSTRACT The ability to excrete an oral water load and the renal diluting mechanism were studied in hypothyroid rats and in age-matched euthyroid controls. Hypothyroid animals excreted a significantly smaller fraction of a 50-ml/kg oral water load than controls, demonstrating the same limited ability to excrete free water as thyroid-deficient man. During hypotonic (0.45%) saline infusion, absolute sodium delivery to the diluting segment and free water clearance were markedly lower in hypothyroid rats. However, both fractional distal sodium delivery and fractional free water clearance were similar in hypothyroid and control animals, suggesting that the reduced absolute free water formation in hypothyroid rats was due to decreased net distal delivery. In support of this hypothesis was the observation that fractional distal sodium reabsorption was equal or higher in thyroid-deficient rats, which indicates that the sodium reabsorptive capacity of the diluting segment was preserved in these animals. The results cannot be attributed to incomplete suppression of antidiuretic hormone (ADH) since they were identical in diabetes insipidus rats, nor to different rates of non-ADH-dependent backflux of filtrate since tissue osmolality and solute concentrations in the cortex, medulla, and papilla were similar in hypothyroid and control rats of both Sprague-Dawley and Brattleboro strains.

The functional integrity of the diluting segment in hypothyroid rats was further demonstrated in experiments in which distal delivery was increased by contralateral nephrectomy or by administration of carbonic anhydrase inhibitors which decrease proximal sodium reabsorption. In both studies, fractional free water clearance increased markedly reaching levels significantly greater than in euthyroid controls.

These results demonstrate that the impaired ability of the hypothyroid rat to excrete a water load is not due to incomplete suppression of ADH or decreased reabsorptive capacity of the diluting segment but results from decreased filtrate delivery to this site secondary to reduced GFR.

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INTRODUCTION

Hypothyroid man exhibits numerous renal functional defects, including decrements in glomerular filtration rate (GFR),[1] effective renal plasma flow (RPF), maximal urine osmolality, and free water generation and reabsorption (1, 2). In the rat, hypothyroidism is associated with similar reductions in GFR and RPF, along with a defect in tubular sodium reabsorption and a limited renal concentrating ability (3, 4). Data on urinary dilution in the hypothyroid rat are not available.

The most important clinical consequence of the renal functional defects in hypothyroid man is water retention and dilutional hyponatremia, due to the limited ability of the kidney to generate free water. The mechanism underlying this defect remains undefined, being variously attributed to inappropriate secretion of or increased tubular sensitivity to vasopressin (5–8), relative deficiency of adrenocortical hormones (9), defective distal sodium reabsorption (1), and decreased delivery of filtrate to the distal diluting segment (1, 2).

The present study was designed to evaluate the renal diluting process in hypothyroid rats and to examine in this species some of the pathogenetic mechanisms ad-

[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; C_{H_2O}, free water clearance; C_{In}, inulin clearance; C_{Na}, sodium clearance; $C_{Na} + C_{H_2O}$, distal sodium delivery; C_{osm}, osmolar clearance; C_P, phosphate clearance; DI, diabetes insipidus; GFR, glomerular filtration rate; RPF, renal plasma flow; $T^c_{H_2O}$, free water reabsorption; U_KV, potassium excretion; $U_{Na}V$, sodium excretion; U_{osm}, urine osmolality; V, urine volume.

vanced to explain the defect in free water formation in human myxedema. The results demonstrate an impaired ability of hypothyroid rats to excrete a water load, while sodium handling in the diluting segment and medullary solute concentrations were similar in thyroid-deficient and normal rats. It is suggested that the limitation of free water formation in hypothyroid animals is not due to incomplete suppression of antidiuretic (ADH) or decreased reabsorptive capacity of the diluting segment, but results primarily from decreased filtrate delivery to this site, probably secondary to reduced GFR.

METHODS

Male and female adult Sprague-Dawley or congenital diabetes insipidus (DI) rats of the Brattleboro strain[2] were used in all experiments. Hypothyroidism was induced by surgical thyroidectomy (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) or by intraperitoneal injection of 1 mCi $Na^{131}I$ (New England Nuclear, Boston, Mass.) at least 4 wk before study at which time control rats matched for sex, age, and weight were selected. All animals were fed a standard rat-chow diet and had free access to tap water until the day preceding the experiment. To insure adequate hydration, 24 h before each study except in the free water reabsorption experiments, they were provided with a solution of 2% dextrose in water. Hypothyroid and control rats were handled identically and studied simultaneously. Thyroid function was evaluated by measuring plasma total thyroxine and free thyroxine index in several groups of randomly selected rats.[3]

Oral water-loading experiments. A 50-ml/kg load of distilled water was administered by gavage under light ether anesthesia. After voiding, the rats were placed in individual metabolic cages and three consecutive hourly urine collections under mineral oil were obtained. Each collection was terminated by ether sniffing and light suprapubic pressure to insure bladder emptying.

Free water formation in hypothyroid and control rats. Animals were anesthetized with Inactin (Promonta, Hamburg, Germany) 100 mg/kg body wt intraperitoneally. A tracheostomy was performed and the bladder, both jugular veins, and one carotid artery were cannulated with polyethylene PE 50 tubing. The rats were placed on a heated board and their rectal temperatures, monitored by a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, Ohio), were maintained between 36° and 38°C. During surgery, isotonic saline equal to 1% of the body wt (Sprague-Dawley rats) or 0.45% saline, 2% of the body wt (DI rats) was infused through a jugular vein to replace estimated fluid losses. After a priming dose, a sustaining infusion of 10% inulin in isotonic saline calculated to maintain plasma inulin levels at approximately 50 mg/100 ml was given at a rate of 20 μl/min. During the 45-min equilibration period, an intravenous water load equivalent to 10% body wt was administered as 2.5% dextrose in water at a rate of 1.15 ml/min through the second jugular catheter. The resulting diuresis was sustained and augmented by the infusion of hypotonic saline (0.45%) at constant rates ranging between 0.2 and 1.15 ml/min. All solutions were administered with constant infusion pumps (model 975, Harvard Apparatus Co., Inc., Millis, Mass.). During each experiment, 3-10 urine samples were collected in tared plastic tubes and their volume determined by weighing to the nearest 0.1 mg. Urine aliquots were analyzed for osmolality and for reducing substances (Clinitest, Ames Co., Div. of Miles Lab, Inc., Elkhart, Indiana). Samples with osmolality in excess of 100 mosmol/kg H_2O or more than a trace of reducing substances were excluded.[4] Free flowing tail blood was collected in heparinized capillary tubes during alternating collecting periods. Since relatively large plasma volumes were required for duplicate osmolality determinations, 1.2 ml of blood was withdrawn from the carotid artery before the first urine collection and immediately replaced with an equal volume of blood from an identically treated rat. The donor rats were age- and sex-matched hypothyroid or control animals of the same strain submitted to the same surgical procedure and receiving identical infusions as the experimental rats. A second osmolality determination was done on blood obtained at the end of the experiment. The same protocol was used in Sprague-Dawley and DI animals.

Free water formation by hypothyroid Sprague-Dawley rats during increased sodium delivery to the diluting site. Distal sodium delivery per kidney was increased in hypothyroid rats by contralateral nephrectomy or by administration of the carbonic anhydrase inhibitors benzolamide[5] and acetazolamide. The nephrectomy was performed under ether anesthesia through a left flank incision and the animals were studied at least 3 wk postoperatively after the protocol described above. In studies using carbonic anhydrase inhibitors all animals received 0.3 ml/min of a 0.45% saline solution. After two base-line urine collections, benzolamide (2 mg/kg) or acetazolamide (10 mg/kg) dissolved in 0.45% saline was injected rapidly and followed by a sustaining infusion delivering 2 mg/kg/h of benzolamide or 10 mg/kg/h of acetazolamide at the same infusion rate while three additional urine collections were obtained. These doses were selected to insure adequate renal with minimal systemic carbonic anhydrase inhibition (10). The sustaining solutions contained 37.5 meq/liter sodium chloride and 37.5 meq/liter sodium bicarbonate to prevent the acidosis which results from the profound bicarbonate leak caused by these agents. Blood pH after the infusion ranged between 7.40 and 7.50. Increased phosphate excretion was considered to be an indication of decreased proximal sodium reabsorption (11, 12), and results from animals failing to show increments in phosphate clearance following administration of the two diuretics were excluded.

Tissue osmolality and solute concentrations in kidneys of hypothyroid and control rats during water diuresis. Animals were prepared as outlined above. When maximal water diuresis was established, blood samples and two timed urine collections were obtained to allow clearance calculations. Both kidneys were then rapidly removed and the tissue

[2] Kindly supplied by Drs. John Forrest and Rex Jamison.

[3] We are indebted to Dr. Samuel Refetoff for performing these studies.

[4] Since maximal ADH suppression in non-DI rats is prerequisite for the use of free water clearance (C_{H_2O}) as an index of sodium reabsorption in the diluting segment, urine samples with osmolality in excess of 100 mosmol/kg H_2O were excluded. Urine samples showing more than trace reducing substances were also excluded since the presence of glucose in the end-proximal tubular fluid would tend to limit sodium reabsorption and C_{H_2O}.

[5] 2-Benzenesulfonamido-1,3,4-thiadiazole-5-sulfonamide supplied through the courtesy of Dr. Robert T. Kunau, Jr.

TABLE I
Thyroid Function

Group	*n*	Total thyroxine	Free thyroxine index
		μg/100 ml	
Controls	9	3.74±0.25	6.32±0.37
Hypothyroid	9	0.89±0.12	0.97±0.23
P		<0.001	<0.001

processed according to a modification of the method of Appelboom, Brodsky, Tuttle, and Diamond (13). The capsules were stripped and the kidneys cut along the longitudinal axis. Samples of papilla, medulla, and cortex from individual animals were excised with iridectomy scissors, lightly blotted on filter paper, placed in tared tubes, and weighed to the nearest 0.1 mg (wet wt). The papilla included the papillary tip along with most of the inner medulla, which is recognizable by its gray-yellow color, while the medullary samples consisted entirely of the red outer medulla. Cortical tissue was obtained by tangential cuts close to the kidney surface. Immediately after weighing, the samples were submerged in boiling distilled water and kept in a boiling water bath for at least 60 min. The time interval between excision of the kidneys and beginning of boiling ranged between 4 and 6 min. After cooling to room temperature, the samples were reweighed and osmolality, urea, sodium, and potassium were determined in the supernate. Dry weight was measured after desiccation to constant weight at 105°C. Tissue electrolyte, urea, and osmolal concentrations were calculated per kilogram tissue water or per 100 grams dry solids. The measured osmolality was corrected for dilution by utilizing the appropriate osmotic coefficients for sodium and potassium chloride (14). The same procedures were used in Sprague-Dawley and DI rats.

Free water reabsorption in hypothyroid and control rats. Animals were prepared as in the free water clearance experiments described above. Vasopressin was added to the sustaining infusion of inulin in amounts calculated to deliver 40 mU/kg/h. After the equilibration period, 3% saline was infused at constant rates ranging between 0.2 and 1.15 ml/min and appropriate blood and urine samples were collected for clearance determinations.

TABLE II
Excretion of a 50-ml/kg Body Wt Oral Water Load by Hypothyroid and Control Rats

Group	*n*	% of water load excreted (cumulative) 1 h	2 h	3 h
Sprague-Dawley				
Hypothyroid	18	25.7	67.6	92.9
		±3.0	±4.5	±3.9
Controls	18	44.8	86.6	100.4
		±3.4	±4.3	±3.2
P		<0.001	<0.005	NS*
Brattleboro				
Hypothyroid	7	28.1	61.7	86.2
		±5.2	±9.6	±7.3
Controls	6	51.5	85.3	98.3
		±7.3	±10.7	±10.1
P		<0.025	NS	NS

* Not significant.

GFR was calculated from the clearance of inulin. Since blood was obtained during alternate periods only, interim values were derived by interpolation. Osmolar (C_{osm}), free water (C_{H_2O}), sodium (C_{Na}), and phosphorus (C_P) net and fractional clearances and free water reabsorption ($T^c_{H_2O}$) were calculated from standard formulae. Distal sodium delivery was approximated from the sum of $C_{Na} + C_{H_2O}$ and the fractional distal sodium reabsorption derived from the formula $C_{H_2O}/(C_{Na} + C_{H_2O}) \times 100$. Inulin was measured by a semimicro modification of the anthrone method (15), sodium and potassium were determined by flame photometry using lithium as internal standard (IL 343 digital flame photometer, Instrumentation Laboratory, Inc., Lexington, Mass.), phosphorus was measured by the

TABLE III
Results from Period of Maximal Free Water Clearance during the Infusion of 0.45% Saline

Group	*n*	C_{in}		U_{osm}	V	C_{H_2O}	C_{Na}
		ml/min	*ml/min/kg body wt*	*mosmol/kg H_2O*		*ml/min*	
1. Control	7	3.71	8.41	85	0.585	0.403	0.101
		±0.12	±0.33	±4	±0.016	±0.012	±0.013
P (1 vs. 2)		<0.001	<0.02	NS	<0.001	<0.001	<0.001
2. Hypothyroid	6	1.60	6.28	72	0.275	0.201	0.030
		±0.21	±0.74	±5	±0.036	±0.028	±0.005
P (2 vs. 3)		<0.05	<0.001	<0.02	<0.01	<0.025	<0.01
3. Hypothyroid	7	1.06	5.10	89	0.221	0.147	0.041
+uninephrectomy		±0.06	±0.82	±3.0	±0.017	±0.012	±0.007
P (3 vs. 1)		<0.001	<0.01	NS	<0.005	<0.005	NS

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

method of Fiske and SubbaRow (16), urea by a modification of the Berthelot method (17), and total thyroxine by the competitive binding assay of Murphy (18). The free thyroxine index (19), 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. Osmolality was determined by cryoscopy with a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Mass.).

Results are presented as mean±SEM. The statistical significance of changes in individual animals was assessed by analysis of paired differences and that of differences between group means by the Student's t test. P values less than 0.05 were considered significant. Regression lines were calculated by the method of least squares.

RESULTS

Thyroid function was markedly decreased in hypothyroid rats (Table I), in which the mean free thyroxine index was 15% of that measured in intact animals.

Oral water loading experiments (Table II)

Water excretion was delayed in hypothyroid animals which excreted in the first 2 h a significantly smaller fraction of the load received than their controls. Urine osmolality during each collection period was similar in hypothyroid and intact animals of both strains.

Free water formation in hypothyroid and control animals

Sprague-Dawley rats. Results obtained during the period of maximal free water clearance in all experiments in which distal delivery exceeded 10% of GFR are summarized in Table III. Urine osmolality was similar in control and hypothyroid rats, averaging 85±4 and 75±5 mosmol/kg H_2O, respectively. GFR was significantly lower in thyroidectomized animals and, when corrected for body wt, was approximately 75% of that observed in euthyroid controls (6.28±0.74 vs. 8.41±0.33 ml/min/kg body wt). While urine flow, free water clearance, and distal sodium delivery were significantly lower in hypothyroid rats, their fractional counterparts were similar in the two groups. Furthermore, fractional distal sodium reabsorption at similar delivery rates was slightly higher in hypothyroid animals, averaging 87% as compared to 80% in intact controls.

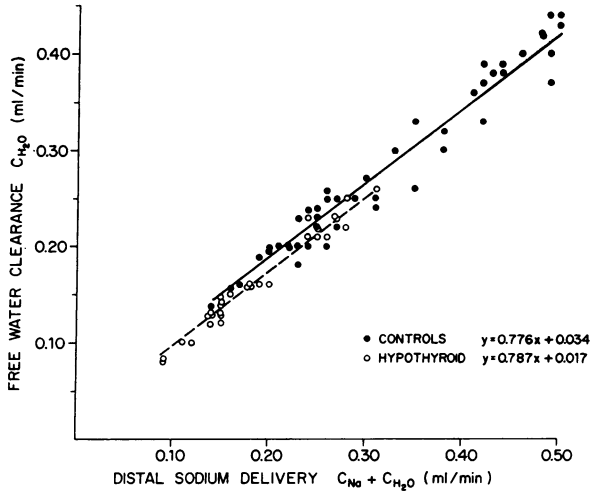

FIGURE 1 Relation of free water clearance to distal sodium delivery in control and hypothyroid Sprague-Dawley rats during hypotonic saline diuresis. Slopes of the two curves are essentially identical, suggesting that the lower absolute free water formation in thyroid-deficient rats is due to reduced distal sodium delivery.

The relation of free water clearance to distal sodium delivery is shown in Fig. 1. Although hypothyroid animals formed less free water, they did so at substantially lower distal sodium delivery rates. Regression analysis

*in Control Animals and Intact and Uninephrectomized Hypothyroid Sprague-Dawley Rats**

$C_{H_2O}+C_{Na}$	$\frac{C_{H_2O}\times 100}{C_{H_2O}+C_{Na}}$	$U_{Na}V$	U_KV	$\frac{V\times 100}{GFR}$	$\frac{C_{H_2O}\times 100}{GFR}$	$\frac{C_{H_2O}+C_{Na}\times 100}{GFR}$	$\frac{C_{Na}\times 100}{GFR}$
ml/min	%	*μeq/min*			*ml/min/100 ml GFR*		
0.504	80.10	13.54	2.83	15.79	10.88	13.62	2.74
±0.020	±1.8	±1.58	±0.41	±0.55	±0.37	±0.54	±0.31
<0.001	<0.02	<0.001	<0.005	NS	NS	NS	NS
0.231	87.1	3.75	0.79	17.51	12.74	14.62	1.87
±0.032	±1.6	±0.65	±0.16	±1.17	±0.08	±0.97	±0.25
<0.02	<0.01	<0.01	<0.001	<0.05	NS	NS	<0.01
0.188	79.1	5.13	0.95	20.85	13.88	17.65	3.77
±0.017	±1.9	±0.85	±0.06	±0.98	±0.65	±1.06	±0.49
<0.01	NS	NS	<0.05	<0.001	<0.005	<0.01	NS

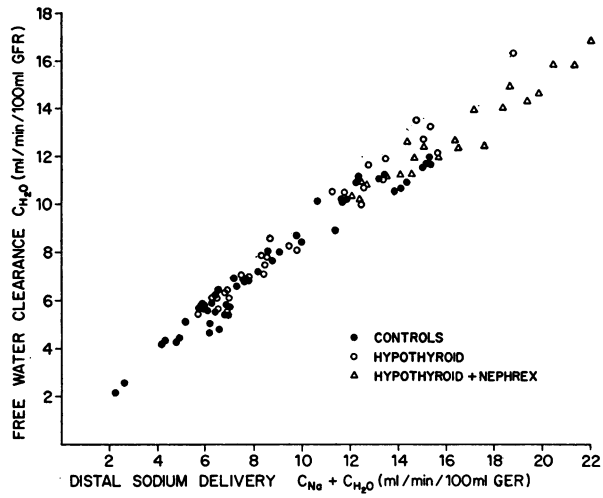

FIGURE 2 Relation of fractional free water clearance to fractional distal sodium delivery during hypotonic saline diuresis in Sprague-Dawley rats. Fractional C_{H_2O} was similar in normal and thyroid-deficient animals, while uninephrectomy in hypothyroid rats increased both fractional distal delivery and fractional free water clearance to levels higher than in intact controls.

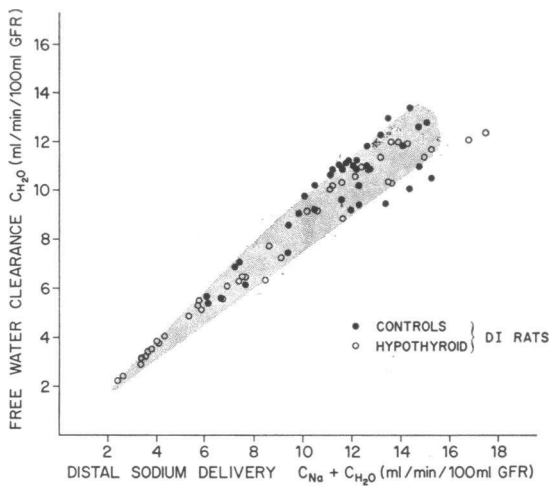

FIGURE 3 Relation of fractional free water clearance to fractional distal sodium delivery during hypotonic saline diuresis in control and hypothyroid diabetes insipidus rats. The shaded area depicts this relationship in Sprague-Dawley rats. Fractional C_{H_2O} was similar for any given fractional distal delivery in all groups studied.

of the two curves generates essentially identical slopes, suggesting that the reduction in absolute free water formation in thyroid-deficient rats might be due to the lower distal sodium delivery in these animals.

Fig. 2 depicts the relation of fractional free water clearance to fractional distal sodium delivery in the two groups. Correction for differences in GFR between hypothyroid and intact rats also eliminates the observed difference in free water formation. The curve relating the fractional functions is linear at distal delivery rates below 10% of the glomerular filtrate and becomes curvilinear thereafter, progressively deviating from the line of complete distal sodium reabsorption without, however, reaching a maximum.

DI rats. Results were essentially identical to those obtained in Sprague-Dawley rats, and are summarized in Table IV and Fig. 3. GFR was significantly lower in hypothyroid rats, and when corrected for body wt was 75% of that measured in euthyroid animals (6.74±0.35 vs. 8.98±0.75 ml/min/kg body wt). During the period of maximal free water clearance, fractional distal delivery, free water and sodium clearances, and distal sodium reabsorption, as well as urine osmolality, were similar in hypothyroid and control animals.

Free water formation by hypothyroid Sprague-Dawley rats during increased sodium delivery to the diluting site

Clearance data of hypothyroid, uninephrectomized rats during the period of maximal fractional free water clearance are summarized in Table III. Although absolute urine flow, free water clearance, and distal sodium delivery were lower than those of intact, euthyroid ani-

TABLE IV

Results from Period of Maximal Free Water Clearance during the Infusion of 0.45% Saline, and Tissue Osmolality in Control and Hypothyroid Diabetes Insipidus Rats

Group	n	C_{in}	U_{osm}	$\frac{V \times 100}{GFR}$	$\frac{C_{H_2O} \times 100}{GFR}$	$\frac{C_{Na} \times 100}{GFR}$	$\frac{C_{Na} + C_{H_2O} \times 100}{GFR}$	$\frac{C_{H_2O} \times 100}{C_{H_2O} + C_{Na}}$	Tissue osmolality			Water content		
									Cortex	Medulla	Papilla	Cortex	Medulla	Papilla
		ml/min/kg body wt	*mosmol/kg H_2O*	*ml/min/100 ml GFR*				%	*mosmol/kg tissue water*			%		
Control	5	8.98 ±0.75	74 ±7	15.74 ±0.69	11.53 ±0.71	2.20 ±0.53	13.73 ±0.64	84.0 ±3.7	339 ±7	364 ±9	398 ±13	75.3 ±0.6	83.9 ±1.5	86.1 ±0.9
Hypothyroid	5	6.74 ±0.35	69 ±10	14.36 ±1.19	10.68 ±0.94	2.61 ±0.67	13.29 ±1.41	81.0 ±3.1	328 ±9	362 ±19	382 ±11	78.1 ±1.7	84.8 ±0.5	88.2 ±1.2
P		<0.05	NS*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

TABLE V

Composition of Renal Papilla, Medulla, and Cortex of Hypothyroid and Control Sprague-Dawley Rats during Maximal Water Diuresis

Group	n	Water content	Na	Na	K	Urea	Osmolality	
							Measured	Calculated*
		%	*meq/100 g dry solids*	*meq/kg H_2O*	*meq/kg H_2O*	*mmol/kg H_2O*	*mosmol/kg tissue water*	
Papilla								
Control	8	86.8	92.0	138.0	60.7	34.6	439	432
		±0.3	±7.2	±9.7	±2.0	±4.3	±14	±20
Hypothyroid	11	86.9	103.3	147.9	59.2	32.3	430	446
		±0.6	±9.0	±5.5	±3.5	±2.5	±21	±15
P		NS	NS	NS	NS	NS	NS	NS
Medulla								
Control	8	83.1	52.7	111.9	73.8	14.6	403	386
		±0.5	±3.7	±8.3	±2.9	±2.2	±20	±19
Hypothyroid	11	81.6	65.1	137.5	73.0	15.4	444	437
		±0.9	±4.8	±6.7	±3.5	±1.5	±25	±17
P		NS	NS	<0.05	NS	NS	NS	NS
Cortex								
Control	8	77.8	23.3	65.9	80.7	13.5	308	307
		±0.4	±1.3	±4.0	±2.1	±1.1	±9	±8
Hypothyroid	11	77.2	26.1	78.9	82.3	14.5	331	338
		±0.5	±1.3	±3.3	±3.3	±0.9	±14	±17
P		NS	NS	<0.05	NS	NS	NS	NS

* Osmolality calculated as 2(Na + K) + urea.

mals even when calculated for one kidney, the same parameters became significantly higher in the uninephrectomized hypothyroid rats when expressed as fractions of the glomerular filtrate. Uninephrectomy increased further both fractional distal delivery and fractional free water clearance (Fig. 2). As in the intact animals, no true maximum for fractional free water clearance was observed even when delivery rates were as high as 22% of GFR.

Benzolamide and acetazolamide increased absolute and fractional phosphate clearance from 0.151±0.074 to 0.254±0.083 ml/min and from 6.04±2.20 to 12.69±2.67 ml/min/100 ml GFR, respectively. Both differences were highly significant ($P < 0.001$). Fig. 4 presents the effect of these drugs on fractional distal delivery and the resulting augmentation of fractional free water clearance. Mean $C_{H_2O}/GFR \times 100$ increased after benzolamide from 10.57±1.09 ml/min to 14.13±0.45 ml/min/100

TABLE VI

Results from Period of Maximal Free Water Reabsorption during the Infusion of 3% Saline in Control and Hypothyroid Rats

Group	n	C_{in}		U_{osm}	V	C_{osm}	C_{Na}	T^eH_2O	$U_{Na}V$	U_KV	$\frac{C_{Na} \times 100}{GFR}$	$\frac{C_{osm} \times 100}{GFR}$	$\frac{T^eH_2O \times 100}{GFR}$
		ml/min	*ml/min/kg body wt*	*mosmol/kg H_2O*	*ml/min*				*μeq/min*		*ml/min/100 ml GFR*		
Control	1	4.44	12.37	567.5	0.618	0.973	0.895	0.356	151.46	5.91	20.06	21.83	8.06
		±0.18	±0.51	±14.2	±0.074	±0.083	±0.086	±0.014	±15.79	±0.29	±1.78	±1.66	±0.20
Hypothyroid	13	2.16	9.06	560.8	0.455	0.649	0.590	0.194	112.30	2.91	27.80	30.52	9.07
		±0.09	±0.34	±11.2	±0.051	±0.050	±0.052	±0.006	±11.24	±0.39	±2.43	±2.31	±0.24
P		<0.001	<0.001	NS	NS	<0.005	<0.01	<0.001	NS	<0.001	<0.02	<0.005	<0.005

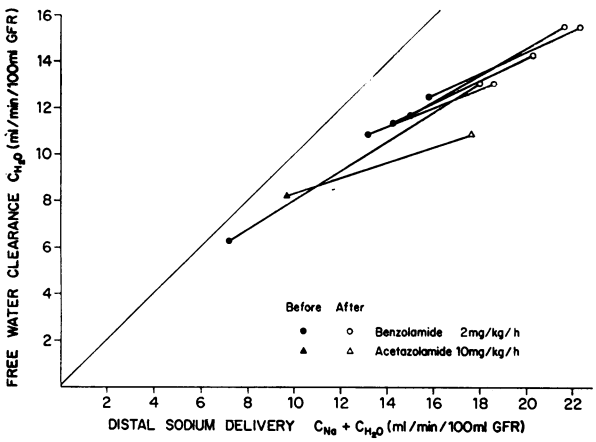

FIGURE 4 Effect of carbonic anhydrase inhibitors on fractional free water clearance and fractional distal sodium delivery in hypothyroid Sprague-Dawley rats. Increase in fractional distal delivery resulted in augmentation of fractional free water clearance.

ml GFR ($P < 0.001$), although distal fractional sodium reabsorption became less efficient as evidenced by the slopes of lines connecting measurements in the same animal.

Tissue osmolality and solute concentrations during water diuresis in kidneys of hypothyroid and control rats (Tables IV and V)

Tissue osmolality and solute concentrations were measured at the peak of water diuresis when fractional C_{H_2O} and plasma and urine osmolality were similar in hypothyroid and control rats. These measurements did not differ significantly in the two groups of either strain of rats in any of the three regions of the kidney. Tissue osmolality, calculated as the sum of solute concentrations [2(Na + K) + urea] correlated satisfactorily with the measured osmolality, attesting to the validity of the methods of tissue analysis employed in this study. Water content of the tissue samples increased progressively from cortex to papilla in agreement with previously reported results (20).

Free water reabsorption in hypothyroid and control rats

Results obtained during the period of maximal free water reabsorption are presented in Table VI. GFR, osmolal clearance, and free water reabsorption were significantly lower in hypothyroid rats. Maximal fractional $T^c_{H_2O}$, on the other hand, was greater in these animals, but was measured at higher osmolal clearance rates. Mass plot of results from all experiments (Fig. 5) indicates that when expressed as a function of fractional osmolal clearance, fractional free water reabsorption is similar in thyroid-deficient and normal rats.

DISCUSSION

These experiments demonstrate a renal diluting defect similar to that described in human myxedema in thyroid-deficient rats, which excreted a significantly smaller fraction of an oral water load and showed markedly lower absolute free water clearance rates than normal animals.

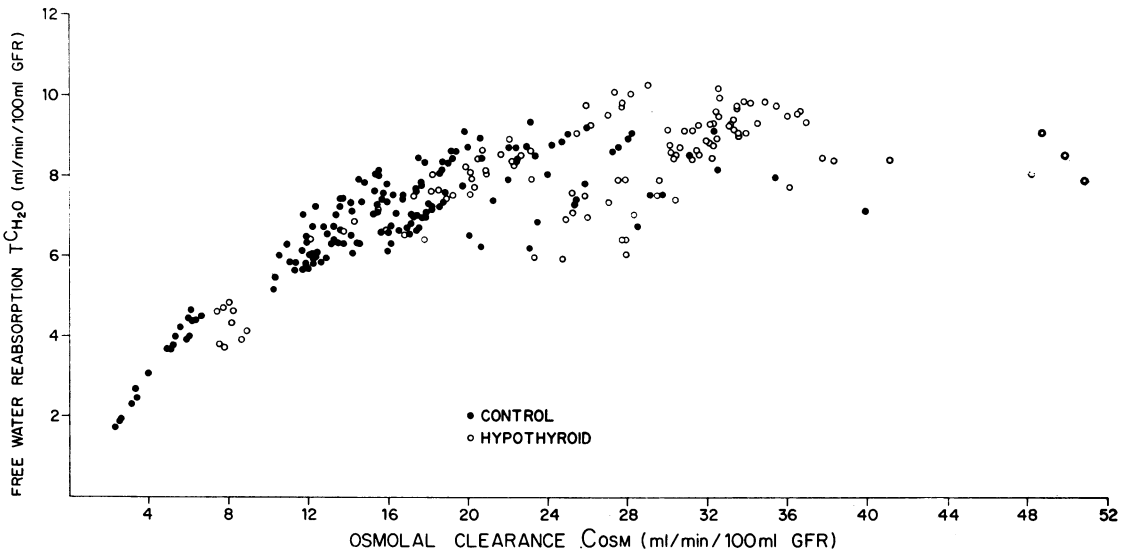

FIGURE 5 Relation of fractional free water reabsorption to fractional osmolar clearance in control and hypothyroid rats during hypertonic saline diuresis. Results in the two groups were similar.

Sodium chloride transport by the ascending limb of Henle's loop is a cardinal feature of the renal diluting mechanism. Several authors have noted a sodium leak in the hypothyroid rat, which raises the possibility that the limited ability of hypothyroid animals to generate free water might be due to an intrinsic defect in the transport of sodium by the ascending limb of Henle's loop. Another possibility is that the dilution defect could be due to decreased delivery of filtrate to the diluting site, an explanation suggested by the proportional reduction in C_{H_2O} and GFR observed in this study.

To distinguish between these alternatives we analyzed the relationship between free water formation and distal delivery in hypothyroid and control rats. Although both C_{H_2O} and $C_{H_2O} + C_{Na}$ were markedly lower in hypothyroid animals, the curves relating these functions (Fig. 1) had identical slopes, suggesting that the decreased C_{H_2O} was due to decreased net distal delivery. This impression was substantiated when free water formation and distal delivery expressed as fractions of the GFR (Figs. 2 and 3, Tables III and IV) were found to be similar in the two groups. Furthermore, fractional distal sodium reabsorption in the period of maximum free water formation was equal or higher in the thyroid-deficient rats, an observation which suggests strongly that the reabsorptive capacity of the diluting segment was preserved in these animals.

To further evaluate the functional integrity of the diluting segment, experiments were designed to test whether hypothyroid animals can generate additional C_{H_2O} in response to increased distal delivery. When the latter was increased by uninephrectomy, fractional free water formation rose further, and was significantly higher than in control animals. Similarly, hypothyroid rats increased C_{H_2O} appreciably when distal delivery was augmented by benzolamide and acetazolamide, two inhibitors of carbonic anhydrase which decrease proximal sodium reabsorption (11, 12, 21). It is noteworthy that C_{H_2O} rose despite the fact that a considerable proportion of the additional sodium delivered to the diluting segment was probably accompanied by relatively nonreabsorbable bicarbonate (22, 23). Demonstration that hypothyroid rats can generate substantially more free water when delivery of filtrate to the diluting segment is increased offers additional support to the hypothesis that reduced distal delivery plays a central role in generating the renal diluting defect in hypothyroidism.

If free water generation were impaired in hypothyroid rats because of decreased delivery of filtrate to the ascending limb, a similar defect should be demonstrable when measuring free water reabsorption, since the latter is also dependent indirectly on sodium chloride transport in the loop of Henle. Indeed, absolute $T^c_{H_2O}$ during hypertonic saline diuresis was lower in hypothyroid rats (Table VI). However, fractional $T^c_{H_2O}$ was slightly higher than in normal controls indicating that the reduced $T^c_{H_2O}$ was due to decreased filtrate delivery to the loop, analogous to the situation in water diuresis.

Besides the functional integrity of the ascending limb and the delivery of filtrate to this site, renal diluting capacity depends on the permeability of the collecting duct to water. Inappropriate secretion of ADH (5–7), or increased sensitivity of the renal tubule to its effect (8) have been suggested to explain impaired water excretion in hypothyroidism. In the present study, results obtained in animals with congenital diabetes insipidus indicate that, at least in the hypothyroid rat, abnormal vasopressin activity does not account for the limited ability of the kidney to form free water.

Next we considered the possibility that differences in vasopressin-independent water reabsorption distal to the diluting site might have contributed to the results observed. Jamison and colleagues (24, 25) recently demonstrated in Brattleboro rats that a substantial amount of water can be reabsorbed from the collecting duct by transtubular osmotic driving force even in the absence of antidiuretic hormone. Thus, lower tissue osmolality in the kidney of hypothyroid rats, by presenting a reduced drive for back-diffusion of water along the cortical and/or medullary segments of the collecting duct, could have produced an artificial increase in fractional free water excretion independent of the events in the ascending limb of Henle's loop. This possibility was ruled out by measurements of tissue osmolality and solute concentrations (Tables IV and V) which, during peak water diuresis, were identical in hypothyroid and control rats of both Sprague-Dawley and Brattleboro strains in all three regions of the kidney. These observations suggest therefore that any underestimation of distal delivery and free water formation was probably similar in hypothyroid and normal animals.

Several interesting features of these studies deserve further comment. During hypertonic saline loading, both normal and hypothyroid rats achieved very high osmolal clearance rates, greater than previously reported in this species (23, 26–28). As a result, it appears that fractional $T^c_{H_2O}$ in both groups reached a plateau when C_{osm} equaled approximately 24% of GFR, as no further increments could be detected even when fractional osmolal clearances were as high as 40–50 ml/min/100 ml GFR. Although no such apparent maximum for fractional C_{H_2O} was observed in the water diuresis experiments, it should be noted that distal delivery in these studies never exceeded 22% of GFR. Also of interest is the confirmation of a "sodium leak" in hypothyroid animals (3, 4), in which fractional sodium clearance was higher than in controls during administration of massive sodium loads. However, the significance of this observation is not

clear since in the same experiments net sodium excretion was not increased, and both net and fractional sodium excretion were lower in hypothyroid rats during water diuresis experiments in which the amount of sodium infused was smaller.

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