Effects of Hypothyroidism on Vascular ^{125}I-Albumin Permeation and Blood Flow in Rats

Ronald G. Tilton, Giuseppe Pugliese, Katherine Chang, Amanda Speedy, Michael A. Province, Charles Kilo, and Joseph R. Williamson

Effects of hypothyroidism on vascular ^{125}I-albumin permeation and on blood flow were assessed in multiple tissues of male Sprague-Dawley rats rendered hypothyroid by dietary supplementation with 0.5% (wt/wt) 2-thiouracil or by thyroidectomy. In both thiouracil-treated and thyroidectomized rats, body weights, kidney weight, arterial blood pressure, and pulse rate were decreased significantly *v* age-matched controls. After 10 to 12 weeks of thiouracil treatment, ^{125}I-albumin permeation was increased significantly in the kidney, aorta, eye (anterior uvea, choroid, retina), skin, and new granulation tissue, remained unchanged in brain, sciatic nerve, and heart, and was decreased in forelimb skeletal muscle. A similar pattern was observed in thyroidectomized rats, except that increases in ^{125}I-albumin permeation for all tissues were smaller than those observed in thiouracil-treated rats, and ^{125}I-albumin permeation in retina did not differ from controls. In both thiouracil-treated and thyroidectomized rats, changes in blood flow (assessed with 15-μm, ^{85}Sr-labeled microspheres) relative to the decrease in arterial blood pressure were indicative of a decrease in regional vascular resistance except in the choroid and in the kidney, in which vascular resistance was increased significantly. Glomerular filtration rate was decreased, but filtration fraction and urinary excretion of albumin remained unchanged by thiouracil treatment and thyroidectomy. These results indicate that vascular hemodynamics and endothelial cell barrier functional integrity are modulated in many different tissues by the thyroid. In view of the correspondence of hypothyroid- and diabetes-induced vascular permeability changes, these results raise the possibility that altered thyroid function in diabetes may play a role in the pathogenesis of diabetic vascular disease.

IT IS WELL KNOWN that thyroid hormones influence the maturation, growth, and function of tissues through modulation of numerous metabolic pathways and several endocrine systems. Thyroid dysfunction is associated with marked alterations in cardiovascular function. In hypothyroidism, cardiac output, myocardial contractility, and oxygen consumption are decreased, time-to-peak tension in the left ventricle is prolonged, peripheral resistance (mean arterial blood pressure/cardiac output) is increased, and the effects of catecholamines on blood pressure and heart rate are smaller than in euthyroid patients.[1-4] In general, the converse occurs in hyperthyroid patients. In addition, hypercholesterolemia and albuminuria,[5] as well as decreases in tissue capillary density[6] and capillary surface area[7] have been reported in hypothyroidism.

Although previous studies have shown that hypothyroidism decreases blood flow in the kidney, adipose tissue, and gastrointestinal tract and increases blood flow in the liver,[5,8-10] relatively few studies have assessed effects of hypothyroidism on endothelial cell barrier functional integrity. Increased vascular permeation by small molecules such as fluorescein has been observed in the skin,[11,12] and an increased (whole body) transcapillary escape rate for plasma proteins such as albumin[13,14] has been reported in hypothyroid patients. These changes are consistent with the generalized edema and exudates (pericardial, pleural, and peritoneal) in patients with myxedema. Parving et al[13] reported an increased transcapillary escape rate of albumin, an increase in the extravascular mass of albumin, and a longer mean-transit time through the extravascular spaces in primary myxedema *v* other states of edema, and suggested that inadequate lymphatic drainage may explain the presence of exudates.

Several vascular structural changes associated with hypothyroidism are similar to changes observed in diabetes. Mesangial hypertrophy and glomerular capillary basement membrane thickening similar to that occurring in diabetic renal disease have been reported in patients with hypothyroidism.[15] Thickening of capillary basement membrane in the heart of hypothyroid patients also has been reported.[16] Likewise, thyroid insufficiency has been reported to mimic the functional and biochemical abnormalities seen in peripheral nerve in experimental diabetes, and administration of thyroid hormone restores motor nerve conduction velocity and phosphatidylinositol synthetase and Na^+/K^+-ATPase activities in diabetic rats.[17]

Because increased blood flow and increased vascular leakage of plasma proteins are early functional alterations of diabetic vascular disease in eyes, aorta, kidney, and nerve of humans and experimental animals,[18-20] the present study was undertaken to investigate whether hypothyroidism (induced with dietary thiouracil or by thyroidectomy) also affects regional ^{125}I-albumin permeation of vessels, blood flow, and vascular resistance in rats.

MATERIALS AND METHODS

Animal Protocols

Male Sprague-Dawley rats initially weighing ~200 g were divided into three groups: control, thiouracil-treated, or thyroidectomized.

From the Departments of Pathology and Medicine, and the Division of Biostatistics, Washington University School of Medicine, St Louis.

Supported by National Institutes of Health Grant Nos AM07296, AM20579, and HL30678, and by the Kilo Diabetes and Vascular Research Foundation.

Address reprint requests to Ronald G. Tilton, PhD, Washington University, Department of Pathology, Box 8118, 660 South Euclid Ave, St Louis, MO 63110.

0026-0495/89/3805-0015$03.00/0

All rats were fed a powdered diet (purchased from Ralston Purina, Richmond, IN) containing: dextrin, 50%; casein, 21%; sucrose, 8.65%; solka floc, 3.0%; corn oil, 5.0%; lard, 5.0%; Dl-methionine, 0.15%; choline chloride, 0.2%; RP vitamin mix, 2.0%; RP mineral mix #10, 5.0%. Rats were allowed food and water ad libitum. Thyroidectomized rats were purchased from Sasco, (Omaha). On day 0, 2-thiouracil (0.5% wt/wt) was added to the diet of group 2 rats, and at this time, all rats received a subcutaneous (dorsolateral) implant of a 1-cm^2 piece of sterile polyester fabric to induce angiogenesis and the formation of new granulation tissue. Body weights were measured weekly; plasma glucose and total cholesterol concentrations, 24-hour urine volume, food consumption, and arterial blood pressure and pulse rate were measured monthly. Rats were killed for the permeability and blood flow studies after 10 to 12 weeks of hypothyroidism.

Radiolabeling of Tracers

Purified, monomer bovine serum albumin (BSA) was iodinated with 1 mCi ^{125}I by the lactoperoxidase method.[21] Before use, ^{125}I-BSA was dialyzed extensively against Krebs Henseleit buffer (pH 7.0, 4°C) and filtered through 0.2-μm Acrodisc filters. ^{57}Co-EDTA was prepared by the method of Bridge et al.[22] Immediately before use, an aliquot was passed through a 4-cm column of Dowex 50 (Sigma Chemicals; St Louis) to remove trace amounts of free $^{57}Co^{2+}$. Rat erythrocytes were labeled with ^{51}Cr as described previously.[23,24] After chromation, erythrocytes were washed with PBS containing 1% BSA and 0.1% dextrose until free ^{51}Cr activity in the supernatant was less than 1% of the ^{51}Cr activity of the packed erythrocytes. The ^{51}Cr-labeled erythrocytes were stored in PBS at a hematocrit of 40% at 4°C until used (within 24 hours), at which time they were again washed with PBS.

Experimental Protocol

Rats were anesthetized intraperitoneally with Inactin (~100 mg/kg body weight), the left femoral vein and both iliac arteries were cannulated with polyethylene tubing (0.58 mm ID) filled with heparinized saline, and the animals were placed on a small rodent respirator for continuous ventilatory support. The right iliac artery cannula was connected via a three-way stopcock to a saline-filled Statham P50 pressure transducer for continuous recording of blood pressure, and the left iliac artery cannula was connected to a withdrawal pump. ^{51}Cr-RBC (~1.0 mL) was injected through the femoral vein cannula, followed five minutes later, at time 0, by 100 μL of ^{125}I-BSA. An arterial blood sample (~200 μL) was obtained from the right iliac artery cannula one and 15 minutes after injection of ^{125}I-BSA. Approximately 20 minutes after injecting ^{125}I-BSA, the chest wall was opened and the heart exposed for injection of microspheres. Twenty-eight minutes after time 0, 100 μL of ^{57}Co-EDTA was injected intravenously and the arterial withdrawal pump was started at 0.34 mL/min. Thirty seconds later, ^{85}Sr-microspheres were injected slowly over a 10- to 15-second interval into the left ventricular chamber of the heart. Ninety seconds later (30 minutes after the injection of ^{125}I-BSA), the heart was quickly excised to stop all blood flow, the withdrawal pump was stopped, and the different tissues were removed as described below.

Eyes were removed, cleaned of all extraneous tissue and rinsed briefly in saline to remove any blood contaminating tissue surfaces. Each eye was cut with fine dissecting scissors into anterior and posterior segments just posterior of the ora serrata, the retina was dissected free from the choroid and attached sclera, and both tissues, along with the anterior segment containing iris, ciliary body, and ciliary process (aqueous fluid was removed and counted separately; the lens was discarded), as well as the whole brain, heart, forelimb skeletal muscle, skin, aorta, kidney, sciatic nerve, and fabric implant (containing the new granulation tissue) were prepared for gamma spectrometry. Tissues from both eyes were pooled before counting. The heart and aorta were rinsed in saline to remove all blood contaminating surfaces.

Data Acquisition and Analysis

Tissue and arterial whole blood and plasma samples were counted for ^{125}I, ^{57}Co, ^{51}Cr, and ^{85}Sr activities in a gamma counter interfaced to a Hewlett-Packard computer system in which the counting data were stored and subsequently corrected for background and spill-over. In the choroid, anterior uvea, brain, heart, skeletal muscle, and kidney, greater than 5,000 counts were obtained for each isotope. Fewer counts were obtained in the retina, sciatic nerve, and aorta (500 to 1000) so that the counting accuracy was reduced.

Two methods were used to assess effects of hypothyroidism on vascular permeation by ^{125}I-albumin. The index of albumin permeation obtained by the first, nongravimetric method is referred to as the tissue-to-blood isotope ratio (TBIR), and has been described in detail elsewhere.[24] This ratio is obtained by dividing the ratio of ^{125}I-BSA/^{51}Cr-RBC counts in the tissue by the corresponding ratio of counts in the arterial blood sample obtained from the withdrawal pump syringe, ie,

$$\text{TBIR I/Cr} = \frac{^{125}\text{I-BSA}/^{51}\text{Cr-RBC(tissue)}}{^{125}\text{I-BSA}/^{51}\text{Cr-RBC(blood)}}$$

A TBIR >1 is indicative of permeation of the vasculature by albumin into the extravascular space. Because microvessel hematocrits are lower than those of large arteries,[25] minimum estimates of TBIR I/Cr will be >1. The TBIR ^{57}Co-EDTA/^{51}Cr-RBC (TBIR Co/Cr) also is determined. ^{57}Co-EDTA is a much smaller molecule than ^{125}I-BSA and it permeates vessel walls much more readily. If only the TBIR I/Cr is determined, and it is low or close to 1, it is impossible to know whether the low value is due to the permeability characteristics of the vessel, the result of a limited extravascular fluid space, or because blood flow was so low that little albumin was available to access the extravascular space. However, a low TBIR I/Cr in the face of a high TBIR Co/Cr indicates that permeation of ^{125}I-BSA was limited by the vessel wall.

The second method provides a more quantitative assessment of ^{125}I-albumin permeation and is obtained by calculating vascular-corrected plasma gram equivalents of ^{125}I-BSA per gram of tissue by use of the formula:

$$\text{Plasma gram equivalents per g tissue} = \frac{\dfrac{\text{CPM } ^{125}\text{I-BSA}}{\text{g tissue}} - \left(\dfrac{\text{CPM } ^{51}\text{Cr}}{\text{g tissue}} \times \dfrac{^{125}\text{I blood}}{^{51}\text{Cr blood}}\right)}{\text{CPM } ^{125}\text{I-BSA per g plasma}}$$

The tissue activity of ^{125}I-BSA was corrected for the vascular content of ^{125}I-BSA by using ^{51}Cr-RBC activity in the tissue as an indicator of blood volume and the ratio of ^{125}I to ^{51}Cr activities in a reference arterial blood sample (tissue hematocrits are assumed to be equivalent to those of large vessels). Although this approach provides a quantitative index of albumin permeation, it is subject to gravimetric errors that may be significant for small tissue samples, ie, retina.

Assessment of Renal Function

Renal clearance of ^{125}I-BSA. Renal plasma clearance of ^{125}I-BSA (mg plasma/kidney/min) was calculated using the formula:

$$\frac{\left[\dfrac{\text{CPM } ^{125}\text{I-BSA}}{\text{kidney}} - \left(\dfrac{\text{CPM } ^{51}\text{Cr}}{\text{kidney}} \times \dfrac{^{125}\text{I blood}}{^{51}\text{Cr blood}}\right) + \dfrac{\frac{1}{2}\,\text{CPM } ^{125}\text{I-BSA}}{\text{urine}}\right]}{\text{time-averaged CPM } ^{125}\text{I-BSA/mg plasma}}$$

The ^{125}I-BSA activity in the entire kidney was corrected for the vascular content of ^{125}I-BSA as described above. One-half the ^{125}I-BSA activity in the bladder urine was added to the vascular-corrected kidney activity (^{125}I-BSA activity in the bladder was assumed to be derived equally from both kidneys), which was then divided by a time-averaged arterial plasma ^{125}I-BSA activity (calculated from the multiple arterial plasma samples obtained during the 30-minute ^{125}I-BSA circulation) and by the tracer circulation time (30 minutes). Results were expressed per whole kidney and per gram of kidney wet weight.

The correction of ^{125}I-BSA tissue activity for the vascular content of tracer by use of tissue ^{51}Cr-RBC activity is slightly inaccurate because tissue microvessel hematocrits are lower than those of large arteries.[25] Because total kidney and urine ^{125}I-BSA activities are measured, an advantage of this method is that total ^{125}I-BSA filtration is determined rather than the difference between filtered and reabsorbed tracer (which is measured in conventional 24-hour urine collections on intact rats). In view of evidence that ^{125}I-BSA reabsorbed by tubules clears the kidney slowly (significant amounts are still demonstrable autoradiographically 60 minutes after injection of radiolabeled albumin[26]), substantial amounts, if not most, of the reabsorbed tracer are still present in the tubular epithelial cells by the end of the 30-minute tracer circulation time. Estimates of renal ^{125}I-BSA clearance also include permeation of the tracer across peritubular capillaries into the interstitial space.

Glomerular filtration rate (GFR). GFR was calculated as renal ^{57}Co-EDTA clearance (g plasma/kidney/min) by substituting ^{57}Co-EDTA activities in place of ^{125}I-BSA in the above equation. The time-averaged arterial plasma ^{57}Co activity was obtained from the withdrawal pump blood and the circulation time was two minutes. In the kidney, errors in vascular tracer correction do not affect ^{57}Co-EDTA as much as ^{125}I-BSA because the vascular content of the former tracer represents only 2% to 3% of the total kidney activity even after a two-minute circulation time.

Urinary albumin excretion. Rats were placed in individual metabolic cages with free access to food and water. After an initial 12-hour adaptation period, total urinary output was collected for 24 hours into jars containing sodium azide and protease inhibitors. Urinary albumin excretion rates were quantified from an aliquot of the urine by radial immunodiffusion[27] using monospecific rabbit antisera to rat albumin as described by Mauer et al.[28] Highly purified monomer rat albumin (Miles Laboratories; Naperville, IL) standards were diluted to ~250, 125, 60, 30, and 15 μg/mL, then assayed for protein content. A 1% agarose gel containing the rabbit antisera to rat albumin was poured into RID plates; 24 hours later, 4-mm diameter wells were punched in the gel and 15-μL samples of albumin standards or urine samples were placed in separate wells. Three different standards were run on each plate. Three days later, ring diameters were recorded and urinary albumin concentrations were calculated from the albumin standard curve. If necessary, urine was concentrated by lyophilization and reconstitution with distilled water.

Measurement of Regional Blood Flows

Regional blood flows (mL/min/g wet weight) were calculated using the formula:

$$\text{Blood flow} = \frac{\dfrac{\text{CPM }^{85}\text{Sr tissue}}{\text{CPM }^{85}\text{Sr blood}} \times \text{pump withdrawal rate}}{\text{g tissue}}$$

where CPM ^{85}Sr blood represents the total ^{85}Sr activity in the arterial sample collected by the withdrawal pump. Results were normalized per gram of wet weight of tissue. Estimates of vascular resistance were obtained as mean arterial pressure/flow rate per unit tissue mass, assuming that mean arterial pressure is equivalent to organ input pressure and represents pressure drop across the organ vasculature.

Measurement of Blood Pressure and Pulse Rate

Mean arterial blood pressure was measured in conscious rats using the tail-cuff method[29] and in anesthetized rats during the blood flow and permeability studies by connecting one iliac artery cannula to a P50 Statham pressure transducer and Gould recorder. Pulse rate was obtained from the blood pressure recordings.

Statistical Analyses

Except for urinary protein data, all data are expressed as mean ± SD; urinary albumin excretion data are expressed as the antilogs of the mean of $\log_e$ transformed data (mean ±1 SD in parentheses). ANOVA of all parameters was performed using the SAS general linear models procedure. Overall differences among groups for each parameter were assessed by the Van Der Waerden test, which makes no assumptions regarding data distribution. Because of the large variance in some parameters, a nonparametric (rank order) Blom transformation of the data was performed before assessment of differences between groups by least square means.

RESULTS

The Van Der Waerden test indicated that final body weight, blood pressure, pulse rate, and plasma cholesterol differed significantly between experimental groups at $P <$ .0002 or better (Table 1). Control rats gained weight continuously, reaching levels 1.9× initial body weights after 10 weeks, whereas body weights of thiouracil-treated and thyroidectomized rats plateaued after 1 to 2 weeks at levels 1.3× and 1.1× initial weights, respectively. Arterial blood pressure was reduced ~28% in conscious thiouracil-treated and 23% in thyroidectomized rats. When measured under Inactin anesthesia, blood pressure was lower in thiouracil-treated than in thyroidectomized rats. Changes in pulse rate tended to parallel blood pressure changes. Plasma glucose values did not differ between experimental groups, whereas plasma cholesterol concentration was increased significantly in the thiouracil-treated and thyroidectomized rats. Food consumption did not differ among the three experimental groups.

Van Der Waerden tests for group differences in ^{125}I-BSA to ^{51}Cr-RBC tissue-to-blood isotope ratios (TBIR I/Cr) were significant at $P < .0001$ for choroid, anterior uvea, kidney, aorta, and skeletal muscle, at $P < .0007$ for retina and skin, and at $P < .004$ for new granulation tissue. Group differences were not statistically significant for brain, sciatic nerve, and heart. As shown in Table 2, TBIR I/Cr values for thiouracil-treated animals were increased significantly v controls in kidney (2.5×), anterior uvea (2.1×), choroid (1.7×), new granulation tissue (1.4×), aorta (1.3×), retina (1.2×), and skin (1.1×), and decreased significantly in forelimb skeletal muscle (0.9×). A similar pattern of TBIR I/Cr changes was observed in thyroidectomized rats, although values were consistently lower than in thiouracil-treated rats. The only exception was the TBIR I/Cr value for the retina, which did not differ significantly from controls. The ^{57}Co-EDTA to ^{51}Cr-RBC tissue-to-blood isotope ratios were much larger than the TBIR I/Cr values (even though the tracer circula-

Table 1. Effects of 2-Thiouracil and Thyroidectomy on Body Weight, Blood Pressure, Pulse Rate, Plasma Glucose and Cholesterol Concentrations, and Food Consumption

	Control	2-Thiouracil	Thyroidectomy
Body weight (g)			
Initial	197 ± 18 (23)	202 ± 18 (28)	189 ± 14 (11)
Final	392 ± 72 (23)	254 ± 34 (28)†	208 ± 21 (11)†§
Blood pressure*			
Conscious	120 ± 11 (17)	86 ± 10 (20)	92 ± 7 (10)†
Anesthetized	119 ± 21 (14)	84 ± 20 (18)†	108 ± 13 (9)§
Pulse rate (beats/min)			
Conscious	277 ± 14 (5)	178 ± 7 (7)‡	—
Anesthetized	293 ± 33 (7)	129 ± 35 (10)†	168 ± 39 (9)‡‖
Plasma glucose (mg/dL)	136 ± 18 (17)	128 ± 14 (20)	130 ± 9 (11)
Plasma cholesterol (mg/dL)	82.2 ± 14.4 (15)	145.2 ± 26.7 (17)†	124.8 ± 27.3 (11)†‖
Food consumption (g/24 h/100 g BW)	3.4 ± 1.1 (17)	3.7 ± 1.2 (28)	3.8 ± 0.8 (11)

Values are given as mean ± SD; number of animals in parentheses.
*Systolic pressure in mmHg.
†$P < .0001$ *v* controls.
‡$P < .0005$ *v* controls.
§$P < .005$ *v* thiouracil-treated.
‖$P < .05$ *v* thiouracil-treated.

tion time was two minutes for ^{57}Co-EDTA *v* 30 minutes for ^{125}I-BSA) and did not differ between experimental groups (data not shown).

The extravascular content of ^{125}I-BSA was measured as plasma gram equivalents of tracer per gram of wet weight of tissue following subtraction of the ^{125}I-BSA activity attributable to the content of the tracer in blood vessels of each tissue (Table 3). This vascular-corrected value varied significantly between different tissues of controls with highest values in the kidney and heart, intermediate values in aorta, anterior uvea, and choroid, and lowest values in retina, skeletal muscle, and skin. Van Der Waerden tests for overall differences among experimental groups were significant at $P <$ .005 for choroid, kidney, and new granulation tissue, and at $P < .05$ for anterior uvea and aorta. Group differences were not significant for retina, brain, sciatic nerve, skin, heart, and skeletal muscle.

The Van Der Waerden test indicated that blood flow differed significantly between experimental groups for the kidney ($P < .0004$), choroid ($P < .009$), anterior uvea ($P < .002$), and brain ($P < .04$), and that vascular resistance differed significantly between groups in the kidney ($P < .0007$), anterior uvea ($P < .005$), and skin ($P < .03$). In both thiouracil-treated and thyroidectomized rats, blood flow was decreased in the choroid and new granulation tissue (significantly different from controls only for thiouracil-

Table 2. Effects of 2-Thiouracil and Thyroidectomy on the ^{125}I-Albumin to ^{51}Cr-RBC Tissue-to-Blood Isotope Ratio (TBIR I/Cr)

	Control	2-Thiouracil	Thyroidectomy
Eye			
Retina	2.02 ± 0.22 (18)	2.41 ± 0.33 (26)*	2.00 ± 0.22 (7)‡
Choroid	2.17 ± 0.18 (18)	3.68 ± 0.35 (25)*	3.52 ± 0.33 (9)*
Anterior uvea	3.01 ± 0.15 (18)	6.46 ± 1.10 (25)*	4.19 ± 0.31 (9)*§
Kidney	3.52 ± 0.31 (18)	8.87 ± 2.76 (25)*	5.06 ± 0.39 (9)*§
Aorta	3.00 ± 0.09 (18)	3.94 ± 0.43 (25)*	3.20 ± 0.21 (9)‖§
Brain	1.78 ± 0.19 (18)	1.73 ± 0.19 (25)	1.73 ± 0.32 (9)
Sciatic nerve	1.86 ± 0.33 (10)	1.91 ± 0.41 (17)	1.71 ± 0.59 (9)
Granulation tissue	1.98 ± 0.11 (5)	2.73 ± 0.28 (7)†	2.65 ± 0.20 (9)‖
Skin	2.15 ± 0.11 (17)	2.44 ± 0.22 (26)*	2.34 ± 0.26 (8)‖
Heart	2.39 ± 0.19 (18)	2.45 ± 0.39 (26)	2.16 ± 0.28 (9)¶
Skeletal muscle	2.24 ± 0.15 (18)	2.03 ± 0.18 (26)*	1.95 ± 0.14 (9)*

Values are given as mean ± SD; number of animals in parentheses. TBIR I/Cr is derived from the formula:

$$\text{TBIR I/Cr} = \frac{^{125}\text{I-BSA}/^{51}\text{Cr-RBC (tissue)}}{^{125}\text{I-BSA}/^{51}\text{Cr-RBC (blood)}}$$

*$P < .0001$ *v* controls.
†$P < .0005$ *v* thiouracil-treated.
‡$P < .005$ *v* thiouracil-treated.
§$P < .0001$ *v* thiouracil-treated.
‖$P < .005$ *v* controls.
¶$P < .05$ *v* thiouracil-treated.

Table 3. Effects of 2-Thiouracil and Thyroidectomy on Vascular-Corrected Plasma Gram Equivalent of ^{125}I-BSA

	Control	2-Thiouracil	Thyroidectomy
Eye			
Retina	0.27 ± 0.05 (4)	0.39 ± 0.13 (6)	0.32 ± 0.15 (9)
Choroid	0.76 ± 0.17 (5)	1.74 ± 0.12 (5)*	1.66 ± 0.37 (9)*
Anterior uvea	0.92 ± 0.18 (4)	2.78 ± 0.77 (6)†	2.82 ± 1.03 (8)*
Kidney	5.14 ± 0.31 (5)	13.85 ± 1.80 (7)‡	11.19 ± 1.78 (8)*‖
Aorta	1.03 ± 0.20 (5)	2.06 ± 1.11 (5)§	1.48 ± 0.55 (8)§
Brain	0.56 ± 0.10 (5)	0.89 ± 0.33 (6)	0.65 ± 0.36 (9)
Sciatic nerve	0.39 ± 0.07 (5)	0.66 ± 0.24 (6)	0.64 ± 0.21 (5)§
Granulation tissue	0.44 ± 0.08 (5)	1.22 ± 0.43 (6)‡	0.97 ± 0.24 (9)†
Skin	0.40 ± 0.16 (5)	0.42 ± 0.15 (7)	0.42 ± 0.13 (9)
Heart	2.35 ± 0.35 (5)	2.57 ± 0.44 (6)	2.36 ± 0.22 (6)
Skeletal muscle	0.20 ± 0.03 (5)	0.29 ± 0.13 (7)	0.19 ± 0.04 (8)

Values are given as mean ± SD; number of animals in parentheses. Corrected gram equivalents are $\times 10^{-2}$; plasma gram equivalents of tracer are obtained by dividing the ^{125}I-BSA CPM per gram of wet weight of tissue (after correction for the vascular content of tracer) by ^{125}I-BSA CPM per gram of plasma.

*$P < .001$ *v* controls.

†$P < .005$ *v* controls.

‡$P < .0001$ *v* controls.

§$P < .05$ *v* controls.

‖$P < .01$ *v* thiouracil-treated.

treated rats), increased in the anterior uvea and brain (significantly different from controls only for thyroidectomized rats), and remained unchanged in the retina, sciatic nerve, skin, and skeletal muscle (Table 4). Renal blood flow also was markedly decreased in both thiouracil-treated and thyroidectomized rats (Table 5). In the kidney and choroid of hypothyroid rats, the percent decrease in flow rate exceeded the decrease in mean arterial pressure, reflecting an increase in vascular resistance, which was statistically significant in the kidney but not in the choroid. In anterior uvea and brain, decreased vascular resistance was manifested by significant increases in blood flow in hypothyroid rats (Table 4) despite the decrease in arterial blood pressure. The decreased vascular resistance in other tissues of hypothyroid *v* control rats was attested by the finding of little or no change in blood flow even though arterial blood pressure was significantly reduced. These changes in vascular resistance did not achieve statistical significance because of the large variances and small numbers of animals.

Van Der Waerden tests for indices of renal function shown in Table 5 were all significant at $P < .005$ or greater except for urinary albumin excretion, which did not differ signifi-

Table 4. Effects of 2-Thiouracil and Thyroidectomy on Regional Blood Flows and Estimates of Vascular Resistances

	Control	2-Thiouracil	Thyroidectomy
Eye			
Retina	0.47 ± 0.04 (5)	0.46 ± 0.05 (6)	0.43 ± 0.05 (8)
	255 ± 39	215 ± 31	253 ± 39
Choroid	1.25 ± 0.09 (4)	0.96 ± 0.05 (5)*	1.06 ± 0.11 (8)†
	88 ± 19	103 ± 8	103 ± 19
Anterior uvea	0.67 ± 0.06 (5)	1.16 ± 0.12 (5)†	1.40 ± 0.15 (8)§‖
	181 ± 37	86 ± 13*	78 ± 12§
Sciatic nerve	0.15 ± 0.04 (5)	0.15 ± 0.03 (6)	0.13 ± 0.02 (8)
	850 ± 310	679 ± 115	872 ± 189‖
Granulation tissue	0.13 ± 0.02 (5)	0.11 ± 0.01 (8)‡	0.12 ± 0.01 (8)
	905 ± 193	865 ± 80	908 ± 159
Skin	0.03 ± 0.01 (5)	0.03 ± 0.01 (5)	0.03 ± 0.01 (8)
	4263 ± 1223	3045 ± 526†	4031 ± 635‖
Brain	0.49 ± 0.06 (5)	0.57 ± 0.11 (6)	0.60 ± 0.04 (8)†
	248 ± 55	192 ± 28‡	180 ± 24‡
Skeletal muscle	0.06 ± 0.01 (5)	0.06 ± 0.01 (5)	0.06 ± 0.01 (8)
	2156 ± 318	1802 ± 264	1905 ± 520

Regional blood flows, (mL/min)/g wet weight; mean ± SD; number of animals in parentheses. Estimates of vascular resistances, mmHg/(mL/min)/g wet weight; mean ± SD.

*$P < .005$ *v* controls.

†$P < .01$ *v* controls.

‡$P < .05$ *v* controls.

§$P < .0002$ *v* controls.

‖$P < .02$ *v* thiouracil-treated.

cantly between experimental groups (Table 5). The kidneys were smaller (whether expressed as absolute kidney weight or as a percentage of final body weight) in thiouracil-treated and in thyroidectomized rats as compared with controls. Renal ^{125}I-BSA clearance, which averaged 1.6 mg plasma/min/g kidney in control rats, was increased threefold in thiouracil-treated rats, and twofold in thyroidectomized rats. When albumin clearance was expressed per whole kidney (rather than normalized per gram of kidney wet weight), it was still increased 1.6× controls in thiouracil-treated rats, but did not differ from controls in thyroidectomized rats (despite the fact that kidney weight was only 43% of controls). GFR (per gram of kidney) was decreased significantly in thiouracil-treated and thyroidectomized rats as compared with controls. The decreases were even greater if GFR was expressed per whole kidney. Albumin fractional clearance (^{125}I-BSA clearance/GFR) was increased 4.5× in thiouracil-treated and 3.2× in thyroidectomized rats *v* controls. While urine volumes (mL/24 h/kidney) were increased 2.2× in thiouracil-treated rats and 2.1× in thyroidectomized animals, urinary albumin excretion rates were unaffected.

DISCUSSION

These observations confirm and extend previous evidence that vascular hemodynamics and endothelial cell barrier function are modulated by thyroid function in multiple tissues. Although plasma levels of thyroid hormones were not measured, the absence of growth, hypercholesterolemia, increased thyroid weight in thiouracil-treated rats (data not shown), and decreased blood pressure, pulse rate, and GFR attest to the hypothyroid state of these rats.

While arterial blood pressure was decreased by approximately 25% in hypothyroid rats and there was a substantial (35% to 55%) reduction in pulse rate, regional blood flows remained unchanged or varied only slightly in most tissues. The only exceptions were a marked increase in blood flow in the anterior uvea and a substantial decrease in the choroid and in the kidney. These observations are consistent with a decrease in regional vascular resistance for most tissues examined except the kidney and choroid. This evidence of a decrease in vascular resistance is consistent with the generalized decrease in peripheral vascular resistance in rats with diabetes of comparable duration. It is of interest, however, that the decrease in vascular resistance in diabetic rats is associated with significant increases in blood flow in many tissues, while systemic blood pressure does not change.[30]

The finding that albumin permeation was increased in many different tissues in both thiouracil-treated and thyroidectomized rats is consistent with an important role of thyroid hormones in modulating endothelial cell barrier functional integrity directly and/or an effect of thyroid hormone on tissue metabolism that would indirectly modulate vascular permeability. The observation that albumin permeation tended to be higher in many tissues of thiouracil-treated *v* thyroidectomized rats (significantly higher in retina, anterior uvea, kidney, and aorta) suggests that thiouracil exerts an additional effect either on vascular endothelium directly or on tissue metabolism. The nature of this putative effect remains to be elucidated.

The tissues in which vascular permeability is increased by

Table 5. Effects of 2-Thiouracil and Thyroidectomy on Kidney Weight, Renal Blood Flow, and Filtration Function

	Control	2-Thiouracil	Thyroidectomy
Kidney weight			
g	1.27 ± 0.21 (5)	0.70 ± 0.08 (7)†	0.55 ± 0.06 (9)§¶
g/100 g BW	0.33 ± 0.01 (5)	0.28 ± 0.02 (6)†	0.27 ± 0.02 (9)§
Blood flow			
(mL/min)/g kidney	4.19 ± 0.03 (5)	2.63 ± 0.25 (7)†	2.62 ± 0.34 (8)‖
(mL/min)/kidney	5.31 ± 0.91 (5)	1.84 ± 0.27 (7)†	1.40 ± 0.18 (8)§¶
Vascular resistance [mmHg/(mL/min)/g kidney]	29 ± 4 (5)	37 ± 5 (7)‡	42 ± 9 (8)†
Albumin clearance			
mg plasma/min/g kidney	1.6 ± 0.2 (5)	4.8 ± 0.6 (6)§	3.4 ± 0.7 (9)‖¶
mg plasma/min/kidney	2.0 ± 0.5 (5)	3.2 ± 0.3 (6)†	1.9 ± 0.4 (9)#
GFR			
g plasma/min/g kidney	0.667 ± 0.033 (5)	0.435 ± 0.057 (7)‖	0.447 ± 0.047 (9)†
g plasma/min/kidney	0.851 ± 0.175 (5)	0.301 ± 0.032 (7)†	0.244 ± 0.011 (9)§#
Fractional clearance (Albumin/GFR)	0.0024 ± 0.0002 (5)	0.0109 ± 0.0008 (6)§	0.0076 ± 0.0015 (9)§#
Urine output (mL/24 h/kidney)	3.45 ± 1.56 (17)	7.73 ± 3.20 (24)§	7.10 ± 2.84 (10)†
Urinary albumin* (mg/24 h/100 g BW)	0.135 (0.057-0.321) (6)	0.158 (0.083-0.304) (8)	0.112 (0.056-0.222) (10)

Values are given as mean ± SD; number of rats evaluated are in parentheses.

*Data are expressed as antilog of mean of $\log_e$ transformed data (mean ± 1 SD).

†$P < .005$ *v* controls.

‡$P < .05$ *v* controls.

§$P < .0001$ *v* controls.

‖$P < .0005$ *v* controls.

¶$P < .001$ *v* thiouracil-treated.

#$P < .0002$ *v* thiouracil-treated.

the hypothyroid state correspond, in general, to tissues affected by diabetes.[18,19] In particular, increased vascular permeation by ^{125}I-BSA in hypothyroid rats was greatest in the kidney, in the different vasculatures of the eye, and in the aorta, the sites of important vascular complications in diabetes. ^{125}I-BSA permeation, expressed as vascular-corrected plasma gram equivalents of tracer per gram of wet weight of tissue, also was increased in the sciatic nerve of hypothyroid rats (significantly different from controls only in thyroidectomized rats), which is similar to that observed in diabetic animals.[18] The increased ^{125}I-BSA permeation in the skin of hypothyroid rats (*v* controls), however, is in contrast to diabetic rats, in which changes in albumin permeation in the skin tend to be minimal or not detectable.

The similarity between the vascular permeability changes in these hypothyroid rats and corresponding changes in diabetic rats raises the possibility that a common mechanism may contribute to loss of endothelial cell barrier function in both conditions and that thyroid hormones may modulate the development and/or severity of diabetic vascular disease. The possibility that the common mechanism might be a reduction in vascular Na^+/K^+-ATPase levels/activity is suggested by evidence that tissue levels of this enzyme are markedly reduced by hypothyroidism[31-34] as well as by diabetes (peripheral nerve,[35,36] skeletal and cardiac muscle,[36] glomeruli isolated from diabetic rats[37]), and by in vitro incubation of rat aorta with elevated glucose levels.[38] Although no studies have linked a decrease in endothelial cell Na^+/K^+-ATPase activity to loss of barrier functional integrity, numerous studies have demonstrated that short-term intravascular infusion of ouabain, an inhibitor of Na^+/K^+-ATPase, is associated with an immediate increase in vascular resistance in a variety of tissues.[39-42] We are not aware of studies on the impact of long-term impairment of Na^+/K^+-ATPase activity by ouabain (ie, comparable to the duration of hypothyroidism in the present experiments) on vascular resistance. It is of interest, however, that whereas vascular resistance is decreased in poorly controlled diabetes of short duration as well as in most tissues in hypothyroid animals in the present experiments, resistance is increased in many tissues of diabetics with disease of long duration and with vascular complications.

Renal ^{125}I-BSA clearance (expressed per gram of kidney) and albumin fractional clearance were increased in the kidney of hypothyroid rats, although urinary albumin excretion rates did not differ from controls. This discordance between ^{125}I-BSA clearance and excretion of endogenous albumin may reflect the fact that the former measurement reflects filtration rather than excretion of protein. Thus, increased tubular reabsorption could compensate for increased glomerular filtration of albumin. In addition, we do not know how much of the tracer clearance represents glomerular filtration *v* albumin permeation of peritubular capillaries into the interstitium. The increase in renal ^{125}I-BSA clearance in these hypothyroid rats must be viewed as evidence of an increase in overall renal filtration of radiolabeled tracer. It is of great interest, however, that ^{125}I-BSA renal filtration was increased in the face of marked decreases in renal blood flow and GFR in hypothyroid rats. In general, an increase in tissue blood flow would increase the rate of vascular permeation of plasma constituents that normally permeate the microvasculature by transmitting arterial pressure further downstream into terminal arterioles and capillaries (without any change in the permeability characteristics of the vessel wall per se, ie, in the number and/or size of vascular pores). Therefore, the discordance between blood flow and albumin permeation changes in the kidney (and choroid) suggest that an additional mechanism(s) independent of hemodynamic changes contributes to the loss of barrier functional integrity of the capillary wall in hypothyroid rats.

It should be recognized that the TBIR I/Cr values for aorta, which probably include albumin permeation across small adventitial vessels as well as across aortic endothelium, are expressed in terms of the ^{51}Cr-RBC contained in the former vessels only. Although the relative contribution of each route of albumin permeation to the increased albumin content of aorta in hypothyroid rats was not assessed, it is of interest that the increased albumin content occurred without any change in the ^{51}Cr-RBC content of the aorta (data not shown) and in the presence of a significant decrease in arterial blood pressure. In previous studies, TBIR I/Cr values for adventitial connective tissue scraped from the aorta were much lower than those of the inner wall of the aorta.[18] In view of these observations, it is likely that the increase in TBIR I/Cr values for aortas of hypothyroid rats reflects increased albumin permeation across the intimal surface. The observation that albumin permeation is increased in the aorta of hypothyroid rats raises the possibility that permeation of atherogenic lipoproteins into the intima and media of large arteries also may be increased, and suggests an explanation for the accelerated rate of development of atherosclerosis in hypothyroid patients.

In conclusion, these observations indicate that vascular hemodynamics and endothelial cell barrier function in many different tissues are markedly altered in hypothyroid rats, and the correspondence between hypothyroid-induced changes in vascular permeability, in general, with those in diabetic rats suggests that a common pathogenetic mechanism, possibly impaired Na^+/K^+-ATPase activity, may contribute to impaired vascular functional integrity in both conditions.

REFERENCES

1. Benforado JM, Wiggins LL: Contractility, heart rate, and response to norepinephrine of isolated rat myocardium following ^{131}I-induced hypothyroidism. J Pharmacol Exp Ther 147:70-75, 1965

2. McDonough KH, Chen V, Spitzer JJ: Effect of altered thyroid status on in vitro cardiac performance in rats. Am J Physiol 252 (Heart Circ Physiol 21):H788-H795, 1987

3. Buccino RA, Spann JR Jr, Pool PE, et al: Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. J Clin Invest 46:1669-1682, 1967

4. Whitsett JA, Pollinger J, Matz S: β-adrenergic receptors and catecholamine sensitive adenylate cyclase in developing rat ventricular myocardium: Effect of thyroid status. Pediatr Res 16:463-469, 1982

5. Freedberg AS, Hamolsky MW: Effects of thyroid hormones on certain nonendocrine organ systems, in Greer MA, Solomon DH (eds): Handbook of Physiology, Endocrinology, vol 3. Baltimore, Waverly, 1974, pp 435-468

6. David S, Nathaniel EJH: Development of brain capillaries in euthyroid and hypothyroid rats. Exp Neurol 73:243-253, 1981

7. Zondek H, Michael M, Kaatz A: The capillaries in myxedema. Am J Med Sci 202:435-440, 1941

8. Riedel W: Effects of propylthiouracil, and of bacterial endotoxin (LPS), on thyroid hormones, respiratory rate, cutaneous and renal blood flow in rabbits. Pflugers Arch 399:11-17, 1983

9. Wennlund A, Linde B: Influence of hyper- and hypothyroidism on subcutaneous adipose tissue blood flow in man. J Clin Endocrinol Metab 59:258-262, 1984

10. Gillum DM, Falk SA, Hammond WS, et al: Glomerular dynamics in the hypothyroid rat and the role of the renin-angiotensin system. Am J Physiol 253 (Renal Fluid Electrolyte Physiol 22):F170-F179, 1987

11. Lange K: Capillary permeability in myxedema. Am J Med Sci 208:5-15, 1944

12. McGavack TH, Schwimmer D: Problems in the treatment of cardiac failure in myxedema. J Clin Endocrinol 4:427-439, 1944

13. Parving H-H, Hansen JM, Nielsen SL, et al: Mechanisms of edema formation in myxedema-increased protein extravasation and relatively slow lymphatic drainage. N Engl J Med 301:460-465, 1979

14. Wheatley T, Edwards OM: Mild hypothyroidism and oedema: Evidence for increased capillary permeability to protein. Clin Endocrinol 18:627-635, 1983

15. DiScala VA, Salomon M, Grishman E, et al: Renal structure in myxedema. Arch Pathol 84:474-485, 1967

16. McFadden PM, Berenson GS: Basement membrane changes of myocardial capillaries in myxedema. Circulation 42:52, 1970 (abstr, suppl 3)

17. Clements RS, Kassira W, Garcia AR, et al: Thyroid status and motor nerve function in experimental diabetes. Diabetes 35:13, 1986 (abstr)

18. Williamson JR, Chang K, Tilton RG, et al: Increased vascular permeability in spontaneously diabetic BB/W rats and in rats with mild versus severe streptozotocin-induced diabetes. Prevention by aldose reductase inhibitors and castration. Diabetes 36:813-821, 1987

19. Williamson JR, Tilton RG, Chang K, et al: Microvascular changes in diabetes: Experimental aspects, in Alberti KGMM, Krall LP (eds): The Diabetes Annual, vol 4. Amsterdam, Elsevier, 1988, pp 394-410

20. Parving HH: Increased microvascular permeability to plasma proteins in short- and long-term juvenile diabetics. Diabetes 25:884-889, 1976 (suppl 2)

21. Krohn KA, Welch MJ: Studies of radioiodinated fibrinogen. I: Lactoperoxidase iodination of fibrinogen and model compounds. Int J Appl Radiat Isot 25:315-323, 1974

22. Bridge JHE, Bersohn MM, Gonzalez F, et al: Synthesis and use of radiocobaltic EDTA as an extracellular marker in rabbit heart. Am J Physiol 242:671-676, 1982

23. Donahue DM, Motulsky AG, Giblett ER, et al: The use of chromium as a red cell tag. Br J Haematol 1:249-263, 1955

24. Kilzer P, Chang K, Marvel J, et al: Tissue differences in vascular permeability changes induced by histamine. Microvasc Res 30:270-285, 1985

25. Gibson JG, Seligman AM, Peacock WC, et al: The distribution of red cells and plasma in large and minute vessels of the normal dog, determined by radioactive isotopes of iron and iodine. J Clin Invest 25:848-857, 1946

26. Maunsbach AB: Absorption of ^{125}I-labeled homologous albumin by rat kidney proximal tubule cells. J Ultrastruct Res 15:197-241, 1966

27. Mancini G, Carbonara AO, Heremans JF: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235-254, 1965

28. Mauer SM, Brown DM, Matas AJ, et al: Effects of pancreatic islet transplantation on the increased urinary albumin excretion rates in intact and uninephrectomized rats with diabetes mellitus. Diabetes 27:959-964, 1978

29. Pfeffer JM, Pfeffer MA, Frohlich ED: Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. J Lab Clin Med 78:957-962, 1971

30. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int 19:410-415, 1971

31. Blumenthal SJ, McConnaughey MM, Iams SG: Hypothyroidism lowers blood pressure, adenylate cyclase and Na^+, K^+- and K^+, Ca^{++}-ATPase activities in normotensive and spontaneously hypertensive rats. Experientia 38:707-709, 1982

32. McConnaughey MM, Jones LR, Watanabe AM, et al: Thyroxine and propylthiouracil effects on alpha- and beta-adrenergic receptor number, ATPase activities, and sialic acid content of rat cardiac membrane vesicles. J Cardiovasc Pharmacol 1:609-623, 1979

33. Sharma VK, Banerjee SP: Specific [^{3}H] ouabain binding to rat heart and skeletal muscle: Effects of thyroidectomy. Mol Pharmacol 14:122-129, 1978

34. Curfman GD, Crowley TJ, Smith TW: Thyroid-induced alterations in myocardial sodium- and potassium-activated adenosine triphosphatase, monovalent cation active transport, and cardiac glycoside binding. J Clin Invest 59:586-590, 1977

35. Greene DA, Lattimer S, Ulbrecht J, et al: Glucose-induced alterations in nerve metabolism: Current perspective on the pathogenesis of diabetic neuropathy and future directions for research and therapy. Diabetes Care 8:290-299, 1985

36. Kjeldsen K, Braendgaard H, Sidenius P, et al: Diabetes decreases Na^+-K^+ pump concentration in skeletal muscles, heart ventricular muscle, and peripheral nerves of rat. Diabetes 36:842-848, 1987

37. Cohen MP, Dasmahapatra A, Shapiro E: Reduced glomerular sodium/potassium adenosine triphosphatase activity in acute streptozocin diabetes and its prevention by oral sorbinil. Diabetes 34:1071-1074, 1985

38. Winegrad AI: Dose a common mechanism induce the diverse complications of diabetes? Diabetes 36:396-406, 1987

39. Harrison LA, Blaschke J, Phillips RS, et al: Effects of ouabain on the splanchnic circulation. J Pharmacol Exp Ther 169:321-327, 1969

40. Treat E, Ulano HB, Jacobson ED: Effects of intra-arterial ouabain on mesenteric and carotid hemodynamics. J Pharmacol Exp Ther 179:144-148, 1971

41. DeMots H, Rahimtoola SH, McAnulty JH, et al: Effects of ouabain on coronary and systemic vascular resistance and myocardial oxygen consumption in patients without heart failure. Am J Cardiol 41:88-93, 1978

42. Robinson BF, Phillips RJW, Wilson PN, et al: Effect of local infusion of ouabain on human forearm vascular resistance to potassium, verapamil, and sodium nitroprusside. J Hypertension 1:165-169, 1983