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Enhanced Binding of 3,5,3′-L-Triiodothyronine by Rat Kidney Microsomes After Acclimation to Cold[1,2] (38022)

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The thyroid hormones, triiodothyronine and thyroxine, are distributed and apparently metabolized differentially in tissues in the rat (1, 2). Triiodothyronine is more diffusely distributed in rat tissues, while thyroxine is concentrated largely in plasma, liver and kidney (1, 2). Extrahepatic tissues appear to predominate in the overall deiodination of triiodothyronine, whereas, the liver is considered the principal site of deiodination of thyroxine (2, 3). In a previous series of investigations, we have demonstrated that increased deiodination of triiodothyronine and thyroxine in the rat following cold adaptation was associated with increased binding of these hormones by diverse tissues, suggesting the induction of hormonal deiodinative sites by the cold (4). Increased hormonal binding by liver was due to stimulation of binding by microsomes. Enhanced hepatic microsomal binding was accompanied by generalized stimulation of the smooth endoplasmic reticulum, the enzyme-rich agranular component of microsomes. The present studies were performed to extend our observations regarding the stimulation of the binding of triiodothyronine by tissues after cold exposure utilizing the operational definition of whole-tissue binding proposed by Oppenheimer (2), and to identify the subcellular basis of the cold-enhanced binding of triiodothyronine in extrahepatic tissues as exemplified by kidney.

Materials and Methods. Adult male Sprague Dawley rats[2] weighing 300 g were divided into weight-matched groups. The experimental groups was acclimated to 4°C for a minimum period of three months. The control group was maintained at 27°C during the same period. All animals were fed Purina Lab Chow and supplied drinking water *ad lib.* 3,5,3′ L-triiodothyronine (T_3), labelled in the outer ring with either ^{131}I or ^{125}I was purchased from Abbott Laboratories, North Chicago, Illinois. T_3 was diluted in a 1% human serum albumin solution and dialyzed in buffer for 2 hr to remove radioiodide (2). Less than 0.3% of the dose was ^{125}I-iodide as measured by electrophoresis (5). Weighed aliquots of the hormonal preparation containing 1.25 μCi T_3 were injected into the rat tail vein under light ether anesthesia. Thirty-five minutes postinjection animals were exsanguinated through the abdominal aorta. Tissues were rapidly excised and weighed aliquots digested in concentrated potassium hydroxide. Plasma aliquots were precipitated with trichloroacetic acid (TCA) following the addition of outdated bank

[1] The research reported in this paper was conducted by personnel of the Environmental Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks AFB, Texas. Further reproduction is authorized to satisfy the needs of the U. S. Goverment.

[2] The animals involved in this study were maintained and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences—National Research Council.

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plasma. All specimens were counted in an Autogamma Spectrometer with a dilution of the injected dose and expressed as a percentage of the dose. Tissue-plasma concentration ratios were computed as the quotient of tissue activity (% dose/g) and plasma activity (% dose/ml). The binding of T_3 by plasma proteins (b_P) was assessed by equilibrium dialysis (6). b_P is determined by the expression (1-DF)/DF × 150, where DF is the dialyzable fraction of tracer hormone. The dialyzable fraction is proportional to the percent free hormone in undiluted plasma (6). Tissue binding was calculated as the product of the tissue-plasma concentration gradient and plasma binding: T/P × b_P (2). Whole kidneys were homogenized in 0.32 molar sucrose and subcellular fractions isolated after differential centrifugation according to the method of Schwartz *et al.* (7). Particulate subcellular fractions were dissolved in 0.75% desoxycholate prior to assay of radioactivity and protein concentrations (7).

Results. Table I. Acclimation to cold was associated with diminished body weight; adapted animals weighed 74.2 percent of controls. Despite the lesser body weight of exposed animals, no significant difference was noted in the liver weight of these animals compared to controls. The mean kidney weight of the experimental animals exceeded that of the controls by 19.3%. Despite the smaller body weights, the concentration of TCA precipitable radioactivity in plasma 35 min postinjection of labelled T_3 was slightly, though not significantly, diminished (10%) in adapted animals. Conversely, the concentrations of radioactivity in tissues of these animals were increased. An increase of 10% in the hepatic concentration of radioactivity was statistically significant. Increases of lesser magnitude in the concentrations of radioactivity in kidney and skeletal muscle were not significant. Nevertheless tissue-plasma concentration gradients of radioactive T_3 in liver, kidney, and muscle were all significantly increased in cold-exposed ani-

TABLE I. Effect of Cold Acclimation on the Binding of Triiodothyronine by Tissues.[a]

Parameter	Control	Acclimated	*P*
Body Weight (g)	694 ± 20.5	515 ± 8.65	<.001
Liver Weight (g)	17.1 ± 0.46	17.0 ± 0.46	NS
Kidney Weight (g)	1.66 ± 0.03	1.98 ± 0.03	<.001
Radioactivity Concentrations			
Plasma (% dose/ml)	0.30 ± 0.013	0.27 ± 0.010	NS
Liver (% dose/g)	1.61 ± 0.041	1.77 ± 0.042	<.01
Kidney (% dose/g)	2.43 ± 0.06	2.52 ± 0.51	NS
Muscle (% dose/g)	0.092 ± 0.002	0.096 ± 0.003	NS
Tissue:Plasma Radioactivity Concentration Ratios			
L/P	5.63 ± 0.234	6.76 ± 0.220	<.001
K/P	8.48 ± 0.358	9.49 ± 0.303	<.05
M/P	0.320 ± 0.012	0.367 ± 0.015	<.02
Plasma Binding			
b_P	117 ± 8.03	127 ± 7.78	NS
Tissue Binding			
b_L	627 ± 36.9	855 ± 59.6	<.005
b_K	937 ± 47.6	1185 ± 73.6	<.01
b_M	36.3 ± 2.30	45.6 ± 2.92	<.02

[a] Tissue binding studies were performed in 29 control and 29 cold-acclimated animals. Radioactivity concentrations and tissue-plasma concentration gradients were measured 35 min postinjection of tracer labelled T_3. Tissue-plasma hormone ratios of liver, kidney, and muscle denoted L/P, K/P, and M/P respectively. Hormonal binding by plasma, liver, kidney, and muscle: b_P, b_L, b_K, and b_M. Mean ± SE. Statistical comparison by Student *t*-test. NS: $P > .05$.

mals. Similarly, tissue binding of this hormone calculated after adjustment of tissue-plasma ratios for differential plasma hormonal binding was uniformly stimulated by cold: increases were observed in the binding of T_3 by liver (36.4%), kidney (26.4%) and muscle (25.6%). Table II. The subcellular distribution of injected ^{125}I–T_3 in kidney was examined 35 min postinjection. Following whole-kidney homogenization and differential centrifugation in 0.32M sucrose, nuclear, mitochondrial, microsomal and cytosol fractions were isolated. A decrease of 26.1% was observed in the nuclear radioactivity in the adapted animals. A decrease of only 9.3% in the nuclear protein concentration was observed, suggesting that the major portion of the decrease in nuclear T_3 activity could not be accounted for by possible differential cross-contamination by the mitochondrial fractions in experimental animals. No significant differences in the percent ^{125}I–T_3 or protein associated with mitochondrial or cytosol fractions were noted. A 20% increase in the microsomal binding of tracer T_3 was noted in acclimated animals compared to controls ($P = .025$). This increase in hormonal binding by microsomes was paralleled by a significant increase (12%) in the protein content of the microsomes ($P < .005$).

Discussion. We have demonstrated in previous studies that cold acclimation in the rat is associated with an increased deiodinative clearance of T_3 (4). Increased deiodination of T_3 *in vivo* was accompanied by expansion of the total distribution space of this iodothyronine (4). Since the increased hepatic distribution space of T_3 was relatively small, the increased total space of this hormone was presumably related to an increased extrahepatic tissue space. The demonstration of diffuse stimulation of extrahepatic tissue binding of T_3 following acclimation served to explain the enlarged extrahepatic space of the hormone. The observation of stimulated tissue binding of T_3 after acclimation was consistent with the earlier finding of Albright, Heninger and Larson of elevated concentrations of T_3 in tissues after cold exposure utilizing isotopic equilibration with ingested radioiodide (8). The increased tissue binding of T_3 further suggested the possibility of induction of deiodinative sites in tissues by the cold stimulus. We have observed that the cold stimulated increased whole-tissue binding in liver is related to increased hormonal binding by hepatic microsomes, the subcellular locus of degradative enzymes which deiodinate or conjugate iodothyronines prior to biliary excretion (9–11). Since both the deiodinative and the biliary disposition are increased following adaptation (4, 12–14), increased hepatic microsomal binding of T_3 may reflect induction of microsomal constituents involved in both degradative pathways. In contrast, the detection of increased kidney microsomal

TABLE II. Subcellular Distribution of Injected ^{125}I-L-Triiodothyronine and Partition of Subcellular Proteins in Kidney Following Cold Acclimation.[a]

	Control	Acclimated	*P*
% ^{125}I–T_3 in			
Nuclei	15.3 ± 0.37	11.3 ± 0.59	<.001
Mitochondria	4.70 ± 0.27	5.18 ± 0.33	NS
Microsomes	18.0 ± 1.08	21.6 ± 1.18	=.025
Cytosol	62.0 ± 1.14	61.9 ± 1.13	NS
% Protein in			
Nuclei	28.1 ± 0.56	25.5 ± 0.80	<.02
Mitochondria	13.4 ± 0.50	14.1 ± 0.14	NS
Microsomes	20.7 ± 0.41	23.2 ± 0.66	<.005
Cytosol	37.7 ± 0.33	37.1 ± 0.29	NS

[a] Whole-kidney fractionation performed 35 min after intravenous injection of ^{125}I–T_3 in 17 control and 17 cold-acclimated animals. Mean ± SE.

binding of T_3, observed in the present study, is presumably related to induction of deiodinative sites only.

Oppenheimer *et al.* have noted that hepatic microsomal inducers such as phenobarbital stimulate the metabolism of thyroid hormones differentially, resulting in increased deiodinative and fecal clearances of thyroxine, but in an increased fecal disposal only in the case of triiodothyronine (2). Furthermore, phenobarbital induction did not augment the total distribution space of T_3 or the binding of this hormone by extrahepatic tissues, suggesting that the stimulation of hormonal metabolism by this pharmacological agent was mediated exclusively by the liver (2). In the studies of cold stimulation of the metabolism of T_3, in contrast, the deiodinative clearances and binding of T_3 by extrahepatic tissues are increased. The relationship between hormonal binding of T_3 by tissues and hormonal deiodination observed following both pharmacological and cold induction of metabolism provides evidence of the importance of extrahepatic tissues in the overall deiodination of T_3. Furthermore, the present studies of the effect of cold on the subcellular binding of T_3 in kidney demonstrate that augmented binding by the microsome, the suggested site of hormonal deiodination, is the basis of the increased whole-tissue binding of T_3 in extrahepatic tissues.

The observation of hepatic and kidney microsomal induction following cold adaptation suggests that the cold stimulus effects generalized stimulation of microsomal enzyme systems in tissues. The microsomal constituents involved in the deiodination of triiodothyronine and thyroxine presumably participate in this generalized cold-mediated microsomal induction, resulting in increased deiodination of these hormones after adaptation.

Summary. Whole-tissue binding of triiodothyronine was augmented in liver, kidney and skeletal muscle following adaptation of the rat to cold. The basis of the increased kidney binding of this hormone was increased binding to microsomes, the putative subcellular locus of hormonal deiodination. Cold induction of deiodinative sites in tissues is the suggested etiology of the increased deiodination of triiodothyronine *in vivo* after cold acclimation observed in previous studies.

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