Effect of Experimentally Altered Thyroid States on the Uptake of Monovalent Cations in Liver and Muscle of Rats

G. C. Marshall, W. V. Kessler, and R. J. Vetter
Bionucleonics Department, Purdue University,
West Lafayette, Indiana 47907

Introduction

Altered thyroid states, hypothyroidism and hyperthyroidism, have been shown to produce modifications in bodily functions which include changes in protein synthesis and mitochondrial activity (3, 13) as well as changes in the uptake of metals and minerals (5-7, 10, 11, 16). The mechanism by which the changed uptake of metals occurs is not known. In fact, little is known about the action of thyroxine at the cellular level, although much research has been done in this area (14, 15).

This study was performed to evaluate the effects of experimentally altered thyroid states on the uptake of the monovalent cations Na^+, Rb^+, and Cs^+ in the liver and muscle of rats. These three cations all belong to the alkali metal group and have a large range in size.

Materials and Methods

Male Sprague-Dawley descendent rats (Murphy Breeding Laboratories, Inc., Plainfield, Indiana) with a weight range of 180-200 g were used. They were housed in individual cages by random assignment and were given free access to food and water. A 2-day period of acclimation was allowed before the experiment was begun.

^{22}Na, ^{86}Rb, and ^{137}Cs were used as radiotracers for the cations. Injection solutions were made to contain the same number of atoms for each cation. The Na solution was made to contain 10 μCi of $^{22}NaCl$ and 0.18 mg of carrier NaCl per milliliter of water. The volume was adjusted for each rat to give 0.36 mg NaCl/kg. For Rb and Cs, the quantities were 0.36 mg of RbCl and 32 μCi of $^{86}RbCl$ and 0.50 mg of CsCl and 16 μCi of $^{137}CsCl$ per milliliter of water, respectively. The volumes injected were adjusted to give 0.72 mg RbCl/kg and 1.00 mg CsCl/kg. All radionuclides were determined to be radionuclidically pure. An aliquot of each solution was prepared for a standard so that the total activity injected into each rat could be determined.

Propylthiouracil (PTU) was used to induce hypothyroidism. The dosing solution contained 2 mg PTU/0.5 ml/rat and was made fresh daily. L-Thyroxine was used to induce a state of hyperthyroidism. The dosing solution contained 20 μg L-thyroxine sodium pentahydrate/0.5 ml/rat and was made fresh daily. Euthyroidism was maintained with 0.5 ml of normal saline per rat daily. The drug pretreatments were continued for 15 days and all doses were given by ip injection. In a preliminary experiment with a separate group of rats, a radioimmunoassay kit was used to measure serum thyroxine levels after the 15-day pretreatment. The levels found for each treatment group were similar to those reported in the literature (10).

The studies on each cation were run separately. A total of 162 rats was used with 54 rats being run with each cation. The 54 rats for each cation were randomly assigned to one of three treatment groups, hypothyroid, euthyroid, and hyperthyroid. The 15-day drug pretreatment was begun to attain the desired thyroid state. The daily drug treatment was then continued until each rat was sacrificed since it has been shown that thyroxine levels in the blood decrease during the 24-hr period between thyroxine injections (4). On the 16th day, each rat was given either ^{22}Na, ^{86}Rb, or ^{137}Cs by ip injection. The rats were randomly assigned to sacrifice times with six rats from each drug treatment group being sacrificed at 1, 2, or 5 days after injection with the radionuclide.

The liver and an aliquot of the muscle were the samples taken from each rat. The muscle sample was taken from tissue surrounding the right femur. The samples were wiped to remove external blood and placed in preweighed, prelabeled plastic scintillation tubes so that tissue weights could be determined. The liver was sectioned, due to its large size, and placed in several tubes at a predetermined height which was approximately 1.25 cm below the top of the well of the counting crystal. The geometry error was minimized by placing all samples no higher than a predetermined height in the counting tubes. The tissue samples as well as the standards for the dosing solutions were counted in a small well-type NaI(Tl) scintillation counter. The counting error did not exceed 3%.

Results and Discussion

The percentage of the total activity injected per organ (P) (liver) and the percentage of the total activity injected per gram (A) (liver and muscle) were calculated by use of the activities in the tissues and the activities in the aliquots of the solutions injected. The P and A values for each cation were analyzed separately. The data were transformed when needed to achieve homogeneity of variance by the Foster-Burr test. A two-way analysis of variance was then run to test for treatment and time effects which were both found to be significant ($P < 0.05$) (1).

Since treatment by time interactions were significant, the data could not be pooled across time to analyze for treatment effects nor across treatments to analyze for time effects. Instead, the treatment-time combinations were regarded as nine separate treatments, with these treatments being analyzed with a one-way analysis of variance and a Newman-Keuls Sequential Range Test to determine which combination means were significantly different. The ranking of these treatment-time combinations were hard to interpret from a biological standpoint. It was therefore desirable to translate them back to their original treatment and time framework in which the treatment rankings were examined for each of the three sacrifice times, and the sacrifice time rankings were examined for each of the three treatments. The results are shown in Tables 1 and 2.

For liver, ^{86}Rb and ^{137}Cs were handled in a similar manner with the hypothyroid treatment causing the highest uptake of the radio-

TABLE 1. *Newman-Keuls multiple comparison of the treatment means within each time for liver and muscle.*

Organ	Time (days)	% Activity/organ (P)			% Activity/gram of tissue (A)		
		^{22}Na					
Liver	1	P[a]	S	T	P	S	T
		1.54	1.80	1.87	0.14	0.15	0.16
	2	P	S	T	S	P	T
		1.28	1.36	1.47	0.12	0.13	0.13
	5	S	T	P	S	T	P
		0.91	0.91	0.91	0.08	0.09	0.10
Muscle	1				S	T	P
					0.12	0.13	0.15
	2				T	S	P
					0.11	0.11	0.13
	5				S	T	P
					0.07	0.07	0.10
		^{86}Rb					
Liver	1	T	S	P	T	S	P
		1.39	1.59	1.79	0.13	0.14	0.17
	2	T	S	P	T	S	P
		1.19	1.37	1.57	0.11	0.13	0.16
	5	T	S	P	S	T	P
		0.90	0.98	1.08	0.08	0.10	0.11
Muscle	1				T	S	P
					0.11	0.11	0.11
	2				T	S	P
					0.10	0.10	0.10
	5				S	T	P
					0.07	0.07	0.07
		^{137}Cs					
Liver	1	T	S	P	T	S	P
		3.22	4.19	5.90	0.30	0.36	0.57
	2	T	S	P	T	S	P
		2.06	2.61	3.72	0.21	0.25	0.40
	5	T	S	P	T	S	P
		1.06	1.43	2.01	0.11	0.12	0.21
Muscle	1				P	S	T
					0.25	0.28	0.34
	2				P	S	T
					0.29	0.33	0.40
	5				S	P	T
					0.30	0.32	0.34

[a] The designations represent the drug treatment group (P = propylthiouracil, S = saline, and T = thyroxine). They are arranged in order of increasing magnitude from left to right. Those underlined are not significantly different at $P > 0.05$ level. The mean value for each treatment is shown.

TABLE 2. *Newman-Keuls multiple comparison of the effect of time within each treatment for liver and muscle.*

Organ	Treatment	% Activity/organ (P)			% Activity/gram of tissue (A)		
			^{22}Na				
Liver	Hypothyroid	5[a]	2	1	5	2	1
		0.91	1.28	1.54	0.10	0.13	0.14
	Euthyroid	5	2	1	5	2	1
		0.95	1.36	1.80	0.08	0.12	0.15
	Hyperthyroid	5	2	1	5	2	1
		0.91	1.47	1.87	0.09	0.13	0.16
Muscle	Hypothyroid				5	2	1
					0.10	0.13	0.15
	Euthyroid				5	2	1
					0.07	0.11	0.12
	Hyperthyroid				5	2	1
					0.07	0.11	0.13
			^{86}Rb				
Liver	Hypothyroid	5	2	1	5	2	1
		1.08	1.57	1.79	0.11	0.16	0.17
	Euthyroid	5	2	1	5	2	1
		0.98	1.37	1.59	0.08	0.13	0.14
	Hyperthyroid	5	2	1	5	2	1
		0.90	1.19	1.39	0.10	0.11	0.13
Muscle	Hypothyroid				5	2	1
					0.07	0.10	0.11
	Euthyroid				5	2	1
					0.07	0.10	0.11
	Hyperthyroid				5	2	1
					0.07	0.10	0.11
			^{137}Cs				
Liver	Hypothyroid	5	2	1	5	2	1
		2.01	3.72	5.90	0.21	0.40	0.57
	Euthyroid	5	2	1	5	2	1
		1.43	2.61	4.19	0.12	0.25	0.36
	Hyperthyroid	5	2	1	5	2	1
		1.06	2.06	3.22	0.11	0.21	0.30
Muscle	Hypothyroid				1	2	5
					0.25	0.29	0.32
	Euthyroid				1	5	2
					0.28	0.30	0.33
	Hyperthyroid				1	5	2
					0.34	0.34	0.40

[a] The designations represent the time intervals after injection at which the rats were sacrificed (1, 2, and 5 days). They are arranged in order of increasing magnitude from left to right. Those underlined are not significantly different at $P > 0.05$ level. The mean value for each time is shown.

nuclides for both P and A values (Table 1). No trend was seen for ^{22}Na. In liver tissue, NaK-ATPase causes Na to be pumped out and K to be pumped in. It has been reported that thyroid hormone causes an increase in NaK-ATPase activity in liver tissue (8). These results have been contradicted by Kovtunyak *et al.* (7), whose results showed an increase in ATPase activity in the liver during hypothyroidism. They also showed an increase in K in the liver during hypothyroidism. Since K, Rb, and Cs are transported into cells by a similar mechanism, that being facilitated diffusion (9), and an increase in K has been shown in the liver due to increased ATPase activity during hypothyroidism (7), an increased ATPase activity could be used to explain the facilitated diffusion of Rb and Cs into cells during hypothyroidism. An increase in Rb and Cs in the liver of the hypothyroid rats was found in the present study; therefore, this study supports the finding of Kovtunyak *et al.* A similar statement cannot be made for Na since no definite trend was seen.

In muscle tissue, a difference was shown between the handling of ^{137}Cs and the handling of ^{22}Na and possibly ^{86}Rb (Table 1). The hyperthyroid treatment caused the highest uptake of ^{137}Cs while the hypothyroid treatment caused the highest uptake of ^{22}Na and ^{86}Rb, although the differences seen for ^{86}Rb were not significant. For muscle, an increase in NaK-ATPase activity was reported during hyperthyroidism (2). This increase possibly explains the highest levels of ^{22}Na being seen in muscle when the pump activity was decreased during hypothyroidism and the ^{22}Na was not being pumped out of cells. The higher levels of ^{137}Cs in the hyperthyroid group support the increased pumping activity caused by thyroxine.

The effect of the time of sacrifice (Table 2) showed a similar trend for all three radionuclides for all treatments in the liver with a general trend of the highest concentrations being seen on day 1 and the lowest on day 5. In muscle tissue, the same trend was followed for ^{22}Na and ^{86}Rb, but the results indicated that ^{137}Cs was being bound to some extent in muscle tissue. These findings are supported by the literature (12).

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