Effect of Replacement of Medium Potassium by Sodium, Cesium or Rubidium on *in Vitro* Iodide Transport and Iodoamino Acid Synthesis by Rat Thyroid[1] (37264)

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Both active transport of iodide into the gland and its subsequent oxidation and binding into tyrosyl residues of preformed thyroglobulin are believed necessary for an optimal rate of synthesis of iodoamino acids in the thyroid (1). It is generally believed that there are two main classes of antithyroid agents, each group having a relatively specific inhibitory effect on either the iodide pump or organic binding. However, monovalent anions such as perchlorate and thiocyanate, which primarily inhibit only the iodide pump, in high concentration also inhibit organic iodination (2).

Potassium is apparently important in maintaining an active iodide transport mechanism. There is a marked depression of the thyroid iodide pump when the K^+ in the medium in which thyroid lobes are incubated is decreased or if substances are added which interfere with the Na^+-K^+ATPase dependent pump (3). The present studies were designed to determine whether replacement of medium potassium by other cations would depress organic binding of that iodide which entered the gland or would have an effect only on the thyroid iodide pump.

Materials and Methods. Male Sprague–Dawley rats weighing 180–250 g were maintained on a high iodine diet (Purina Laboratory Chow, approx 3 mg I/kg). They were fed a low-iodine diet (LID, General Biochemicals, approx 30 μg I/kg) or a low-iodine diet containing 0.15% propylthiouracil (PTU) for 1–2 wk before each study to give various degrees of thyroid stimulation. The rats were killed with ether. The thyroid lobes were immediately carefully excised, incubated individually in 50 ml flasks in 4 ml Krebs–Ringer–phosphate solution (KRP) in a shaking water bath at 37°, for 4–5 hr (5). KRP was prepared in the laboratory with glass-distilled deionized water and crystalline solutes, all of analytical grade (Fisher Scientific Co.): 100 ml 0.154 *M* NaCl; 21 ml 0.2 *M* potassium phosphate buffer (pH 7.4); 4 ml 0.154 *M* KCl; 2 ml 0.11 *M* $CaCl_2$; 1 ml 0.154 *M* $MgSO_4$. In potassium-depleted media, potassium in KRP was replaced by equimolar quantities of sodium, rubidium, or cesium. These potassium-replaced media were employed in the preincubation as well as in the final incubations, as indicated below. Rubidium and cesium chloride were obtained from Mann Research Laboratories. Each incubation flask contained 1–10 μCi carrier-free $^{131}I^-$, depending on the anticipated thyroid uptake. Each final incubation in K^+-depleted media was preceded by two 15 min preincubations in fresh media without radioactivity to facilitate K^+ depletion. At the end of incubation, the lobes were rinsed with 0.5 ml 0.154 *M* NaCl and blotted with absorbent paper. Thyroid radioactivity was calculated as a percentage of the $^{131}I^-$ added to each flask present in the lobe at the end of incubation. The relative distribution of labeled iodide, monoiodotyrosine (MIT), and diiodotyrosine (DIT), was determined by ascending paper chromatography in *n*-butanol–ethanol-0.2 *N* ammonia, 5:1:2 (BEA) and/or *n*-butanol–acetic acid–water, 4:1:5 (BAW) after homogenization and pancreatin digestion of the thyroid lobe (4).

Results. Duplicate incubations gave consistent results. Thyroid ^{131}I uptake was markedly diminished by replacement of medium

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TABLE I. Utilization of ^{131}I by Thyroid Lobes Incubated in KRP, K^+ Substituted by Na^+.[a]

No. of expt.	Diet	K^+ in medium	Equimolar substitute	% uptake of ^{131}I	Relative distribution of intrathyroidal ^{131}I				No. of incubations
					I^-	MIT	DIT	MIT/DIT	
1	1 wk LID	+	—	32 ± 3[b]	18 ± 3	64 ± 5	18 ± 3	3.6	8
		—	Na	2 ± 2	64 ± 9	31 ± 5	4 ± 2	7.5	8
2	2 wk LID	+	—	40	15	57	28	2.0	2
		—	Na	3	86	10	3	3.3	2
3	2 wk PTU	+	—	—	43	42	14	3.0	2
		—	Na	—	85	12	2	6.0	2
4	2 wk PTU	+	—	30	40	44	15	2.9	2
		—	Na	2	67	29	4	7.2	2
5	2 wk LID	+	—	38	24	43	32	1.3	2
		—	Na	3	77	18	5	3.6	2
	10 days LID	+	—	39	30	48	21	2.3	2
	4 days PTU	—	Na	4	78	18	3	6.0	2
	11 days LID	+	—	37	19	57	24	2.4	2
	4 days PTU	—	Na	2	79	17	3	5.7	2
6	10 days PTU	+	—	29	24	58	18	3.2	2
	4 days LID	—	Na	2	69	25	5	5.0	2

[a] One thyroid lobe was incubated individually in KRP containing K^+, the contralateral lobes were incubated in KRP in which Na^+ repleted K^+. Incubations were 4–5 hr. ^{131}I uptake was not measured in Expt. 3.

[b] Mean ± SE.

potassium with sodium (Table I). When rubidium or cesium were substituted for potassium, the ^{131}I uptake was nearly normal (Tables II and III). Rubidium usually produced slightly higher uptakes than cesium.

Organically bound ^{131}I was present as MIT and DIT; no iodothyronines were found in any incubations. There were consistently lower (MIT + DIT)/I^- and higher MIT/DIT ratios in the Cs^+ and Na^+ media in those with unsubstituted K^+. There was little difference in these ratios between the K^+ and Rb^+ media. In Expts 3, 4, and 5 (Table I) and 3 and 4 (Table II) 0.15% PTU was administered with LID in the last few days before killing the rats. This amount of PTU inhibits iodoamino acid synthesis *in vivo*. In this study, however, the lobes were leached by two 15 min preincubations which probably removed most of the PTU (4). Similar results were obtained with cation substitution regardless of the dietary regimen and degree of stimulation of the gland (Tables I–III).

Discussion. The present studies confirm earlier reports (3, 5) that replacement of potassium by sodium in the medium depresses the ability of thyroid lobes to concentrate iodide. Our studies additionally show that these conditions depress organic binding of that iodide which does enter the thyroid lobe. Rubidium, and to a slightly lesser extent cesium, can effectively substitute for potassium in maintaining thyroid iodide transport. Rubidium can also substitute for potassium in maintaining organic binding of iodine, but cesium is ineffective in this repect. This indicates that there is a qualitative difference in the ability of the various cations to maintain the thyroid iodide pump and organic binding of iodine.

It is highly unlikely that the depression of thyroid iodide transport caused by replacing potassium with sodium in the medium is solely responsible for the depression of organic binding of iodine. Previous studies have shown that depression of the iodide pump to a low level by monovalent anions such as perchlorate or thiocyanate is not associated with decreased organic binding of that iodide

TABLE II. Utilization of ^{131}I by Thyroid Lobes Incubated in KRP, K^+ Substituted by Na^+, Rb^+, or Cs^+.[a]

No. of expt.	Diet (wk)	K^+ in medium	Equimolar substitute	% uptake of ^{131}I	Relative distribution of intrathyroidal ^{131}I			No. of incubations
					I^-	MIT	DIT	
1	1 LID	+	—	42	34	44	22	2
		—	Rb	32	38	36	26	2
		—	Cs	20	97	21	1	2
		—	Na	1 ± 0[b]	90 ± 4	6 ± 2	4 ± 1	6
2	2 LID	+	—	47	29	43	27	2
		—	Rb	34	25	49	26	2
		—	Cs	21 ± 3	86 ± 1	12 ± 1	1 ± 1	4
		—	Na	1 ± 0	82 ± 2	16 ± 1	1 ± 0	8
3	1 PTU	+	—	40	23	59	18	2
		—	Rb	43	26	61	13	2
		—	Cs	25	75	21	3	2
		—	Na	1 ± 0	73 ± 4	24 ± 3	3 ± 1	6
4	2 PTU	+	—	—	11	60	27	2
		—	Rb	—	10	62	28	2
		—	Cs	—	58	30	12	2
		—	Na	—	59 ± 5	30 ± 2	10 ± 1	6

[a] One thyroid lobe was incubated individually in KRP containing K^+, or equimolar Rb^+ or Cs^+ as a substitute; the contralateral lobes were incubated in KRP with Na^+ as a substitute. Incubations were 4–5 hr. ^{131}I uptake was not determined in Expt. 4.

[b] Mean ± SE.

TABLE III. Utilization of ^{131}I by Thyroid Lobes Incubated in KRP, K^+ Substituted by Rb^+, Cs^+, or Na^+.[a]

K^+ in medium	Equimolar substitute	% Uptake of ^{131}I	Relative distribution of intrathyroidal ^{131}I				
			I^-	MIT	DIT	MIT/DIT	MIT + DIT/I^-
+	—	16.8 ± 4.1[b]	15.5 ± 2.0	61.6 ± 2.1	22.8 ± 0.8	2.7 ± 0.2	5.9 ± 1.1
—	Rb	18.5 ± 2.6	12.9 ± 2.5	65.5 ± 2.6	21.5 ± 0.4	3.0 ± 0.1	7.8 ± 2.0
+	—	10.9 ± 1.1	15.1 ± 3.2	57.3 ± 5.1	27.6 ± 2.5	2.2 ± 0.4	6.5 ± 1.5
—	Cs	10.5 ± 2.1	75.5 ± 1.5[c]	20.3 ± 1.2[c]	4.1 ± 0.4[c]	5.0 ± 0.4[c]	0.3 ± 0.0[c]
+	—	11.4 ± 0.6	18.4 ± 4.0	58.2 ± 1.3	23.4 ± 3.5	2.7 ± 0.4	5.1 ± 1.0
—	Na	2.3 ± 0.2[b]	37.7 ± 6.5[c]	49.7 ± 4.9[d]	12.6 ± 2.6[c]	4.3 ± 0.8[c]	1.7 ± 0.0[d]

[a] Rat thyroid lobes, 4 in each group, were incubated individually in KRP containing K^+, contralateral lobes were incubated in KRP with Rb^+, Cs^+, or Na^+ as an equimolar substitute; all rats were fed LID for 2 wk.

[b] Mean ± SE.

[c] Statistical comparison with K^+ control (analysis of variance): $p < .01$; [d] $p < .05$; no superscript *c* or *d*, $p > .05$.

which enters the thyroid primarily by diffusion (2). Although the quantity of iodide entering the thyroid gland is not further depressed, increasing concentrations of such anions above the level necessary to produce a maximal depression of the iodide pump will progressively inhibit organic binding of newly entering intrathyroidal radioiodine. It is unlikely that such depression of organic binding is due to a reduction of intrathyroidal iodide substrate. If the concentration of medium $^{127}I^-$ is increased several hundredfold compared to "tracer" concentrations, there is no alteration of the pattern of inhibition of transport or organic binding of iodine produced by increasing concentrations of monovalent anions (2). The dissociability of the depression of thyroid iodide transport and organic binding is also supported by the findings in the present study. Substitution of potassium in the medium by either cesium or sodium resulted in a marked depression of organic binding of that $^{131}I^-$ which entered the thyroid. ^{131}I uptake was greatly suppressed in the sodium-substituted medium but was essentially normal in the medium with cesium substitution.

Summary. Rat thyroid lobes were incubated in Krebs–Ringer–phosphate (KRP) solution containing $^{131}I^-$. Their ability to concentrate and to organically bind ^{131}I was compared with thyroid lobes incubated in KRP in which the potassium was replaced by equimolar amounts of sodium, rubidium, or cesium. Both uptake and organic binding of ^{131}I were depressed by sodium substitution. ^{131}I uptake was near normal but organic binding of ^{131}I was markedly depressed with cesium substitution. Rubidium was as effective as potassium in maintaining both these functions. It is concluded that the cation concentration of the medium is important in the maintenance of both iodide transport and organic binding of that iodide which enters the thyroid gland. These two functions differ in their cation requirements since cesium restores the capacity to concentrate iodide to a much greater degree than it does the capacity for organic binding of iodine.

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