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Iodine Metabolism in Leukocytes: Effect of Graded Iodide Concentrations

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INTRODUCTION

The metabolism of iodide and iodocompounds in human leukocytes is interesting from the standpoint of host defense and endocrine malfunctions. Klebanoff has found that iodide exerts a powerful bactericidal effect in human leukocytes (1). Several investigators recently reported that thyroid hormones are metabolized by human leukocytes (2–5). Our data show that the spectrum of iodocompounds which is found in pronase hydrolyzates of human leukocytes is very similar to that found after hydrolysis of the thyroid gland (6).

Because of the scarcity of available information concerned with the iodine metabolism in polymorphonuclear leukocytes, we sought to study the effect of high doses of iodide on iodine uptake and organification. The iodination mechanism in neutrophils offers an interesting model system to study the effect of various compounds which are known to influence the iodine metabolism in the thyroid gland. By comparison of these two iodinating mechanisms operating in different body cells, we might better understand the conditions that lead to malfunction of either system.

MATERIALS AND METHODS

Human neutrophils were isolated and suspended in Krebs-Ringer-Tris isotonic solution containing 1.31 mM calcium and 1 μc ^{125}I per ml and various concentrations of sodium iodide. The cells (80–90% polymorphonuclear leukocytes) were incubated for 15–60 min at 37°C with or without latex microparticles. The ratio neutrophil:particles was 1:900, and phagocytosis was monitored by microscopic examination of leukocyte smears stained with Wright's stain and by dioxane extraction method (7). The neutrophils were centrifuged 3 min at 600 × g after termination of iodination by methimazole and resuspended in phosphate-buffered saline containing 2 mM thiosulfate (PBST). Approximately 2 × 10^7 cells were

sonicated in 0.5 ml PBST containing 20 mM tapazole, 50 mM Tris, and 1 mM manganese sulfate and incubated 18 hr at 37°C with 5 mg of pronase. The hydrolysate was chromatographed in two systems: collidine —3 M ammonia (3:1) and *n*-butanol-acetic acid:water (78:5:17) containing 1 mM thiosulfate. The reference compounds, thyroxine (T4), monoiodotyrosine (MIT), diiodotyrosine (DIT), and iodide (I′) were run on parallel strips of chromatographic paper and detected by Pauly's reaction. The radioactivity in chromatogram segments corresponding to the parallel markers was counted in a Packard well counter. From the known specific activity of iodide in media, the amount of organic iodine accumulated in leukocytes and incorporated into iodocompounds was calculated. The value of nonenzymatic iodination was determined by boiling the leukocyte suspension for 5 min before the incubation of cells with radioiodide and latex microparticles. The values of radioiodine present in chromatogram segments of pronase hydrolyzates of boiled leukocytes were deducted from the experimental data.

RESULTS AND DISCUSSION

As shown in Table 1, ^{125}I-iodine was maximally accumulated in resting and phagocytosing leukocytes at 10 μg iodide/100 ml. The radioiodine found in the pellets of boiled cells represents a higher value than the

TABLE 1
THE EFFECT OF IODIDE ON PER CENT OF ADDED ^{125}I ACCUMULATED AND ORGANICALLY BOUND IN RESTING (R) AND PHAGOCYTOSING (Ph) LEUKOCYTES[a]

Iodide concentration μg/100 ml		Per cent of added ^{125}I/10^7 leukocytes	
		Total accumulated iodine	Organic iodine
1	R	0.39 ± 0.09	0.047 ± 0.012
	Ph	2.22 ± 0.27	1.31 ± 0.42
10	R	0.81 ± 0.28	0.153 ± 0.066
	Ph	5.52 ± 0.33	2.89 ± 0.40
100	R	0.59 ± 0.13	0.057 ± 0.021
	Ph	4.67 ± 0.47	3.07 ± 0.48
1,000	R	0.14 ± 0.02	0.01 ± 0.003
	Ph	0.66 ± 0.05	0.28 ± 0.033
10,000	R	0.08 ± 0.01	0.005 ± 0.002
	Ph	0.22 ± 0.02	0.024 ± 0.004
Boiled cells	R	0.15 ± 0.01	0.01 ± 0.003
	Ph	0.17 ± 0.01	0.01 ± 0.003

[a] Only organic iodine was corrected for the nonenzymatic iodination by deduction of the values found in boiled cells. Mean ± SE for seven experiments.

radioiodine accumulated by resting leukocytes at 1.0 and 10.0 mg iodide/100 ml. Therefore, the values for iodine accumulation in leukocytes were not corrected for nonspecific iodine accumulation. On the other hand, the values found in the pronase hydrolysates of boiled leukocytes incubated with or without latex microparticles were deducted from the experimental data after the chromatography. The organically bound iodine shown in Table 1 represents the sum of all iodocompounds found on the chromatogram in each experimental group. The organically bound iodine reached the maximal value in the resting cells at 10 μg iodide/100 ml, but in the phagocytosing neutrophils the maximal iodine organic binding was found at 100 μg iodide/100 ml.

Table 2 shows the representation of iodocompounds found in pronase hydrolysates of resting and phagocytosing leukocytes incubated with various iodide concentrations in the media. These data represent the enzymatic iodination and the radioactivity found in each chromatogram segment of the pronase hydrolysate of boiled cells was deducted from experimental values. As can be seen, the total organification of ^{127}I-iodine in resting neutrophils rises with increased iodide concentration in the media up to 10 mg/100 ml. On the other hand, the organic binding of iodine in phagocytosing cells increases up to an iodide concentration in media of 100 μg/100 ml and then slightly falls with further increasing iodide concentration in the medium. This is contrary to the thyroid gland where acute doses of iodide sharply depress thyroidal ^{127}I-organification *in vitro* (8) and *in vivo* (9), and this inhibition is commonly known as the Wolff–Chaikoff effect (see review, Ref. 10). Our data suggest that there is no Wolff–Chaikoff effect in the resting human neutrophils. The phagocytosing cells demonstrate much higher organic binding of iodine then the resting neutrophils, and it is difficult to assume that the slight decrease of organic iodine found between 100 and 10,000 μg iodide/100 ml represents the Wolff–Chaikoff effect. This assumption is supported also by the increase of DIT/MIT ratio in both resting and phagocytosing leukocytes in relation to the graded iodide concentration in the media (Table 2). It is interesting that a similar find was reported by Shimoda, Inoue, and Greer (11) who studied DIT/MIT ratio in isolated thyroid cells in relation to increasing iodide concentrations in the incubating buffer. In the thyroid gland, the DIT/MIT ratio rises with subinhibitory doses of iodide (8, 12). The same phenomenon was observed with chemical iodination of proteins (13). The changes in DIT/MIT ratio probably indicate the number of binding sites for iodide and the spacial configuration of iodinated and noniodinated tyrosyl groups in the protein molecule. Generally, the DIT/MIT ratio decreased in the thyroid gland at high iodine concentrations.

TABLE 2

THE EFFECT OF IODIDE ON ORGANIC IODINE AND IODOCOMPOUNDS FORMATION IN RESTING (R) AND PHAGOCYTOSING (Ph) HUMAN NEUTROPHILS[a]

Iodide concentration μg/100 ml		ng $^{127}I/10^7$ Leukocytes						
		Total organic iodine	Origin	MIT	DIT	T4	Front	DIT/MIT
1	R	0.014 ± 0.004	0.0016	0.0087	0.0016	0.0008	0.0013	0.194 ± 0.039
	Ph	0.415 ± 0.121	0.034	0.275	0.030	0.014	0.063	0.089 ± 0.020
10	R	0.458 ± 0.197	0.030	0.283	0.045	0.034	0.065	0.167 ± 0.043
	Ph	8.67 ± 1.19	0.63	6.19	0.65	0.32	0.88	0.096 ± 0.018
100	R	1.71 ± 0.62	0.08	1.03	0.23	0.15	0.22	0.188 ± 0.039
	Ph	92.3 ± 14.5	5.83	61.9	17.4	2.38	4.75	0.273 ± 0.043
1,000	R	3.13 ± 0.81	0.22	2.14	0.34	0.19	0.24	0.251 ± 0.084
	Ph	83.5 ± 10.3	6.0	59.5	12.7	2.13	3.21	0.214 ± 0.021
10,000	R	14.3 ± 5.62	1.84	8.60	1.29	1.72	0.88	0.350 ± 0.120
	Ph	73.5 ± 11.8	4.76	51.1	11.9	3.83	1.91	0.315 ± 0.100

[a] The leukocytes were incubated 30 min at 37°C in 3.0-ml portions of Krebs-Ringer-Tris medium. Phagocytosis was induced by latex microparticles. The values of iodine found in chromatogram segments of pronase hydrolyzed leukocytes that were boiled for 5 min were deducted from experimental data. Mean ± SE for six experiments.

A significant portion of iodocompounds found in pronase hydrolysates of leukocytes is MIT, which amounts to 60–71% of total iodine. At any level of iodide concentration in the medium the per cent representation of MIT is always higher in the phagocytosing neutrophils than in the resting ones. The highest per cent representation of DIT (13 and 19%) was found in resting and phagocytosing leukocytes at 100 μg iodide/100 ml.

As shown in Table 3, the addition of iodide up to 10 mg/100 ml depletes radioiodide from the leukocytes by isotopic dilution but has little effect on organically bound iodine already preformed in the cells. It also seems that high iodide concentration in the medium does not have an inhibitory effect on the release of organic iodine from leukocytes. Thirty minutes after the raising of the iodide level to 10 mg/100 ml, we found 2.64% of the ^{125}I added dose/10^7 cells and after additional 15 min this value increased to 2.80% of the A.D./10^7 leukocytes. On the other hand, the organic iodine in the medium decreased from 3.23–2.64% of the A.D./10^7 cells during 30 min of incubation after the addition of iodide to the cell suspension. The explanation for this phenomenon is unknown.

Although the Wolff–Chaikoff phenomenon was not observed in human neutrophils, we cannot conclude that the iodination mechanism in leukocytes differs from that in the thyroid gland. It has been shown that escape from the Wolff–Chaikoff effect occurs in the thyroid gland of rats after prolonged treatment with large amounts of iodide (14). The result of this adaptation is renewed ^{127}I accumulation and organic binding in the thyroid, in spite of a high ^{127}I level in the medium. Braverman and Ingbar (14) suggested that thyroidal adaptation to the inhibitory

TABLE 3

THE EFFECT OF IODIDE ON IODINE ACCUMULATION AND RELEASE FROM PHAGOCYTOSING HUMAN LEUKOCYTES[a]

Total incubation time (min)	Iodide added up to 10 mg/100 ml	Per cent of added $^{125}I/10^7$ leukocytes		
		Total accumulation of iodine in cells	Total organically bound iodine in cells	Organically bound iodine in media
15	–	3.14 ± 0.30	1.05 ± 0.10	3.23 ± 0.27
45	–	3.93 ± 0.27	1.47 ± 0.27	3.87 ± 0.10
45	+	2.30 ± 0.19	1.10 ± 0.09	2.64 ± 0.23
60	–	3.47 ± 0.27	1.41 ± 0.27	4.60 ± 0.13
60	+	1.96 ± 0.10	1.00 ± 0.06	2.80 ± 0.25

[a] The cells were incubated with radioiodide at 10/μg ^{127}I/100 ml for 15 min and then the iodide concentration in the medium was raised up to 10 mg ^{127}I/100 ml. The incubations were terminated after 30 and 45 min. Corrections were made for nonenzymatic iodine accumulation and organic binding. Mean ± SE for seven experiments.

effect of graded iodide concentrations is due to the decreased iodide transport into the thyroid.

In phagocytosing leukocytes the iodide transport and accumulation is very similar to the thyroid gland. As shown in Table 2, 0.415 ng ^{127}I is organically bound in the phagocytic neutrophils at 1 μg ^{127}I/100 ml, or approximately 0.09 μg ^{127}I/30 min $\times$ g of neutrophils.[1] Similar data were reported for the thyroid gland (0.2 μg ^{127}I/hr $\times$ g of thyroid tissue) (15). The iodide represents approximately 40% or 0.06 μg ^{127}I per g of cells of total iodine in the phagocytic neutrophils (Table 1). At 1 μg ^{127}I/100 ml of medium the C/M ratio[2] is 6. The T/S[2] ratio in the unblocked mouse and rat thyroid gland was approximately 6–19 (16, 17). It is evident that, although iodide transport and uptake are very similar in unblocked phagocytosing neutrophils and the thyroid gland, the high iodide does produce a different effect.

SUMMARY

Increasing concentrations of iodide in the medium produce a rise in the ^{127}I newly bound to iodocompounds in the resting and phagocytosing human leukocytes. Organic iodine rises in resting leukocytes as the iodide concentration in the medium is increased from 0.001 to 10 mg iodide/100 ml. There is no additional increase in organic iodine in phagocytosing leukocytes between 0.1 and 10 mg iodide/100 ml; however, the organic iodine is not significantly lowered at the highest iodide concentration in the media. These data prove that the Wolff–Chaikoff phenomenon is not demonstrable in human leukocytes.

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[1] The volume of one neutrophil is 450 μm^3. One gram (ml) of neutrophil is approximately 2.22×10^9 cells. No recalculation for specific gravity of cells was made.

[2] C/M and T/S ratio represents the amount of iodide present in an equal volume of cells (C), medium (M), thyroid (T), or serum (S). C for packed neutrophils was obtained after deduction of iodide present in the pellets of boiled leukocytes.

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