STIMULATION OF IODOPROTEINS AND THYROXINE FORMATION IN HUMAN LEUKOCYTES BY PHAGOCYTOSIS

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Received July 27, 1971

SUMMARY

Phagocytosis of inert latex microparticles increases the iodide uptake and organic binding in human leukocytes by several fold in comparison to control cells. Thyroxine, monoiodotyrosine, and diiodotyrosine were found after pronase hydrolysis of leukocyte proteins. An active iodide concentrating mechanism was found in the leukocytes too. The concentration of stable iodine (^{127}I) in leukocytes was found to be 0.003 per cent, and in leukocyte proteins, 0.04 per cent.

It is generally believed that the thyroid gland is the unique site for the production of thyroid hormones - thyroxine and triiodothyronine. Attempts have been made to prove that thyroid hormones are made also extrathyroidally (1) or that the conversion of thyroxine into biologically more active triiodothyronine occurs in athyreotic persons (2).

The significant role of erythrocytes and leukocytes in thyroid hormone metabolism was investigated by several workers. The uptake of thyroid hormones by red blood cells was observed by Ureles and Murray (3) and Hamolsky (4). Siegel and Sachs (5) found that *in vitro* uptake of thyroxine and triiodothyronine is higher by white blood cells than by erythrocytes. The ability of leukocyte homogenates to deiodinate thyroxine was observed by Kurland, et al. (6). Recently Klebanoff and White (7,8)

described another ability of leukocytes to iodinate the cell membranes of ingested bacteria.

While there have been significant attempts to understand the correlation between the thyroid function and blood cell activity, little is known about the nature of organically bound iodine which is formed during some of these processes.

MATERIALS AND METHODS

A 20 ml. sample of peripheral blood from healthy donors was collected in vacutainer tubes containing 0.02 µg./ml. of heparin. The erythrocytes were lysed 20 sec. in 0.2 per cent NaCl, followed by equal volume of chilled hypertonic 1.61 per cent NaCl to restore isotonicity (9). The cells were centrifuged at 400xg for 10 min., and the osmotic lysis was repeated once more. After centrifugation, the cells were twice washed with Earle's balanced salt solution. The total recovery of human leukocytes was approximately 15×10^6 from a 20 ml. blood sample.

The cell suspension contained approximately 80 per cent polymorphonuclear (PMN) leukocytes. The incubations were carried out for different periods of time in 2 ml. portions of Earle's solution under an O_2/CO_2 atmosphere in stoppered flasks. Approximately 2×10^6 leukocytes were employed for the incubation. To study the effect of phagocytosis, 50 µl. of latex polystyrene particles (0.81 µ) (10 per cent solids) were added. The incubamedia contained 10 µc. ^{131}I per ml. and 10 µg. ^{127}I per 100 ml. The iodination was terminated by addition of 100 µl. 0.3 M tapazole. The cells were centrifuged 5 min. at 400xg, resuspended in phosphate buffered saline with 2 mM thiocyanate, and homogenized by sonication.

The aliquots of the cell homogenates were chromatographed in n-butanol-ethanol-3N ammonia, collidine-3N ammonia or in n-

butanol-acetic acid-water systems. The incorporation of ^{127}I into the iodinated compounds was estimated from the known specific activity of ^{131}I. The chromatograms were exposed to x-ray films or run through a Packard chromatogram scanner. Then the radioactive bands were counted in a well-type Packard counter.

RESULTS AND DISCUSSION

As is shown in Fig. 1, the incorporation of radioiodide into the phagocytic leukocytes was very rapid. Although in the resting cells there was not a significant increase of uptake and PBI formation after 30 min. of incubation. in the phagocytizing leukocytes, iodide was accumulated at a rate of approximately 1.5 ng. ^{127}I/min. x 1×10^7 cells, up to 45 minutes of incubation. PBI was increasing at a rate of approximately 1 ng. ^{127}I/min. x 1×10^7 cells.

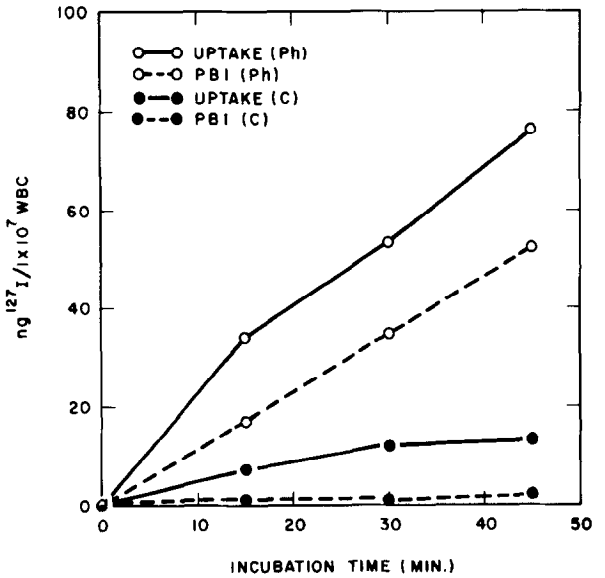

Figure 1. Relationship between time of incubation, uptake of iodine and protein-bound iodine formation (PBI) in control (C) and phagocytic (Ph) human leukocytes. Average of 2 experiments.

Fig. 2 summarizes the data from 11 experiments. It was found that phagocytosis increases the uptake of iodine by

several fold. The resting leukocytes incorporated into proteins 1.8 ± 0.6 ng. $^{127}I/1 \times 10^7$ leukocytes after 45 minutes of incubation, while the phagocytosis of latex particles increased the iodation of proteins by 20 fold. Boiled leukocytes did not show any significant iodinating activity. The latex particles did not stimulate the iodination when 5.2 per cent bovine serum albumin, radioiodide and latex particles were employed (not shown).

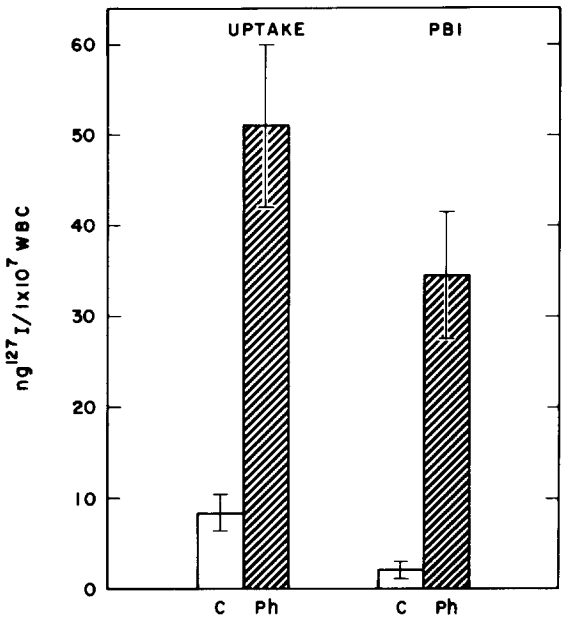

Figure 2. Effect of phagocytosis (Ph) on iodine uptake and protein-bound iodine (PBI) in human leukocytes (control = C) after 45 minutes of incubation. Mean ± SEM of 11 experiments.

After hydrolysis and chromatography of leukocyte homogenates, a similar pattern of iodocompound representation was found, as after the hydrolysis of thyroid tissue. A representative result is shown in Fig. 3. The presence of MIT and DIT is not surprising because these iodinated amino acids were found in chemical iodination of proteins (10). The striking result is the appearance of a labeled thyroxine-like component which ran after rechromatography in 3 different solvents with thyroxine carrier (Fig. 4). As is

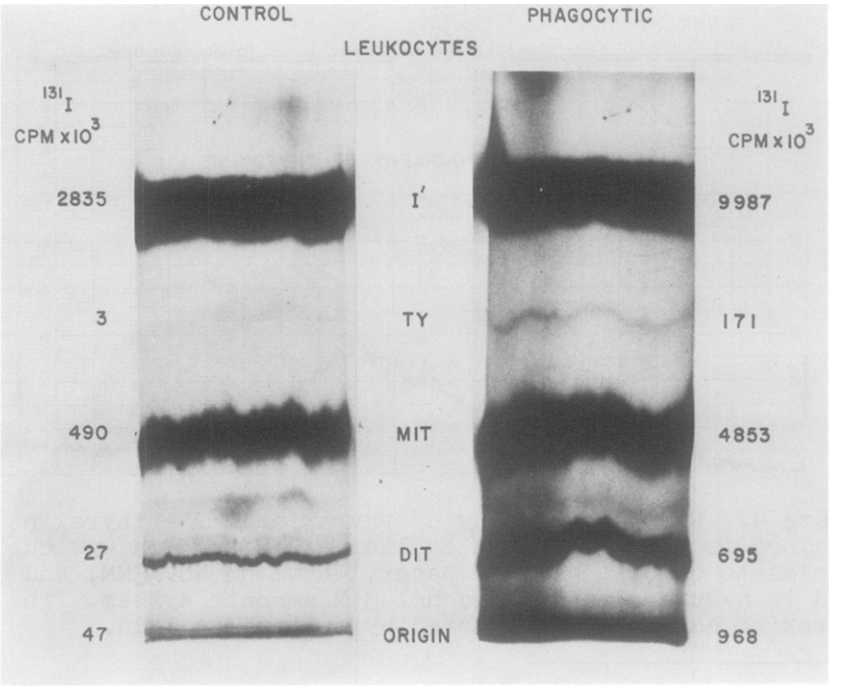

Figure 3. Pronase hydrolysate of human leukocytes. DIT = diiodotyrosine, MIT = monoiodotyrosine, TY = thyroxine, I' = iodide. Chromatographic system: collidine-3 M ammonia.

shown in Fig. 3, the greatest stimulation of phagocytosis was on the formation of thyroxine.

The iodide pump was studied in human leukocytes which were incubated in the presence of 3 mM tapazole, 10 μg. ^{127}I per 100 ml., and radioiodide, ^{131}I. After 1 hour of incubation at 37° C., a C/M ratio was estimated (C = ^{131}I-iodide in 0.1 ml. packed cells; M = ^{131}I-iodide in 0.1 ml. incubation medium). The mean from 4 experiments was 4.2, which suggests an active iodide concentrating mechanism in leukocytes.

The concentration of stable iodine (^{127}I) in leukocytes was estimated by ultramicromethod. The average value from 15 experiments was 0.72 μg. ^{127}I per 10^7 leukocytes (85 per cent PMN). This represents 0.003 per cent of iodine concentration in leukocytes or 0.04 per cent of iodine in leukocyte proteins. For

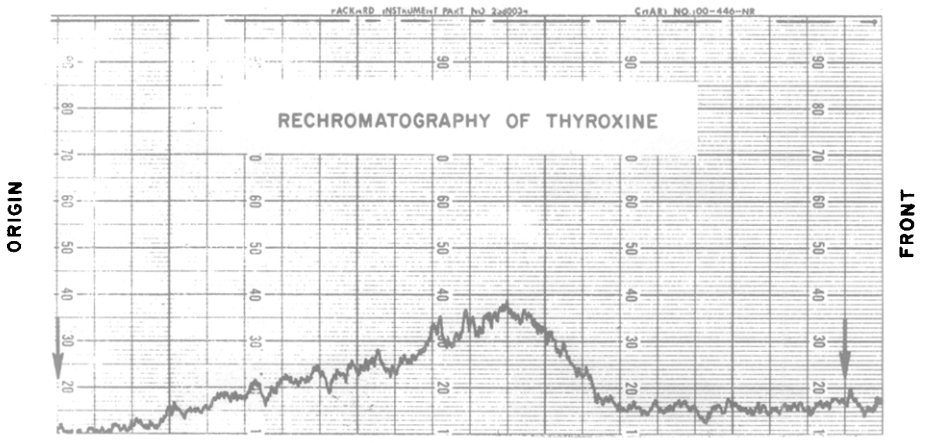

Figure 4. Rechromatography of thyroxine. The thyroxine band obtained from first run in collidine-3 M ammonia was cut off, stapled to chromatographic paper, Whatmann No. 3MM, and developed in n-butanol-ethyl alcohol-3 M ammonia system. The thyroxine carrier was detected by sulfanilic acid.

comparison, the iodine concentration in the thyroid gland is an average 0.05 per cent and 0.5 per cent in thyroglobulin molecule. In no other tissue has such a high concentration of ^{127}I-iodine been found.

Until now, we did not have clear evidence that thyroxine is really synthesized in the leukocytes. There is a possibility that thyroxine is accumulated in leukocytes from blood and that the radioiodide is incorporated into pre-existing thyroxine by exchange. Another possibility exists that radioiodide is incorporated into pre-existing triiodothyronine. Nevertheless, the thyroxine-like compound is present in the leukocytes, and there is a possibility that thyroxine or triiodothyronine could be made in other body cells with high peroxidase activity (11) and protein with high tyrosine content or with tyrosines in the positions preferable for the condensing of diiodotyrosines.

As has been shown by several investigators, thyroxine was formed in cell-free systems employing appropriate substrate, peroxide or peroxide generating system, thyroid peroxidase (11),

xantine oxidase (12), chloroperoxidase (13), and lactoperoxidase or myeloperoxidase (14). The presence of myeloperoxidase (15) and peroxide generation in the leukocytes (16) has been very well established. On this basis, the thyroxine formation in leukocytes could be expected.

There are several reports which suggest an extrathyroidal thyroxine formation; for example, the effect of high doses of stable iodide (^{127}I) on growth of young rats and the appearance of thyroxine in thyroidectomized rats (17). High thyroxine concentration in blood was found with low or normal thyroid activity, as is in the cases of choriocarcinoma (18), Graves' disease (19), or acute pneumococcal infection (20).

The formation of thyroxine in leukocytes is supported by the observation of many authors in the past who found a close similarity between thyrotoxicosis and chronic leukemia (21,22, 23,24). Some of the physicians were so impressed by the similarity of these 2 illnesses that attempts were made to treat leukemia with iodine (25). It was reported recently that, in some cases of leukemia, normal thyroid function, hypermetabolism (26), and increased protein-bound iodine were found (27).

Our results and the data of other investigators viewed in their entirety provide strong evidence that thyroxine and other iodinated compounds are made in the human leukocytes.

ACKNOWLEDGMENT

Appreciation is extended to Miss Mary Jo Sargus for her excellent technical assistance. This investigation was supported in part by research grant AM 06439 belonging to Dr. W. Tong.

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