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Mechanisms of Adaptation to Iodine Deficiency in Rats: Thyroid Status Is Tissue Specific. Its Relevance for Man

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Many animals, man included, live in areas providing insufficient iodine (I) for optimal health. Degrees of I deficiency (ID) vary from mild-moderate to very severe, with quali- and quantitatively different negative consequences. To understand the mechanisms involved in adaptation to different grades of ID, we fed rats a low-iodine diet, plus additions resulting in a 250-fold range of I daily available to the thyroid, ranging from 5 μg (adequate) down to 0.02 μg I. We measured thyroid weight, total I, T_4, T_3, and type I 5′ iodothyronine deiodinase (D1) activity, TSH, T_4, free T_4, and T_3 in plasma, T_4 and T_3 in 11 tissues, and two 5′ deiodinase isoenzymes in four. TSH-independent thyroid autoregulation plays an important role in addition to TSH-dependent mechanisms in the adaptation to ID, avoiding a decrease of T_3 in plasma and most tissues, despite a marked decrease of plasma T_4, whereas extrathyroidal responses of D2 mitigate T_3 deficiency in tissues in which T_3 is mostly generated from T_4. We focused on mild and moderate ID, the least investigated experimentally, despite its current frequency in industrialized countries. The novel and important finding of our study is that thyroid status cannot be defined for the animal as a whole: at all grades of ID, T_3 is simultaneously elevated, normal, and low in different tissues. Present findings in mild-moderate ID draw attention to the importance, for man, of the resulting hypothyroxinemia that may affect mental functions and neurodevelopment of the inhabitants, even when they do not have the increased TSH or clinical hypothyroidism, often wrongly attributed to them. (*Endocrinology* 147: 2098–2108, 2006)

IODINE (I) IS a prerequisite for the synthesis of thyroid hormones, T_4 and T_3. Although relatively abundant in sea water, it is often very scarce in terrestrial areas of the world, in which animals have developed a highly specialized structure, the thyroid follicle, to adapt to situations in which the supply of this element is inadequate to meet thyroid hormone requirements. The thyroid is optimized for the efficient use and storage of I in the form of I-containing compounds and for its intrathyroidal recycling, as a result of which severe systemic deficiency of T_3 is prevented, even during relatively long periods of I deficiency (ID). A chronic and severe ID might, however, lead to a situation in which compensatory autoregulatory mechanisms can no longer avoid a decrease in T_4 and T_3 secretion, and the organism might suffer the ensuing negative consequences.

Today ID is still the single most important cause of preventable mental defects and cerebral palsy. It has been established that more than 1 billion people are living in conditions of chronic ID of varying degrees and are at risk of suffering some, or all, of the various ensuing ID disorders (IDDs) (1, 2). Their severity and irreversibility in man depends on both the degree of the ID and the period during development in which it is suffered. An adequate supply of I to children and pregnant women are now considered basic human rights.

The epidemiological, clinical, and biochemical findings reported from different ID areas are quali- and quantitatively heterogeneous. Definition of different grades of ID (grade I, mild; grade II, moderate; grade III, severe) has been most helpful in clarifying apparently conflicting reports from different regions (3). Although the most severe irreversible IDD, such as the birth of neurological cretins, are usually found in areas with severe (grade III) ID (4), impaired mental functions are frequently found in the general population of even mild to moderate ID areas (5).

Most of our present understanding of the adaptation of human adults to chronic ID has been derived from experimental models that limited the I intake of rats. Such studies have usually involved comparison of results from animals on a low-iodine diet (LID) with those from rats receiving the same diet supplemented with enough I to meet physiological thyroid hormone requirements or eating the stock diet (*i.e.* Refs. 6–13). (For other related references, see supplemental data published on The Endocrine Society's Journals Online Web site at http://endo.endojournals.org.) It is often difficult to compare results because the nutritional composition and I content of the various LID diets differ or are often not even reported and so do the sex and strain of the animals. Few of these previous studies have involved graded degrees of ID, measured the concentrations of T_4 and T_3 in more than a few selected tissues, or evaluated some parameter of thyroid hormone action other than serum TSH. Despite this, such studies have permitted identification of many, but not all, of the intra- and extrathyroidal mechanisms involved in the

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Abbreviations: BAT, Brown adipose tissue; BW, body weight; D1, type 1, 5′ deiodinase; D2, type 2, 5′ deiodinase; DTT, dithiothreitol; FT_3, circulating free T_3; FT_4, circulating free T_4; I, iodine; ID, I deficiency; IDD, ID disorders; LID, low I diet; LID′, low I diet + $KClO_4$ (0.005%); PTU, 6-*N*-propyl-2-thiouracil; Tx, thyroidectomized.

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response to ID, most of which cannot be investigated directly in man for obvious constraints.

We want to point out that the experimental design used here aimed at a difference in the amount of I available to the thyroid as the single controlled variable between groups. It should be an adequate model for inhabitants of areas in which ID is the sole cause of goiter and thyroid tissue is functionally unaffected, even in the neurological cretin. It is not, however, an appropriate model for those ID areas in which other nutritional or environmental factors may lead to thyroid necrosis, primary hypothyroidism, and myxedematous cretinism, such as seen in central Africa (14).

The aim of the present study was to assess the relative roles of intra- an extrathyroidal mechanisms in the response of individual tissues to different grades of chronic ID, from mild to moderate, to severe and very severe, with special focus on the concentrations of T_3. These were measured in a larger number of tissues than previously studied over such a wide range of ID. Although the T_3 concentration was taken as an index of possible thyroid hormone effectiveness at the individual tissue level (15), some biological end points of thyroid hormone action, other than circulating TSH, were also measured in selected tissues. The present approach shows that the thyroid status of ID rats cannot be defined for the animal as a whole because it is eminently tissue specific: at all grades of ID, elevated, normal, and low concentrations of T_3 are simultaneously found in different tissues of the same animal.

Materials and Methods

Experimental design

Young adult female Wistar rats were used. They were housed under humane conditions, with alternating 12-h light, 12-h dark cycles and at 22–24 C, under veterinary control according to European Community guidelines and after approval by the ethics committee of our institution. They were fed a stock pelleted diet for rats (Sandermus; Sanders, S.A., Barcelona, Spain) with an I content varying between 0.15 and 0.35 μg I/g, which would provide 3–7 μg I per day per rat (assuming a daily food intake of 20 g). When they were approximately 50 d of age and weighed 120–150 g, they were switched from the stock diet to a diet with a very low iodine content (LID) and given 1% $KClO_4$ as drinking water for 1 wk to block further uptake of circulating iodide and accelerate depletion of thyroid stores and I-containing compounds (16). This treatment minimized previous differences between animals. From then on, animals were separated into different groups of five to six animals each and fed the different diets described for experiments A and B.

Although we prepare the LID with components obtained from the same commercial sources, its final I content is variable over time. To ensure that at least one experimental group is severely ID, we often include animals fed LID supplemented with very low amounts of $KClO_4$, at a concentration of 0.005% (LID′ group) (17, 18). This concentration is 200 times lower that that used during the initial week before separating the animals into different experimental groups. The purpose is to decrease the availability to the thyroid of the small amounts of I present in the LID and generated during intra-thyroid I recycling.

The LID was prepared by mixing thoroughly 6 kg corn flour (Productos Hercosa, Barcelona, Spain), 2.5 kg wheat gluten (Herman Kröner GMBH, Ibbenbürn, Germany), 1 kg brewer's yeast (Vitalevor, Strasbourg-Neuhof, France), 0.15 kg NaCl, and 0.15 kg $CaCO_3$ (Carlo Erba, Milano, Italy). Sufficient amounts were prepared to cover a complete experiment with the same batch. Daily rations were prepared by mixing the dry LID with distilled water containing the different additions ($KClO_4$ or KI solutions) in a proportion of 0.7:1.0 (water-dry LID) and dividing it into individual aliquots, which were kept frozen until use.

Experiment A. A preliminary experiment was performed to investigate whether these small amounts of $KClO_4$ (0.005%) would exert *per se* effects on thyroid hormone economy other than impairing the uptake of the minute amounts of I contained in the LID and intrathyroid I recycling. For this, two groups of six animals each were fed LID (LID groups) and two other groups LID supplemented with KI (LID + I groups). One each of these two groups received LID + 0.005% $KClO_4$: LID′ and LID′ + I groups, respectively. The amount of KI was such that a daily intake of 20 g of the LID + I mixture would provide 10 μg I per animal.

Experiment B. This comprised five groups of five animals each that drank distilled water and were fed the basic LID diet with the additions specified in Table 1.

The I content of the LID diet itself was below the limit of detection of the analytical procedure used to determine I in food (9). We measured the 24-h urinary I excretion of LID and LID′ rats during 5 consecutive days. Mean values (± SEM) were 0.052 ± 0.012 and 0.083 ± 0.009 μg, respectively. Although these results indicated that the amount of I ingested daily by animals on LID or LID′ was 0.052 ± 0.012 μg, the amount actually available to the thyroid of the LID′ animals was 0.031 μg less, namely 0.021 μg I. Table 1 shows the estimated I intakes of the different groups; they are merely tentative because neither the amount of food ingested individually nor the fecal excretion of I-containing compounds was measured. The amount of I available to the thyroids of the different groups of rats appeared to differ 100-fold or more between the two extreme groups (LID′ *vs.* LID + 5.0).

After 3 months on these different diets, the rats were slightly anesthetized with ether, bled extensively, and perfused (15). Samples of plasma, interscapular pads of brown adipose tissue (BAT), pituitary, brain, cerebral cortex, cerebellum, liver, kidney, heart, lung, adrenals, ovaries, and muscle (musculus quadriceps femoris) were dissected out and frozen for the determination of T_4 and T_3. Aliquots of cerebral cortex, BAT, liver, and lung were kept frozen at −80 C for the determination of iodothyronine deiodinase activities.

The thyroid was dissected out, weighed, divided into three aliquots, and kept frozen for the determination of type I 5′ deiodinase (D1) activity and total I, T_4, and T_3 contents.

Determinations

I content. This was determined in aliquots of thyroid glands, urine, or LID by modifications of a chloric acid digestion procedure (16).

T_4 and T_3 in plasma and extrathyroidal tissues. Total T_4 and T_3 were measured by specific and highly sensitive RIAs, after extraction with methanol and extensive purification of the iodothyronines, as detailed elsewhere (15, 19). T_4 and T_3 concentrations in a given type of sample from the five experimental groups were determined in the same extraction run and in a single RIA for each hormone. To increase recovery of very small tissues (*i.e.* pituitary, adrenals, ovaries), the initial methanol extract was purified directly through the resin columns, omitting the methanol-chloroform extraction and back-extraction procedure.

The plasma free T_4 (FT_4) was calculated from the total T_4 concentration and the percentage of added tracer amounts of [^{125}I]T_4 that was not bound to serum transport proteins. The latter was determined by ultracentrifugation of undiluted samples through Centricon-10 microconcentrators (Amicon GmbH, Witten, Germany) as detailed (20).

T_4 and T_3 in the thyroid. The contents of T_4 and T_3 in the thyroid were measured separately in two different fractions to which we refer here, respectively, as the "Free" T_4 and "Free" T_3 pools and as the "Total" T_4 and T_3 pools. When applied to the thyroid, the adjectives free and total have a different meaning from plasma. In the thyroid, "Free" T_4 and

TABLE 1. Estimation of the amounts of iodine available daily to the thyroid of the rats from the different experiment B groups

Group	Diet + supplements	I, μg/d per rat[a]
LID′	LID′	0.021[b]
LID	LID	0.052[b]
LID + 0.5	LID + 0.5 μg I/20 g	0.50
LID + 1.0	LID + 1.0 μg I/20 g	1.0
LID + 5.0 (controls)	LID + 5.0 μg I/20 g	5.0

[a] Theoretically available for thyroidal uptake.
[b] Based on 24-h urinary I excretion data.

"Free" T_3 correspond to the iodothyronines present in the gland as amino acids, no longer incorporated into proteins by peptidic bonds, and presumably available for secretion as hormones into the bloodstream. "Total" T_4 and T_3 correspond to the iodothyronines residues still incorporated by peptidic bonds into thyroglobulin and other proteins. The concentrations of "Free" T_4 and "Free" T_3 were obtained using the methanol extracts of the thyroid homogenates, then processed as other tissue extracts. The thyroid pools of "Total" T_4 and T_3 were measured in methanol extracts of proteolytic hydrolysates of the pellets remaining after the initial methanol extraction, as described (21)

Iodothyronine deiodinases. D1 activity was assayed in liver and lung homogenates using 400 nM rT_3 and 2 mM dithiothreitol (DTT) for liver and 2 nM rT_3 and 20 mM DTT for lung in 100 mM potassium phosphate buffer (pH 7.0) and 1 mM EDTA. The reaction time was 10 min for liver and 60 min for lung. D1 activity was also assayed in an aliquot of the thyroid gland, using 0.8–1.0 μM rT_3 and 2 mM DTT for 10 min.

Type II 5′-iodothyronine deiodinase (D2) activity was assayed in the cerebral cortex and BAT using 2 nM T_4 + 1 μM T_3 and 20 mM DTT in the presence of 1 mM PTU, and the reaction time was 60 min.

All samples were homogenized in buffer containing 0.32 M sucrose, 10 mM HEPES (pH 7), and 1 (for D1) or 10 mM (for D2) DTT. Before each assay [^{125}I]rT_3 or [^{125}I]T_4 was purified, and the assays were performed as described (22). The protein content was usually 150–200 μg/tube for most tissues but was 10-fold lower when liver or thyroid were assayed.

Circulating TSH. TSH was measured in plasma using immunoreactants kindly provided by the Rat Pituitary Agency of the National Institute of Diabetes, Digestive and Kidney Diseases (National Institutes of Health, Bethesda, MD) as described elsewhere (16). Results are expressed in weight equivalents of the National Institute of Diabetes and Digestive and Kidney Diseases rTSH RP-3 preparation.

Drugs and reagents

T_4, T_3, 3,5-diiodothyronine, 6-*N*-propyl-2-tiouracil, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT_3 and 3′,3-diiodothyronine were obtained from Henning Berlin GMBH (Berlin, Germany).

High specific activity [^{131}I]T_4, [^{125}I]T_3, [^{125}I]T_4, and [^{125}I]rT_3 (3000 μCi/μg) were synthesized in our laboratory, as previously described (19) and used for highly sensitive T_4 and T_3 RIAs, as recovery tracers for plasma and tissues extractions, for the determination of plasma FT_4, and as substrates for D1 and D2.

Statistical analysis

One-way ANOVA and the protected least significant difference *post hoc* test were used for multiple comparisons after validation of the homogeneity of variances by the Bartlett-Box F test. Square root or logarithmic transformations usually ensured homogeneity of variances when this was not found with the raw data. Results are expressed as means ± SEM. $P \leq 0.05$ was considered significant in all comparisons. Whenever it is stated that a difference was found between groups, it implies that it is statistically significant. To be able to compare the degree of changes in different parameters, all present results are expressed as percentages of the mean value obtained for the LID + 5.0 group (also referred to as control, C, group), used as 100%. Multiple regressions and partial correlation analyses between different parameters were also performed. All calculations were done using the SPSS for Macintosh (version 6.1.1; SPSS Inc., Chicago, IL).

Results

Experiment A

T_4, T_3, and TSH concentrations in plasma as well as the concentrations of T_4 and T_3 in liver, brain, BAT, kidney, lung, heart and skeletal muscle, and D2 activities in the cerebral cortex were measured for the four groups. No differences were found between the LID + I *vs.* LID′+I groups. (For detailed data, see table in supplemental data published on The Endocrine Society's Journals Online Web site at http://endo.endojournals.org). The values found in the LID and LID′ groups were significantly different from those of their respective I-supplemented groups, LID + I and LID′ + I. There were also significant differences between values found in LID′, compared with LID animals: plasma T_3 and TSH, liver, brain, kidney, and muscle T_4 and brain, lung, heart, and muscle T_3. Present results confirm that these very small amounts of $KClO_4$ do not have any detectable effect on thyroid hormone status when the I intake is adequate. On the contrary, when the intake is very low, as in rats on LID, thyroid hormone status is affected by the addition of 0.005% of $KClO_4$. The changes observed in LID′ rats *vs.* those on LID are those that would be expected from a greater degree of ID.

Experiment B

Body weight. During the 3 months of treatments, the animals on KI-supplemented LID, namely the LID + 0.5, LID + 1.0, and LID + 5.0 groups, increased similarly in body weight (BW) by 64.4 ± 2.0, 60.80 ± 2.3, and 63.4 ± 8.9 g, respectively. The BW of the LID and LID′ groups increased significantly less, by 51.5 ± 2.0 and 36.2 ± 2.7 g, respectively; the difference between the latter two groups was also significant.

Thyroid gland. Figure 1 shows the results corresponding to the thyroids of the five groups of experiment B. In this and successive figures, the results have been normalized by expressing them as percentages of the mean values for the C group, which are detailed in the figure legend.

All values shown in Fig. 1 for the LID + 1.0 group were already different from those of C animals including the weight of the gland. The exceptions were the "Total" T_3 to "Total" T_4 ratio and the activity of the D1 isoenzyme, which, however, were different from C animals in LID + 0.5 rats. The weight of the thyroid increased up to 2.5-fold in the LID′ rats, with a 2-fold increase being observed already in the LID + 1.0 group. The total I content of the gland decreased with I intakes to an LID′ value that was 5% of that of the C animals. The "Total" T_4 and "Free" T_4 also decreased markedly and to a similar degree, reaching values of 1.5 and 1.8% of the C value, respectively, in LID′ rats. In contrast, the "Total" T_3 and "Free" T_3 followed different patterns of change from those observed for T_4. After a marked decrease in LID + 1.0, compared with C animals, the "Total" T_3 and "Free" T_3 remained relatively constant despite a marked decrease in I content. In LID′ rats, "Total" T_3 and "Free" T_3 were, respectively, 8.5 and 23% of C value, much higher than those for "Total" T_4 and "Free" T_4.

The ratios of both "Total" T_3 and "Free" T_3 to T_4 increased with decreasing I availability. The increase was, however, much more marked (17-fold) for the ratio of "Free" T_3 to T_4 than that found for the ratio between "Total" T_3 to T_4 (7.5-fold). This discrepancy may, at least partly, be accounted for by the increase in D1 activity in the gland that could generate "Free" T_3 from the "Free" T_4 but would not affect the iodothyronine residues still incorporated by peptidic bonds into thyroglobulin and other proteins.

Circulating T_4, FT_4, T_3, and TSH

The changes occurring in the plasma of the different groups of rats as I availability decreases are shown in Fig. 2.

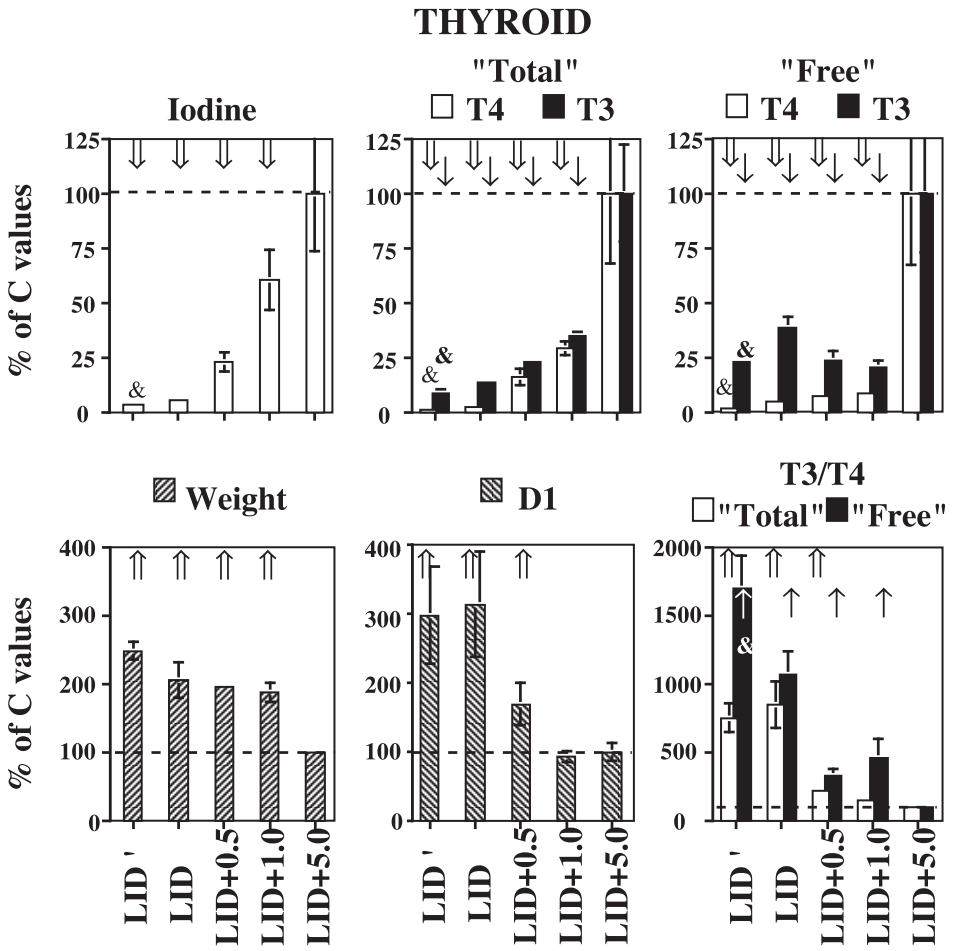

FIG. 1. Changes in thyroid weight, total iodine, T_4, and T_3 contents as well as T_3 to T_4 ratios and total D1 activities in groups of rats with a decreasing iodine intake. "Total" T_4 and T_3 correspond to residues incorporated into thyroglobulin and other proteins by peptidic bonds, whereas "Free" T_4 and "Free" T_3 are those present in the gland as iodoamino acids, available for secretion as hormones into the bloodstream. All values are normalized by taking as 100% the mean value of the corresponding variable in the controls (LID + 5.0 group of animals); weight: 23 ± 1.2 mg; total I: 3.19 ± 0.85 μg/gland; "Total" T_4 and T_3: 4053 ± 1297 and 212 ± 47 ng/gland; "Free" T_4 and "Free" T_3: 139 ± 46 and 15.7 ± 4.3 ng/gland; "Total" T_3 to "Total" T_4 ratio: 0.045 ± 0.007; "Free" T_3 to "Free" T_4 ratio: 0.111 ± 0.026; and D1: 1288 ± 175 pmol/min per gland. *Arrows* identify statistically significant increases or decreases *vs.* the C group; & and **&** identify statistically significant differences between the mean values for LID′ and LID animals. The *black arrows* and *bold &* correspond to the variables shown as *black bars*, the *white arrows* and *&* to those represented by *white* or *striped bars*.

Plasma T_4 is lower in LID + 0.5 rats, compared with controls (LID + 5.0 group), but the difference was not statistically significant for the LID + 1.0 animals. It further decreases with increasing ID, reaching values in LID′ animals that were only 5% of C levels. FT_4 is already decreased in the LID + 1.0 rats, compared with controls. The pattern of changes in circulating T_3 is quite different from that described for T_4. When the I intake decreases from LID + 5.0 to LID + 1.0, circulating T_3 actually increases and then decreases again to C values in the LID + 0.5 and LID groups. Only in the LID′ animals did plasma T_3 decrease, to 45% of C values, a change that contrasts with the much more pronounced decrease observed for circulating T_4. As a consequence, the T_3 to T_4 ratio is already increased above C values in the LID + 1.0 animals and further increased, more than 10-fold, in LID′ rats.

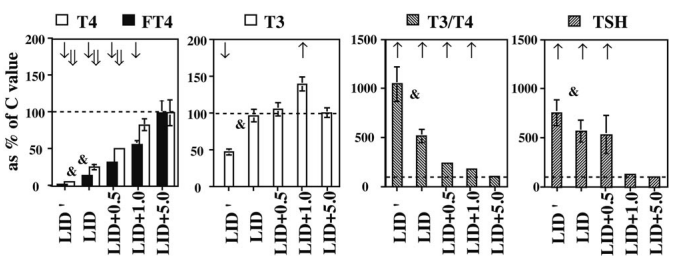

FIG. 2. Changes in plasma T_4, FT_4, and T_3, in the plasma T_3 to plasma T_4 ratio and circulating TSH in groups of rats with decreasing I availability. All values are normalized by taking as 100% the mean value of the corresponding variable in the controls (LID + 5.0 group of animals; T_4: 22.9 ± 3.9 ng/ml; FT_4: 29.6 ± 0.4 pg/ml; T_3: 0.31 ± 0.02 ng/ml; T_3 to T_4: 0.0135 ± 0.0015; TSH: 1.25 ± 0.16 ng rTSH RP-3/ml. The meaning of symbols is the same as in Fig. 1.

The mean circulating TSH value was higher in the LID + 1.0 animals, compared with C values (1.69 ± 0.27 *vs.* 1.25 ± 0.16 ng rTSH RP-3/ml, respectively), but the difference did not reach statistical significance ($P = 0.198$) with present sample sizes. It then increased progressively, 10-fold in LID′, compared with C animals, with a 7-fold increase in the LID + 0.5 group. Plasma TSH was negatively correlated to T_4 ($P = 0.0012$) but not to T_3. Partial correlation analysis disclosed that the relation between circulating TSH and T_4 was independent from T_3 levels. Plasma TSH, however, was negatively correlated to both pituitary T_4 and T_3 ($P < 0.000$ for both). Moreover, the degree of increase in TSH was unexpectedly small, even in the LID′ animals, compared with that observed in rats with a similar degree of hypothyroxinemia caused by primary thyroid failure, when T_4 and T_3 decrease concomitantly, and circulating TSH is negatively and independently correlated to both plasma T_4 and T_3. This is illustrated in Fig. 3, in which the changes in plasma TSH of the present animals are compared with those of sex- and weight-

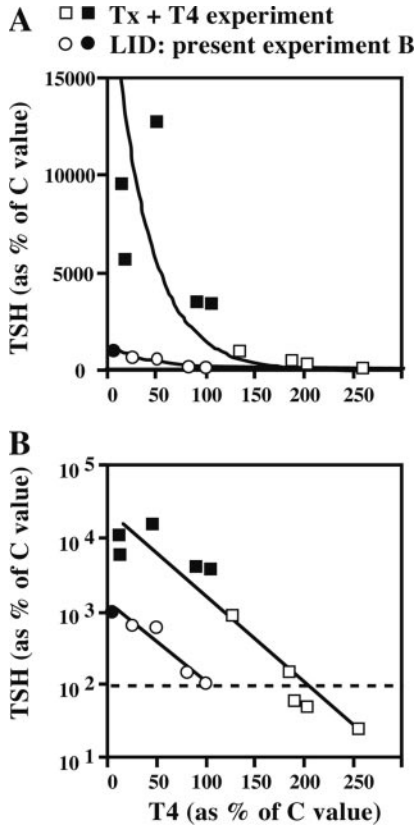

FIG. 3. A, Relationships between plasma TSH and T_4 in rats under two different experimental conditions, namely experiment B and those from a model of primary thyroid failure, namely Tx age- and weight-paired rats infused with different doses of T_4 (15). B, Results on a log scale for TSH. *Symbols in black* identify groups with circulating T_3 that was decreased with respect to the corresponding C value: the LID + 5.0 group for the LID experiment B and intact rats for the Tx + T_4 experiment.

paired animals that had been thyroidectomized (Tx) and infused with different doses of T_4 (15). The latter animals showed plasma T_3 changes parallel to those of T_4, whereas in the LID animals, T_3 was still normal with plasma T_4 and FT_4 levels that decreased to 25 and 15%, respectively, of C values. Figure 3B shows that the plasma TSH increase is blunted, more than 10-fold, in the LID animals, compared with that observed in the Tx rats on T_4.

Extrathyroidal tissues. Figure 4 illustrates the changes of the concentrations of T_4 and T_3 in the liver, lung, brain, and BAT occurring with decreasing I availability as well as the D1 activities in liver and lung and the D2 activities in brain and BAT. In these tissues the concentrations of T_4 decreased following a pattern similar to that observed for plasma T_4 or FT_4. The concentrations of T_3 in liver and BAT followed patterns similar to those of circulating T_3, with an initial increase from the LID + 5.0 to LID + 1.0 animals, normal concentrations in the LID + 0.5 and LID groups, and lower-than-normal levels in the LID′ rats. In the lung the increase in T_3 concentrations persisted in all groups, including the severely iodine-deficient LID′ animals that had a decreased plasma T_3. The pattern of changes for T_3 in the brain were different from those in liver, lung, and BAT: T_3 decreased with decreasing I availability and already did so in groups with normal circulating T_3 but low T_4.

D1 activity in liver and lung showed only small changes in the LID group, which did not persist in LID′ rats. In contrast, the activity of D2 in the cerebral cortex and BAT increased, with the differences between the LID + 1.0 and the C groups being statistically significant. The activities of the D2 isoenzyme increased up to 9.5- and 7-fold in the cerebral cortex and BAT, respectively, of LID′, compared with C rats.

Figure 5 summarizes the changes observed in T_4 and T_3 in cerebellum, pituitary, kidney, ovary, adrenal, heart, and muscle with decreasing I availability. T_4 decreased in all

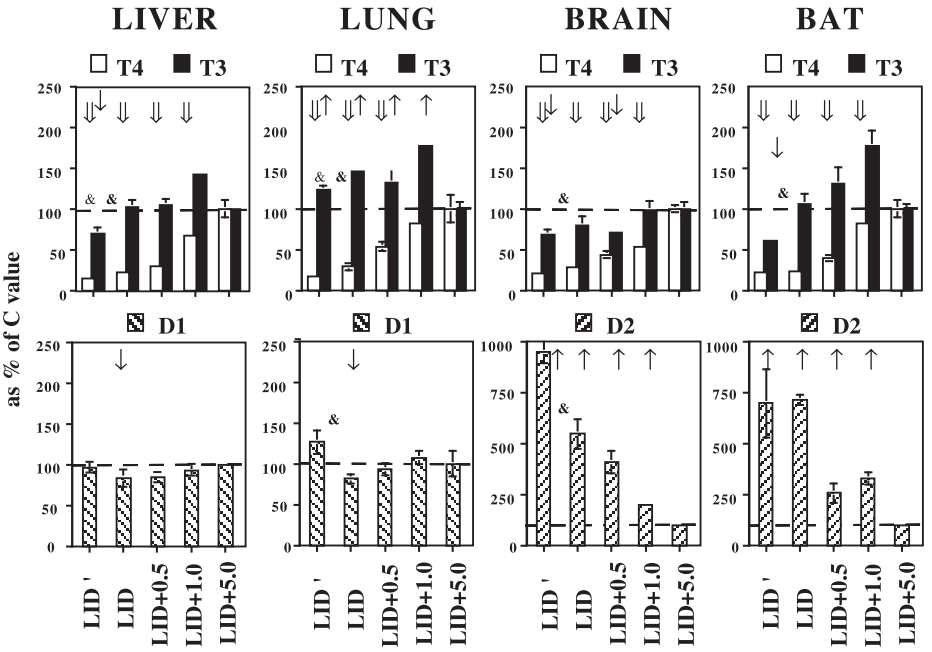

FIG. 4. Changes in the concentrations of T_4 and T_3 and D1 and D2 activities in the liver, lung, brain, and BAT with decreasing I availability. The values corresponding to controls (LID + 5.0 group) were: liver, 40.00 ± 4.23 ng T_4/g, 3.59 ± 0.12 ng T_3/g, 47.5 ± 1.4 pmol/min per mg protein (D1); lung, 8.08 ± 1.35 ng T_4/g, 1.71 ± 0.15 ng T_3/g, 528 ± 84 fmol/h per mg protein (D1); brain, 1.81 ± 0.09 ng T_4/g, 2.44 ± 0.20 ng T_3/g, 10.9 ± 1.4 fmol/h·mg protein (D2); BAT, 2.04 ± 0.21 ng T_4/g, 3.02 ± 0.18 ng T_3/g, 42 ± 6 fmol/h·mg protein (D2). The meaning of symbols is the same as in Fig 1.

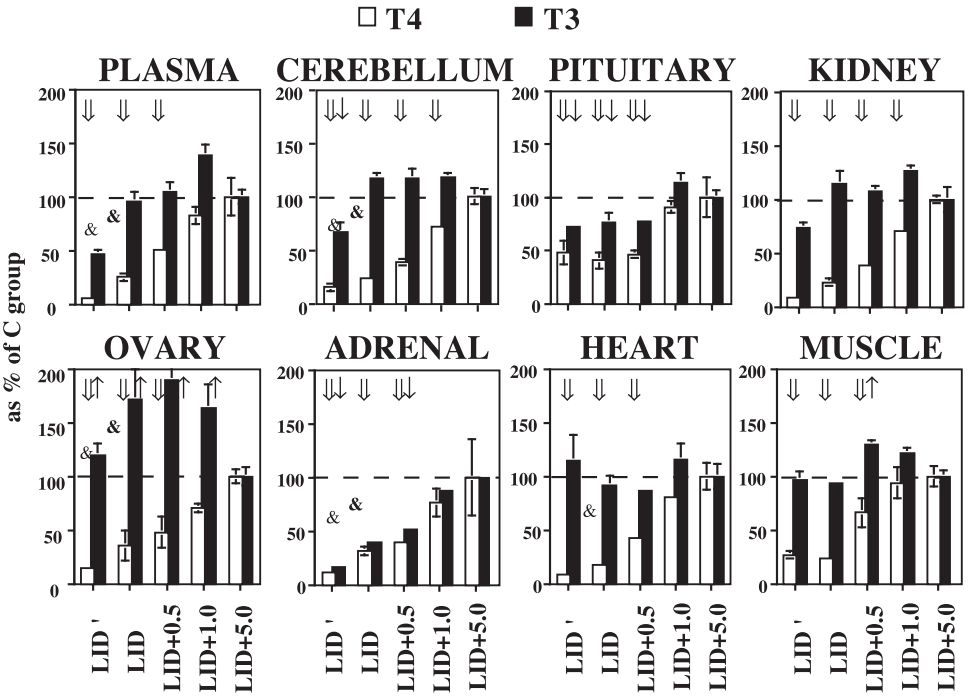

FIG. 5. Changes in the concentrations of T_4 and T_3 in the cerebellum, pituitary, kidney, ovary, adrenal, heart, and muscle with decreasing I availability. The *upper left panel* shows the corresponding plasma concentrations for comparison. The values corresponding to controls (LID + 5.0 group) were: cerebellum, 3.98 ± 0.31 ng T_4/g and 2.18 ± 0.14 ng T_3/g; pituitary, 54 ± 10 pg T_4, 93 ± 6 pg T_3; kidney, 20.70 ± 0.80 ng T_4/g, 7.08 ± 0.79 ng T_3/g; ovary, 1.18 ± 0.07 ng T_4, 0.15 ± 0.01 ng T_3; adrenal, 0.30 ± 0.04 ng T_4, 0.20 ± 0.07 ng T_3; heart, 3.82 ± 0.49 ng T_4/g, 1.54 ± 0.18 ng T_3/g; muscle, 1.91 ± 0.19 ng T_4/g, 1.18 ± 0.06 ng T_3/g. The values shown for the pituitary, ovary, and adrenal are the total T_4 and T_3 contents because the recorded weights were considered unreliable. The meaning of symbols is the same as in Fig. 1.

these tissues following patterns similar to those of plasma T_4 or FT_4. As already described for the liver, lung, brain, and BAT (Fig. 4), the patterns of change in T_3 varied greatly among these other tissues. The greatest difference was found between the patterns for the ovary and adrenal. In the ovary there is a very remarkable increase of T_3 in the animals on LID + 1.0, LID + 0.5, and LID, compared with LID + 5.0, with an almost 2-fold increase in the LID + 0.5 group. Ovarian T_3 is still higher than that of the controls, even in the LID′ group, in which serum T_3 was decreased by about 50%.[1] On the contrary, T_3 in the adrenal decreases steadily with decreasing I availability, almost in parallel to adrenal T_4, and more markedly than circulating T_3: values in the adrenals of LID′ animals are only 17% of those of the C group, whereas plasma T_3 is still 46%. As already described above for the lung, the muscle and heart maintained normal T_3 concentrations, even in LID′ animals. In these animals, T_3 decreased only in the cerebellum, pituitary, and kidney and did so only to 67–73% of C values, less than the decrease in circulating T_3.

Discussion

The experimental design

The I intake of the female rats on LID + 5.0 μg/d was sufficient for adequate growth. The I content of the stock pelleted diet used in our breeding facilities was determined and found to vary between 3 and 7 μg I per 20 g. The daily thyroidal secretion rate of strain-, sex-, and age-paired rats on this diet is estimated as being equivalent to 0.9 μg T_4 + 0.15 μg T_3 per 100 g BW (23), which together contain 0.59 μg I from T_4 + 0.09 μg I from T_3 = 0.68 μg of I per 100 g BW. Present rats weighed less than 300 g at the end of the experiment, and turnover of hormonal I should be 2.04 μg I or less per day. Thus, a daily supplement of 5.0 μg I ought to cover physiological requirements, a conclusion in agreement with previous findings by others (8), namely that an I intake of 3 μg/d or higher already ensures maximum levels of T_4 and T_3 in rat tissues.

The composition of the LID was the same for the five groups of experiment B, with the I content of the daily rations being the single controlled variable from the LID + 5.0 to the LID group. Results of experiment A also support that the amount of I available to the thyroid is the single variable between the LID′ group and the other animals because the very low amount of added $KClO_4$ (1 mg/d) did not have, *per se*, any effect on thyroid hormone status in the I-sufficient (LID + I) groups.

Our experimental design therefore avoided differences between the experimental groups that could be related to sex, strain, and nutritional composition of the diet, all of which are known to affect thyroid hormone status.

As far as could be assessed from the increment in BW, the decrease in I availability did not affect the general condition of the animals until it became severe (LID and LID′ groups). Despite their smaller increase in BW, even the animals of the LID′ group did not show clinical signs of hypothyroidism comparable with those of animals that are thyroidectomized and stop growing within a few weeks. This is in agreement with previous results from this laboratory using the same strain of animals and type of LID (16, 18).

[1] As indicated in *Materials and Methods*, the ovarian extracts were purified by a modified protocol to increase recovery of the iodothyronines. The possibility that interfering substances had been carried over into the T_3 RIA samples was tested. Extracts of the ovaries from the LID′ animals were tested at six different successive 2-fold dilutions. The plot of the specifically antibody-bound tracer *vs.* the log of the aliquot volume was parallel to that of obtained with the T_3 standard. This result confirmed that T_3, and not artifacts, was being measured in the ovaries.

Response to different grades of ID

Present findings fully confirm that the mechanisms involved are clearly dependent on the degree of I availability to the thyroid. For this reason, we will discuss them separately for animals with mild (LID + 1.0 group), moderate (LID + 0.5 group), severe (LID group), and very severe (LID′ group) ID (Table 2).

Mild ID: LID + 1.0 group

Intrathyroidal response mechanisms predominate when the daily I intake is reduced from 5 to 1 μg, no longer sufficient to compensate for daily requirements. This is confirmed by a notable increase in thyroid weight that already accounts for a major part of the total increase observed with decreasing I availability. There is also an increase in the thyroid "Free" T_3 to "Free" T_4 ratios. We wish to point out that these changes occur without an increase in circulating TSH. This finding initially surprised us until we reviewed earlier studies in rats on an I intake similar to that of our LID + 1.0 group (6, 9, 12, 24–27): An increase in vascularity, blood flow, I trapping, acinar cell height, and hyperplasia of the gland have all been described to occur without any significant increase in circulating TSH. The same occurs with the changes in intrathyroid I metabolism, which lead to a preferential synthesis and secretion of T_3 over T_4, and an increased T_3 to T_4 ratio in the circulation. These changes are correlated to the degree of I depletion and are the opposite of the TSH-independent response of the thyroid exposed to a sudden I excess (28). Their independence from serum TSH has been confirmed in hypophysectomized rats fed LID (24, 26). Our present data, therefore, confirm an important role of thyroid autoregulatory responses in the efficient adaptation to a mild degree of ID.

The mechanism(s) involved in these autoregulatory processes have not yet been identified. It is possible that a reduced availability of I decreases the thyroid content of iodolactones, which are involved in TSH-independent hyperplasia of the gland (29). A possible role of the sodium/iodide symporter in ID has hardly been addressed experimentally in rats, with the exception of a report (30) that its expression increased in the thyroid of ID fetuses with normal plasma TSH.

Extrathyroidal response mechanisms involved in these mildly ID rats are less evident than the intrathyroidal autoregulatory ones that result in the higher plasma T_3 to T_4 ratio, resulting mainly from the preferential secretion of T_3 over T_4. The patterns of the changes in the concentrations of T_4 and T_3 in the tissues studied here, however, appear to be tissue specific and not easily predictable from the change in circulating T_4 and T_3. A similar conclusion was reached years ago by Heninger and Albright (8), who measured the concentrations of T_4 and T_3 in several tissues of rats on a diet with an I content similar to that of the present LID + 1.0 group: they also found that T_3 increased in plasma and many tissues but not all (*i.e.* the brain) and that the degree of change was tissue specific. Extrathyroidal responses appear to be involved, as shown by the increased D2 activities in brain and BAT.

The increase in T_3 concentration in many of the tissues studied here, such as the liver, lung, kidney, and muscle, were predictable from their known major dependency on plasma-derived T_3, Similarly, the lack of an increase in the brain, cerebellum, and pituitary would be expected from their dependency on locally generated T_3 by D2 deiodination of T_4 (31). BAT, however, presented some unexpected features, being usually included among the tissues that derive intracellular T_3 locally from T_4 by D2. In these tissues D2 activity increases when T_4 decreases. Indeed, this is what occurred in the BAT of mildly I-deficient rats (see Fig. 4), as expected. Not anticipated, however, was the marked increase in BAT T_3, comparable with that in plasma, and not observed in other experimental models (32). Whichever the mechanisms involved, the increased generation of T_3 in BAT may actually contribute, together with the preferential secretion of T_3 by the gland, to the marked increase in systemic T_3 of the LID + 1.0 rats (33).

An increased activity of D2 in the brain had been expected from the decrease in brain T_4, a response amply shown in rats on LID with markedly decreased plasma T_4 (34), but present results show that D2 responds to a much milder degree of ID than previously described.

The lack of increase of T_3 content of the adrenals had also not been anticipated because this tissue has been considered dependent on serum-derived T_3 (31, 32). The marked increase in the concentration of T_3 in the ovary, a tissue not previously included in other studies (31), suggests its dependence on plasma-derived T_3.

In summary, both intra- and extrathyroidal mechanisms are involved in the response of the rat to mild ID: the former are autoregulatory and very effective in avoiding T_3 deficiency in most tissues, and the latter occur in tissues in which D2 is important for local generation of T_3. In mild ID, hypothyroidism, as inferred from the concentrations of T_3, is avoided in all tissues studied.

TABLE 2. Mean values of thyroid weight and I content and circulating T_4, FT4, T_3, and TSH, expressed as percent of the control values (LID + 5.0 group), used in the present study to define different grades of ID

Grade of ID	Group	I, μg/d[a]	Thyroid weight	Thyroid I	Plasma T_4	Plasma FT_4	Plasma T_3	Plasma TSH
No ID	LID + 5.0 (controls)	5.0	100	100	100	100	100	100
Mild	LID + 1.0	1.0	187 ↑	61 ↓	82	57 ↓	139 ↑	135
Moderate	LID + 0.5	0.50	196 ↑	23 ↓	51 ↓	32 ↓	105	593 ↑
Severe	LID	0.052	204 ↑	5 ↓	25 ↓	15 ↓	96	634 ↑
Very severe	LID′	0.021	248 ↑	6 ↓	5 ↓	2 ↓	46 ↓	976 ↑

[a] Estimated values; see Table 1.

↑ and ↓, Significant increase or decrease *vs.* LID + 5.0 group.

Moderate ID: LID + 0.5 group

There is a further decrease in the I, "Total" T_4, and "Total" T_3 contents of the thyroid to about 25% of control values. A new intrathyroidal-adaptive mechanism becomes evident, namely an increase in D1 activity, which could deiodinate the "Free" T_4 fraction and the plasma T_4 entering the gland. This could prevent a further decrease of the "Free" T_3 content and contribute to maintenance of a normal plasma T_3 as effectively as, or more than, the preferential intrathyroidal synthesis of T_3. We do not know whether the increase in D1 activity is an autoregulatory mechanism or the consequence of the increase in circulating TSH observed in this group. In contrast, thyroid weight is hardly affected by the increase in TSH, a finding consistent with the concept that thyroid growth is mainly determined by autoregulatory processes in both mild and moderate ID.

Plasma T_3 was no longer elevated in the moderately ID animals but remained normal despite a 50% decrease of T_4. T_3 deficiency was prevented in tissues that derive T_3 mostly from plasma and also in BAT and cerebellum. Unexpectedly, despite normal T_3 and very low T_4 in plasma, some tissues maintained high T_3 levels, most notably the lung, ovary, and muscle. The underlying mechanisms have not been identified: in the lung, for instance, an increase in D1 activity was not involved. In the brain T_3 decreased, despite the increased D2 activity, and so did pituitary T_3. As already noted in the mild ID group, T_3 decreased in the adrenal in parallel with T_4.

In summary, the case of moderate ID, intra- and extrathyroidal responses are still adequate to prevent low T_3 levels in plasma and most tissues, despite a reduction of the I intake to 25% of that of controls. Some tissues even maintain elevated T_3 concentrations, whereas others are markedly (adrenal) or moderately (brain and pituitary) T_3 deficient.

Severe I deficiency: LID group

The intra-thyroidal response mechanisms operative in previous groups continue to minimize the effects of a further marked decrease of the I intake and circulating T_3 remains normal. A role is also likely to be played by the marked increase in thyroid D1 activity, which would avoid a further decrease of the "Free" T_3 concentration. This increase occurs without a further concordant increase in TSH; it might be an autoregulatory process, but the influence of the higher than normal serum TSH cannot be excluded.

Despite a major decrease in plasma T_4 to 25% of control values, T_3 concentrations not only remained normal in plasma, but also in most tissues. The role of a further increase of D2 activity is evident in those tissues where it was measured. Again, the most unexpected and striking results are those obtained for the concentration of T_3 in the ovary and lung, where it is much higher, and in the adrenal, where it is much lower, than expected from the normal circulating T_3.

In summary, despite a 100-fold decrease in I availability, a combination of intra- and extrathyroidal adaptive mechanism still mitigates T_3 deficiency and presumably hypothyroidism in most but not all tissues.

Very severe ID: LID′ group

Intrathyroidal adaptive mechanisms are no longer sufficient in LID′ rats to ensure a normal T_3, which decreases in plasma to 45% of C values, and also in many tissues that depend on plasma-derived T_3, including the liver. Despite the marked increase in D2 activity, the brain, cerebellum, pituitary, and BAT are T_3 deficient, probably because of the very low availability of plasma T_4 that has decreased to 5% of normal values. We have previously shown that the brain, pituitary, and liver of such animals are indeed hypothyroid, as assessed from several biological end points of thyroid hormone action (11). Tissue T_3, however, decreases less than would be expected from the circulating T_3 level (adrenals again excepted), and some tissues continue having normal (muscle, heart) or even elevated (lung, ovary) T_3 concentrations.

In summary, the threshold I availability below which most tissues are T_3 deficient appears to be reached when the intra- and extrathyroidal adaptive mechanisms are no longer capable of ensuring a normal circulating T_3. But even then, adaptive mechanisms that protect most tissues, and especially the heart, muscle, and ovary, become operative from the degree of T_3 that would be expected from the decrease in plasma T_3.

In the present study, we did not measure the activities of the iodothyronine deiodinase isoenzymes in most tissues or activities of other enzymes, such as sulfotransferases and sulfatases, or the concentrations of T_4 and T_3 sulfates (35) that might regulate the local availability of T_3 in different tissues. Also not investigated were the possible adaptive roles of changes in tissue uptake and/or exit rates of the iodothyronines (36) that might further minimize T_3 deficiency in tissues. In this context, it is interesting that tissue to plasma T_3 and T_4 ratios were increased in some tissues of the LID rats and in most of those of the LID′ group (data not shown).

T_4, T_3, and TSH feedback in ID

We wish to draw attention to the findings illustrated in Figs. 2 and 3 that clearly show that in conditions of decreased I availability, plasma-derived T_3 plays a very decisive role in the regulation of circulating TSH. The TSH response is one tenth, or less, that observed in rats with primary thyroid failure and comparable degrees of hypothyroxinemia but with parallel decreases in plasma T_3 (15). We believe it is very important and probably quite relevant for inhabitants of areas with mild and moderate ID to realize that a normal plasma TSH does not exclude, *per se*, a selective T_3 deficiency in tissues, such as the brain, that are affected by the decreased availability of T_4 as substrate for the local generation of T_3. There are also other experimental situations, in which serum TSH is more closely correlated to circulating T_3 than T_4 (37). Such findings also point to a more important role of plasma-derived T_3 in the inhibition of TSH release, compared with that of T_3 generated locally from T_4 by D2.

Present results constitute a clear contradiction to the previous idea that findings in I-deficient animals and humans, in which a close negative correlation is found between circulating TSH and T_4, despite normal T_3, are a prime example of the beneficial adaptive role of the local generation of T_3

from T_4: it was, moreover, difficult to explain the adaptive advantage of the observed increase in D2 activity in the pituitary because it would increase locally generated T_3, shutting down the compensatory mechanism, namely an increase in plasma TSH.

Conclusion

ID rats are endowed with numerous and very efficient adaptive mechanisms, most of which require a fully functioning normal thyroid gland, and are thus lost in animals with primary thyroid failure. ID rats are often considered to be either hypothyroid, because of their low circulating T_4 and increased TSH, or euthyroid, because of their normal (or increased) plasma T_3, but present results stress that neither assumption is correct: thyroid hormone status is not only related to the degree of depletion of I availability to the thyroid but is also eminently tissue specific. As discussed elsewhere (15), few tissue-specific direct effects of thyroid hormone action are available, and we measured the concentration of T_3 in the tissues as a first step in assessing their thyroid hormone status. With a moderate, or even severe, I deficiency, most tissues depending on plasma T_3 would have normal, or even slightly elevated, T_3 concentrations, most notably the ovary, lung, heart, and muscle. However, those tissues that depend to an appreciable extent on T_4 for the local generation of T_3 are protected from T_3 deficiency to a lesser degree. As a consequence, in the latter type of tissue, thyroid hormone-sensitive functions are more likely to be affected than those characteristic of tissues depending on plasma-derived T_3. Such is the case of the brain, for instance, and cerebral functions may already be impaired in moderate ID because brain T_3 is already decreased. In the mildly ID group, in which plasma TSH was normal and plasma FT_4 was slightly decreased, total brain T_4 was decreased and D2 activity was increased. This prevented a decrease of T_3 in the total brain but not necessarily in all brain areas (13, 38). Present results also indicate that the degree of increase in D2 activity in different cerebral structures of ID rats does not permit, *per se*, conclusions to be drawn regarding their protection from T_3 deficiency because the latter also involves the amount of T_4 available in each area (13).

Circulating T_3 has to decrease before many T_3-dependent tissues become T_3 deficient. This appears to occur when circulating T_4 decreases from 25 to 5% of normal values. But even under such conditions, the many known and as-yet-undefined intra- and extrathyroidal adaptive mechanisms are efficient enough to maintain euthyroidism in muscle and heart and even slightly elevated T_3 in lung and ovary. The findings in the ovary may underlie the observation that even very severely I-deficient animals are easily mated, do not show decreased fertility, and bear litters of normal size (16, 18), in contrast to Tx or goitrogen-treated hypothyroid females (19).

Possible implications for man

As already pointed out in the introductory text, the present study is relevant only for inhabitants of areas in which ID is the sole cause of goiter but not for areas in which other environmental factors may lead to destruction of the gland and therefore to clinical hypothyroidism. We believe present results in mild and moderate grades of decreased I availability are especially relevant for man because such conditions are still widespread in Western industrialized countries (39).

Many of the findings in rats, described here and by others, have also been described in people from areas with an adequate I intake who are changed to an I-deficient diet or for inhabitants of the ID areas defined above. Thus, for instance, the gland responds within a few days with a striking increase in blood flow, occurring before any change is detected in plasma T_4 or TSH (40). Increased I trapping and circulating T_3 to T_4 ratios have also been shown (3). In simple sporadic goiter, the increase in thyroid volume occurs without a necessary increase in circulating TSH (41–44). Even in very severe ID, the increase in circulating TSH is markedly blunted, compared with that usually observed in hypothyroid patients (43–46). Missler *et al.* (45) reported that in 304 children from an ID area, 60% had an enlarged gland, but only 9% had TSH greater than 4.5 mU/liter. In the seminal studies by Glinoer (47) on thyroid function in pregnant women from a population with mild to moderate ID, TSH levels above the normal reference range were hardly ever found at any stage of pregnancy, even among the women with the lowest first-trimester FT_4 levels. The same was observed in pregnant women from an area with very mild ID (48, 49).

Western-trained physicians dealing mostly with patients with primary thyroid failure rely on an increased circulating TSH for their classification of overt or subclinical hypothyroidism and are often unfamiliar with the concept of hypothyroxinemia: a decreased T_4 without an increase of TSH above normal. The TSH-independent autoregulatory mechanisms controlling thyroid function in ID are often overlooked, as these mechanisms are better known in the case of iodine excess. The present experimental data obtained with mild to moderate grades of ID stress the primary importance of autoregulatory mechanisms that protect many tissues from overt T_3 deficiency. Even in very severe ID (2), people are able to sustain heavy physical work and have normal cardiac function, observations that might be related to the present findings in muscle and heart, which maintain normal T_3 concentrations, even in the LID′ group.

As discussed elsewhere in more detail (49), it is inaccurate to assume that inhabitants of ID areas are clinically hypothyroid individuals. The present experimental model supports the epidemiological findings that inhabitants of ID areas are not clinically hypothyroid individuals because their normal circulating T_3 ensures normal T_3 concentration in most tissues. But, as shown here, this might not avoid selective T_3 deficiency of those tissues, such as the brain, that depend mostly on T_4 for their intracellular T_3 supply. In man, this might already negatively affect mental functions in mild ID (5, 39, 46, 49–51), in which inhabitants are often described as dull (52).

It is often assumed that eradication of severe ID is enough to avoid the most important IDD, including those affecting mental processes. Present experimental results obtained in moderately ID rats stress that this is not so, and countries with areas of moderate ID should actively correct it: an im-

portant proportion of their inhabitants, and their future progeny, may still suffer from easily preventable impairments of mental functions and the consequent socioeconomic implications (49) as well as the increased incidence of thyroid disorders ensuing from thyroid hyperplasia.

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References

1. **Hetzel BS** 1983 Iodine deficiency disorders (IDD) and their eradication. Lancet ii:1126–1129
2. **Hetzel BS** 2002 Eliminating iodine deficiency disorders—the role of the International Council in the global partnership. Bull World Health Organ 80: 410–413
3. **Delange F** 2000 Iodine deficiency. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid. Philadelphia: Lippincott Williams and Wilkins; 295–315
4. **Dunn JT, Delange F** 2001 Damaged reproduction: the most important consequence of iodine deficiency. J Clin Endocrinol Metab 86:2360–2363
5. **Delange F** 2001 Iodine deficiency as a cause of brain damage. Postgrad Med J 77:217–220
6. **Studer H, Greer M** 1965 A study of the mechanisms involved in the production of iodine-deficient goiter. Acta Endocrinol (Copenh) 49:610–628
7. **Silva E** 1972 Disposal rates of thyroxine and triiodothyronine in iodine-deficient rats. Endocrinology 91:1430–1435
8. **Heninger RW, Albright EC** 1975 Alteration in tissue and serum concentrations of TSH, iodide, T4 and T3 induced by various dietary iodide levels. Proc Soc Exp Biol Med 150:137–141
9. **Riesco G, Taurog A, Larsen PR** 1976 Variations in the response of the thyroid gland of the rat to different low-iodine diets, correlation with iodine content of diet. Endocrinology 99:270–280
10. **Okamura K, Taurog A, Krulich L** 1981 Elevation of serum 3,5,3′-triiodothyronine and thyroxine levels in rats fed Remington diets; opposing effects of nutritional deficiency and iodine deficiency. Endocrinology 108:1247–1256
11. **Santisteban P, Obregón MJ, Rodríguez-Peña A, Lamas L, Escobar del Rey F, Morreale de Escobar G** 1982 Are iodine-deficient rats euthyroid? Endocrinology 110:1780–1789
12. **Michalkiewicz M, Huffman L, Connors JM, Hedge GA** 1989 Alterations in thyroid blood flow induced by varying levels of iodine intake in the rat. Endocrinology 125:54–60
13. **Peeters R, Fekete C, Goncalvez C, Legradi G, Tu HM, Harney JW, Bianco AC, Lechan RM, Larsen PR** 2001 Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency. Am J Physiol Endocrinol Metab 281:E54–E61
14. **Contempré B, Morreale de Escobar G, Denef JF, Dumont JE, Many MC** 2004 Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-deficient rat thyroids: a potential experimental model for myxedematous cretinism in central Africa. Endocrinology 145:994–1002
15. **Escobar-Morreale HF, Escobar del Rey F, Obregon MJ, Morreale de Escobar G** 1995 Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. J Clin Invest 96:2828–2838
16. **Escobar del Rey F, Mallol J, Pastor R, Morreale de Escobar G** 1987 Effects of maternal iodine deficiency on thyroid hormone economy of lactating dams and pups: maintenance of normal cerebral 3,5,3′-triiodo-L-thyronine concentrations in pups during major phases of brain development. Endocrinology 121: 803–811
17. **Morreale de Escobar G, Pedraza PE, Escobar del Rey F, Obregón MJ** 1996 Thyroidal and extrathyroidal adaptation to graded degrees of iodine deficiency: an experimental rat model for the study of neurological iodine deficiency disorders (IDD). In: Braverman LE, Köhrle J, Eber O, Langsteger W, eds. Thyroid and trace elements. Vienna: Blackwell; 113–126
18. **Lavado-Autric R, Ausó E, Garcia-Velasco JV, Arufe MC, Escobar del Rey F, Berbel P, Morreale de Escobar G** 2003 Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111:1073–1082
19. **Morreale de Escobar G, Pastor R, Obregón MJ, Escobar del Rey F** 1985 Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues. Endocrinology 117:1890–1901
20. **Calvo RM, Jauniaux E, Gulbis B, Asunción M, Gervy C, Contempré B, Morreale de Escobar G** 2002 Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. Possible consequences of maternal hypothyroxinemia. J Clin Endocrinol Metab 87: 1768–1777
21. **Calvo R, Morreale de Escobar G, Escobar del Rey F, Obregón MJ** 1997 Maternal non-thyroidal illness and fetal thyroid hormone status, as studied in the streptozotocin-induced diabetes mellitus rat model. Endocrinology 138: 1159–1169
22. **Obregón MJ, Ruiz de Oña C, Calvo R, Escobar del Rey F, Morreale de Escobar G** 1991 Outer ring iodothyronine deiodinases and thyroid hormone economy: responses to iodine deficiency in the rat fetus and neonate. Endocrinology 129:2663–2673
23. **Escobar-Morreale HF, Escobar del Rey FE, Obregon MJ, Morreale de Escobar G** 1996 Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. Endocrinology 137:2490–2502
24. **Chapman A** 1941 The relation of the thyroid and pituitary glands to iodine metabolism. Endocrinology 29:680–685
25. **Vanderlaan WP, Caplan R** 1954 Observations on a relationship between total thyroid iodine content and the iodide-concentrating mechanism of the thyroid gland of the rat. Endocrinology 54:437–447
26. **Halmi NS, Sirtos BN** 1955 Analysis of the modifying effects of dietary iodine levels on the thyroidal response of hypophysectomized rats to thyrotropin. Endocrinology 56:157–162
27. **Greer MA, Rockie C** 1969 Effect of thyrotropin and the iodine content of the thyroid on the triiodothyronine:thyroxine ratio of newly synthesized iodothyronines. Endocrinology 85:244–250
28. **Nagataki S, Yokoyama N** 1996 Autoregulation: effects of iodide. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid. Philadelphia: Lippincott-Raven Publishers; 241–247
29. **Gärtner R, Dugrillon A, Bechtner G** 1996 Iodolipids and thyroid function and growth. In: Nauman J, Glinoer D, Braverman LE, Hostalek U, eds. The thyroid and iodine. Stuttgart, Germany: Schattener; 19–30
30. **Schröder-van der Elst J, van der Heide D, Kastellin J, Rousset B, Obregón MJ** 2001 The expression of the sodium/iodide symporter is up-regulated in the thyroid of fetuses of iodide-deficient rats. Endocrinology 142:3736–3741
31. **van Doorn J, Roelfsema F, van der Heide D** 1985 Concentrations of thyroxine and 3,5,3′-triiodothyronine at 34 different sites in euthyroid rats as determined by an isotopic equilibrium technique. Endocrinology 117:1202–1208
32. **Escobar-Morreale HF, Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 1999 Tissue-specific pattern of changes in 3,5,3′-triiodo-L-thyronine concentrations in thyroidectomized rats infused with increasing doses of the hormone. Which are the regulatory systems? Biochimie (Paris) 81:453–462
33. **Silva JE, Larsen PR** 1985 Potential of brown adipose tissue type II thyroxine 5′-deiodinase as a local systemic source of triiodothyronine in rats. J Clin Invest 76:2296–2305
34. **Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 2005 The effects of iodine deficiency on thyroid hormone deiodination. Thyroid 15:917–929
35. **Visser TJ** 1994 Sulfation and glucuronidation pathways of thyroid hormone metabolism. In: Wu S, Visser TJ, eds. Thyroid hormone metabolism. London: CRC Press; 117
36. **Friesema EC, Jansen J, Visser TJ** 2005 Thyroid hormone transporters. Biochem Soc Trans 33:228–232
37. **Emerson CH, Lew R, Braverman LE, DeVito WJ** 1989 Serum thyrotropin concentrations are more highly correlated with serum triiodothyronine concentrations than with serum thyroxine concentrations in thyroid hormone-infused thyroidectomized rats. Endocrinology 124:2415–2418
38. **Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TH, Hume R, Morreale de Escobar G** 2004 Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab 89:3117–3128
39. **Vitti P, Delange F, Pinchera A, Zimmermann M, Dunn JT** 2003 Europe is iodine deficient. Lancet 361:1226
40. **Arntzenius AB, Smit LJ, Schipper J, van der Heide D, Meinders AE** 1991 Inverse relation between iodine intake and thyroid blood flow: color Doppler flow imaging in euthyroid humans. J Clin Endocrinol Metab 73:1051–1055
41. **Barakat RM, Ingbar SH**1965 The effect of acute iodine depletion on thyroid function in man. J Clin Invest 44:1117–1124
42. **Vagenakis A, Koutras D, Burger A, Malamos B, Ingbar S** 1973 Studies of serum triiodothyronine, thyroxine and thyrotropin concentrations in endemic goiter in Greece. J Clin Endocrinol Metab 37:485–488

43. **Patel YC, Pharoah OD, Hornabrook RW, Hetzel BS** 1973 Serum triiodothyronine, thyroxine and thyroid-stimulating hormone in endemic goiter: a comparison of goitrous and nongoitrous subjects in New Guinea. J Endocrinol Metab 37:783–789
44. **Pharaoh POD, Lawton NF, Ellis SM, Williams ES, Ekins RP** 1973 The role of triiodothyronine (T3) in the maintenance of euthyroidism in endemic goitre. Clin Endocrinol (Oxf) 2:193–199
45. **Missler U, Gutekunst R, Wood WG** 1994 Thyroglobulin is a more sensitive indicator of iodine deficiency than thyrotropin: development and evaluation of dry blood spot assays for thyrotropin and thyroglobulin in iodine-deficient geographical areas. Eur J Clin Chem Clin Biochem 32:137–143
46. **Huda SN, Grantham-McGregor SM, Rahman KM, Tomkins A** 1999 Biochemical hypothyroidism secondary to iodine deficiency is associated with poor school achievement and cognition in Bangladesh. J Nutr 129:980–987
47. **Glinoer D** 1997 The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev 18:404–433
48. **Morreale de Escobar G, Escobar del Rey F** 2003 Consequences of iodine deficiency for brain development. In: deVijlder J, Morreale de Escobar G, eds. The thyroid and the brain. Stuttgart, Germany: Schattauer Verlag; 33–56
49. **Morreale de Escobar G, Obregón MJ, Escobar del Rey F** 2004 Role of thyroid hormone during early brain development. Eur J Endocrinol 151:U25–U37
50. **Vitti P, Aghini-Lombardi F, Chiovato L, Ferretti G, Pinchera A** 2003 Neuropsychological assessment in humans living in mild to moderate iodine deficiency. In: DeVijlder J, Morreale de Escobar G, eds. The thyroid and the brain. Stuttgart, Germany: Schattauer Verlag; 57–63
51. **Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, Castagna MG, Violi MA, Crisà A, Artemisia A, Trimarchi F** 2004 Attention deficit and hyperactivity disorders (ADHD) in the offspring of mothers exposed to iodine deficiency: a possible novel iodine deficiency disorder in developed countries? J Clin Endocrinol Metab 89:6054–6060
52. **Dunn JT** 1992 Iodine deficiency. The next target for elimination? N Engl J Med 326:267–268

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