EFFECTS OF STREPTOZOTOCIN-DIABETES AND L-THYROXINE TREATMENT ON TSH AND GH, AND CIRCULATING GLUCOSE AND GLYCEROL IN THYROIDECTOMIZED RATS

T. JOLÍN, C. GONZÁLEZ and E. HERRERA

Departamento de Endocrinologia Experimental, Instituto G. Marañón, Madrid, Spain and Departamento de Investigación, Centro Ramón y Cajal, Madrid, Spain. Telephone 729 00 00 (ext. 1077)

(*Received* 14 *January* 1983)

Abstract—1. The response to exogenous thyroxine in thyroidectomized rats made diabetic by treatment with streptozotocin was greatly impaired, as shown by their growth retardation and the lack of increase in plasma GH and pituitary GH and TSH concentrations.

2. Insulin administration partially compensated for these endocrine alterations in diabetic thyroidectomized rats. When these animals received enough exogenous thyroxine to normalize their plasma PBI and TSH levels, insulin administration did not decrease their augmented glucose and glycerol concentrations.

3. These findings show the permissive action between thyroid hormones and insulin although some effects of the former counteract those of insulin.

INTRODUCTION

Intense thyroid hormone deficiency in young rats results in profound impairment in growth rate (Evans, 1924; Evans *et al.*, 1958; Solomon and Greep, 1959) as a consequence of severe reduction in anterior pituitary function, except for TSH secretion which is actually augmented (Contopoulos *et al.*, 1958; Daughaday *et al.*, 1968; Escobar del Rey *et al.*, 1968; Greenspan *et al.*, 1949; Griesbach and Purves, 1945; Halmi, 1950; Knigge, 1958; Lewis *et al.*, 1965; Peake *et al.*, 1973). Thyroxine administration to the animals blocks these changes but sensitivity of the response in the different parameters is not always identical (Evans *et al.*, 1964; Griesbach and Purves, 1945). The thyroxine dose needed to restore normal growth and number of pituitary acidophils in thyroidectomized rats was much lower than that required completely to normalize the pituitary basophils. It has recently been shown (González *et al.*, 1980) that sensitivity to the inhibitory effect of thyroxine administration on the pituitary TSH secretion is augmented in thyroidectomized diabetic rats. The present study was performed to investigate whether this altered response to exogenous thyroxine in thyroidectomized rats made diabetic by treatment with streptozotocin is also present in other parameters such as circulating glucose, glycerol, protein-bound iodine levels, and both pituitary and plasma concentrations of GH and TSH. The work was extended to determine the effects on these parameters of small doses of exogenous insulin administered to the diabetic animals.

Correspondence should be addressed to: Dr. Emilio Herrera, Departamento de Investigación, Centro Ramón y Cajal, Crtra. Colmenar Km 9, Madrid-34, Spain.

MATERIALS AND METHODS

Animals

Male Wistar rats bred in our laboratory were used. They were kept in a constant temperature environment (22 ± 2°C) with automatic 12 hr light and dark cycles. A Remington low iodine diet (0.05 ± 0.01 μg ^{127}I/g of diet) and distilled water were administered *ad lib*. Surgical thyroidectomy was performed under ether anesthesia.

Experimental design

About 160 rats were thyroidectomized when weighing 90–100 g. They were weighed once a week and 30 days after the operation, those of stable weight were fasted for 24 hr and divided into two groups. One group of animals was made diabetic by i.p. injection of streptozotocin (Upjohn Co., Kalamazoo, Michigan): 4 mg/100 g body wt, freshly dissolved in citrate buffer, pH 4.5. This group was designated diabetic-thyroidectomized (D-Thx). The other group was treated with buffer and used as controls (C-Thx). Following these treatments, the animals were re-fed and 1 week later each group was divided into four sub-groups of matched body wt which for 7 days received a daily i.p. injection of either saline or an L-thyroxine (L-T_4) dose of 0.5, 1.0 or 2.0 μg/100 g body wt. The L-T_4 was dissolved in a drop of 0.1 N NaOH and diluted in saline containing 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri). During treatment with L-T_4, half of the D-Thx animals were injected daily s.c. with bovine insulin (Novo Industri A/S, Copenhagen), 0.5 IU/100 g body wt. Treatments with both L-T_4 and insulin were always performed between 0800 and 0900 hr. A group of age- and sex-matched intact animals, injected with saline, was studied in parallel with the thyroidectomized rats. All animals were sacrificed by decapitation 1 hr after the last injection. Blood was collected into heparinized tubes, centrifuged and the plasma was removed and stored at −20°C until assayed. Pituitaries were rapidly dissected and the anterior lobes weighed, homogenized in 2 ml of distilled water and kept at −20°C until assayed.

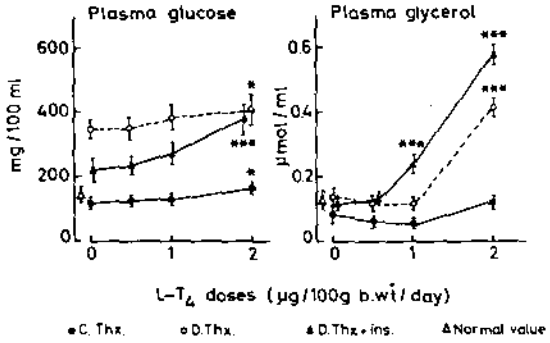

Fig. 1. Effects of replacement doses of L-thyroxine on blood glucose and glycerol levels in thyroidectomized control (●——●), thyroidectomized diabetic (○– – –○) and thyroidectomized diabetic rats treated with insulin (▲——▲) and normal intact controls (△). Values correspond to means ± SEM of 8–10 rats/group. Comparison between each group treated with thyroxine and the same group without this treatment is shown by asterisks: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Other statistical comparisons are indicated in the text.

Analytical procedures

Plasma aliquots were deproteinized (Somogyi, 1945) for glucose (Hugget and Nixon, 1957) and glycerol (Garland and Randle, 1962) evaluation by enzymatic procedures. Plasma protein-bound iodine (PBI) determinations were performed by using the Zak chloric acid digestion method (Benotti and Benotti, 1963). Pituitary and plasma GH and TSH contents were measured by the specific RIAs developed for the rat by NIAMDD of the NIH. Data are expressed in terms of the corresponding NIAMDD rat reference preparations. All samples for plasma and pituitaries were run in the same assay and estimated from the same GH and TSH standard curve.

Calculations

Data shown are means ± SEM. The degree of significance of differences between groups was calculated using the Student's *t*-test.

RESULTS

Values of plasma glucose and glycerol concentrations in the different groups are summarized in Fig. 1. Treatment of the thyroidectomized (Thx) animals with a single dose of streptozotocin made them diabetics (D-Thx), as shown by the significant increment in their plasma glucose levels as compared with the values in both intact normal controls (C-N), $P < 0.001$ and in Thx animals not treated with the drug (C-Thx), $P < 0.001$. Plasma glucose levels were significantly reduced in the D-Thx when injected with 0.5 IU of bovine insulin/100 g body wt for 7 days prior to sacrifice (D-Thx + Ins.), $P < 0.001$. Treatment of the Thx with either 0.5 or 1 µg of L-T_4 did not cause any significant change in their glycemia, but a 2 µg dose produced an increase in all groups (Fig. 1). This effect was greatest in the D-Thx + Ins. animals in which plasma glucose reached the levels of the D-Thx group. Plasma glycerol levels did not differ among the groups not receiving L-T_4 or treated with 0.5 µg of L-T_4. The 1 µg dose produced a significant increment in this parameter in the D-Thx + Ins. animals while the 2 µg dose produced a further increase in this group and a significant enhancing effect in the D-Thx animals, as compared with values in the same group without L-T_4 treatment (Fig. 1).

As shown in Fig. 2, body wts and plasma and pituitary concentrations of GH were much lower ($P < 0.001$) in the C-Thx, D-Thx and D-Thx + Ins. animals than in the C-N ones, while values did not differ among the first three groups. Responses to exogenous L-T_4 were quite different. In the C-Thx animals, the 0.5 µg dose produced a significant increment in body wt and in pituitary and plasma GH concentrations. This effect of pituitary GH concentration was progressively greater with doses of 1 and 2 µg, while plasma GH levels rose with 1 µg and did not increase further with the 2 µg dose. In the D-Thx animals, L-T_4 did not affect body wt, and only the

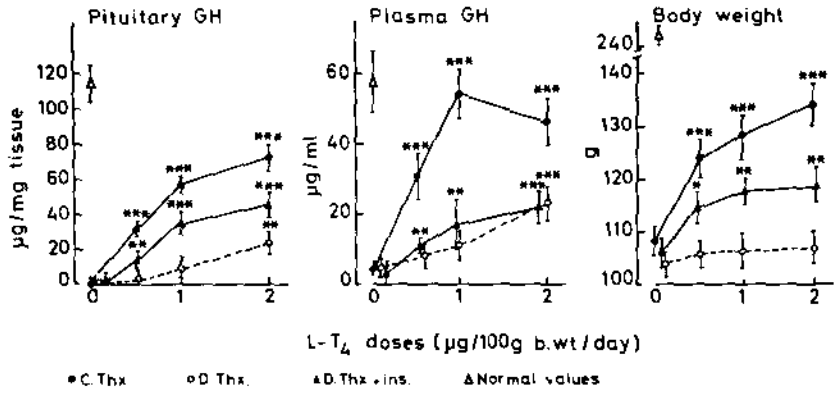

Fig. 2. Effects of replacement doses of L-thyroxine on body wt increase and pituitary and plasma GH concentrations in thyroidectomized control (●——●), thyroidectomized diabetic (○– – –○) and thyroidectomized diabetic rats treated with insulin (▲——▲) and normal intact controls (△). Values correspond to means ± SEM of 8–10 rats/group. Comparison between each group treated with thyroxine and the same group without this treatment is shown by asterisks: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Other statistical comparisons are indicated in the text.

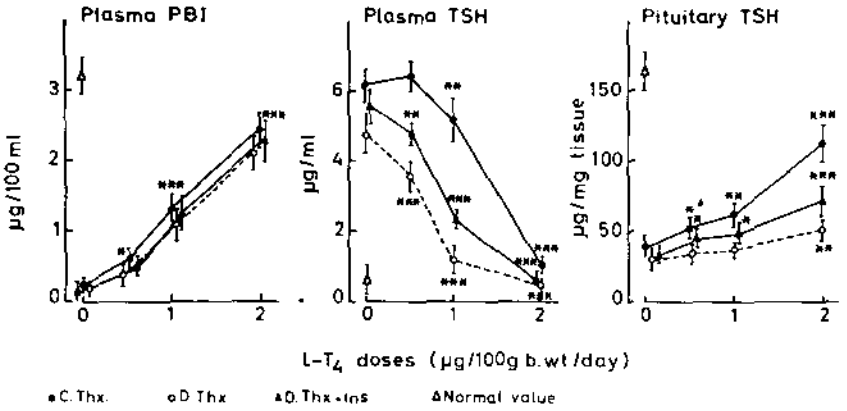

Fig. 3. Effects of replacement doses of L-thyroxine on plasma protein bound iodine (PBI) and plasma and pituitary TSH concentrations in thyroidectomized control (●——●), thyroidectomized diabetic (○- - -○) and thyroidectomized diabetic rats treated with insulin (▲——▲) and normal intact controls (△). Values correspond to means ± SEM of 8–10 rats/group. Comparison between each group treated with thyroxine and the same group without this treatment is shown by asterisks: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Other statistical comparisons are indicated in the text.

2 μg dose caused significant increases in GH levels (in both pituitary and plasma). Insulin administration to the D-Thx rats (D-Thx + Ins.) partially restored their response to L-T_4 in these parameters although the observed values were always below those of the C-Thx (Fig. 2).

To determine whether these differences correspond to differences in the thyroidal status of the animals, plasma PBI and TSH levels and pituitary TSH concentration were also measured (Fig. 3). Plasma PBI levels were much lower in all Thx groups than in the C-N animals ($P < 0.001$), with no difference between D-Thx and D-Thx + Ins. vs the C-Thx. In these groups, L-T_4 administration produced a similar dose–response rise in the plasma PBI values although their levels did not reach those of the C-N, even with the highest dose of L-T_4 administered (2 μg). Plasma TSH levels rose significantly in the three Thx groups as compared with the C-N ($P < 0.001$). The greatest sensitivity involving plasma TSH reduction after L-T_4 administration was shown by the D-Thx rats in which levels were already significantly reduced by the 0.5 μg dose and reached the values of the C-N with the 1 μg dose. In C-Thx rats, however, the 1 μg dose of L-T_4 was needed to show significant reduction in their plasma TSH levels and 2 μg to obtain the levels of C-N. The sensitivity of the D-Thx + Ins. animals to the L-T_4 effect, decreasing the plasma TSH levels, was intermediate to that of the other Thx groups studied (Fig. 3). Pituitary TSH concentration was much lower in the Thx groups than in the C-N ($P < 0.001$) and although the L-T_4 administration produced an increment in this parameter in all Thx groups, their values never reached those of the C-N. Among the Thx groups, this L-T_4 response was greatest in the C-Thx animals with significant effects beginning with the 0.5 μg dose. It was intermediate in the D-Thx + Ins., although effects were also significant from the 0.5 μg dose, while they were lowest in the D-Thx in which pituitary TSH content was only slightly enhanced with 2 μg of L-T_4.

DISCUSSION

The marked growth retardation in thyroidectomized animals coincides with reduced plasma and pituitary GH levels and corresponds to their intense hypothyroidism and increased TSH circulating levels. This endocrine pattern confirms other reported conditions in intense thyroid deficiency (Escobar del Rey *et al.*, 1968; Evans *et al.*, 1964; Greenspan *et al.*, 1949; Lewis *et al.*, 1965; Peake *et al.*, 1973). The unchanged circulating levels of glucose and glycerol in hypothyroid animals also coincides with previously described metabolic situations (Aranda *et al.*, 1972; Llobera *et al.*, 1978) suggesting that, in this endocrine condition, a new balanced equilibrium has been established in which parallel reductions in both anabolic and catabolic pathways allow for the maintenance of unaltered steady state concentrations of circulating metabolites (Metzger and Freinkel, 1971). Administration of exogenous thyroxine to thyroidectomized rats progressively restored to normal most of the observed changes, although the response sensitivity depended on the parameter studied. Thus, although the 0.5 μg dose was enough to initiate a marked recuperation in most endocrine parameters, TSH plasma levels were maintained even higher than in thyroidectomized animals not receiving thyroxine. This may not be explained by a higher threshold level of the thyroid hormones effect, reducing the circulating levels of TSH as compared to their effective modification of other parameters. The plasma levels of TSH in untreated thyroidectomized animals are probably lower than would correspond to their markedly deficient levels of thyroid hormones, due to their negligible pituitary TSH content. In agreement with this interpretation in these animals, 0.5 μg of L-T_4 promotes increased pituitary TSH content, and consequently facilitates its release to the circulation. It has been reported that goiters in intensely hypothyroid individuals are no larger than in milder cases of thyroid hormone

deficiency (Escobar del Rey *et al.*, 1968), and this may be caused by a mechanism similar to that indicated above. In intense goiter and mild thyroid deficiency, animal growth is normal (Castro *et al.*, 1972). This is also the case in our thyroidectomized rats treated with doses of 0.5 or 1 μg thyroxine in which circulating TSH levels were still elevated but growth parameters were recovered.

When thyroidectomized animals were made diabetic these parameters were greatly affected. In spite of the same thyroid hormone deficiency (as shown by the plasma PBI levels), the animals appeared less "hypothyroid" than the non-diabetics, as indicated by their lower TSH plasma levels. It required a smaller dose (0.5 μg) of exogenous thyroxine in the diabetic rats to reduce plasma TSH than in the thyroidectomized controls. This effect was similar to previous findings (González *et al.*, 1980) and although it could be interpreted as an enhanced sensitivity to thyroxine in the hypothyroid diabetics, it coincides with the reduced ability of this hormone to enhance the pituitary TSH content. On the basis of these results the greater effect of thyroxine decreasing circulating TSH levels in diabetic thyroidectomized animals may be interpreted as a consequence of reduced pituitary TSH release due to its decreased availability in the gland, rather than by an augmented sensitivity of the TSH–thyroxine feedback effect. Growth parameters also showed less sensitivity to the exogenous thyroxine treatment in the diabetic thyroidectomized rat, indicating that their pituitary function was considerably impaired.

These endocrine changes were partially restored in the diabetic animal when supplemented with insulin, demonstrating that they were primarily caused by insulin deficiency. From our data it may be concluded that the juxtaposition of insulin and thyroid hormones is required for the pituitary to maintain its proper function. This permissive effect of insulin and thyroid hormones was previously seen for the changes in plasma amino acid levels in animals kept under similar conditions as those used in the present study (Jolin and Herrera, 1982) and it was also seen here for the metabolic parameters studied. The diabetic animals appeared less "diabetic" when intensely hypothyroid, as shown by their blood glucose and glycerol levels which were lower in animals not supplemented with thyroxine than in those treated with it. On the other hand, insulin administration did not reduce the blood glucose levels and only slightly reduced those of glycerol in diabetic animals receiving the thyroxine dose that normalized circulating levels of PBI and TSH. In this condition thyroxine produces its gluconeogenetic and/or its glycogenolytic as well as lipolytic effects (Llobera and Herrera, 1980; Montoya and Herrera, 1974), overcompensating for the opposite effects known to be produced by insulin. Thus, insulin was needed in order for thyroxine to manifest its metabolic effects although they are opposite to those of insulin.

Acknowledgements—The authors wish to express their gratitude to Caroline S. Delgado for her editorial help, Margarita González and Amparo Aguilar for their excellent technical assistance, the Rat Pituitary Program of NIAMDD for supplying the rat TSH and GH RIA kits and the Upjohn Co. for supplying streptozotocin.

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