Thyroid function in diabetic rats by the administration of streptozotocin*

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Para estudiar las variaciones de la función tiroidea en ratas diabéticas, ratas mantenidas con una dieta pobre en yodo se inyectaron con estreptozotocin y fueron comparadas con controles inyectadas con el medio y alimentadas con la misma dieta. Las ratas tratadas con estreptozotocin mostraron una intensa diuresis e hiperglucemia durante los 29 días después del tratamiento. Estos animales presentaban una concentración normal de ^{127}PBI plasmático, pero una captación tiroidea de ^{131}I y una concentración de ^{131}PBI plasmático, reducidas a las 24 horas de la inyección del trazador. Los animales tratados con estreptozotocin mostraron un elevado contenido en ^{127}I en sus tiroides y una alterada distribución porcentual de iodoaminoácidos en la glándula, con una aumentada proporción de DIT y una disminuida proporción de T_4 y T_3. Los resultados permiten sugerir que la síntesis y secreción de las hormonas tiroideas se encuentran reducidas en los animales diabéticos. Esta disminuida función tiroidea concuerda con la disminuida efectividad hormonal del TSH descrita por otros autores.

THYROID FUNCTION IN DIABETIC RATS BY THE ADMINISTRATION OF STREPTOZOTOCIN

To study the changes in the thyroid function in diabetic rats, streptozotocin was injected i.p. in rats on a low iodine diet. They were compared with controls which were fed the same diet and injected with medium. The streptozotocin treated animals showed an intense diuresis and hyperglycemia during the 29 days after the treatment. These animals had a normal plasma ^{127}PBI concentration but reduced uptake of ^{131}I by their thyroids and plasma concentration 24 hours after the injection of the tracer. The rats treated with streptozotocin showed an increased content of ^{127}I in their thyroids and an altered distribution of the percentages of radioactive iodoaminoacids, with an augmented proportion of DIT and reduced concentrations of T_4 and T_3. These results suggest that the synthesis and release of thyroid hormones are reduced in the diabetic animals. This decreased thyroid function agrees with the reduced hormonal effectiveness of TSH described by other authors.

INTRODUCTION

It is well documented that the function of the pituitary-thyroid axis is altered in rats with insulin deficiency, as shown in basal conditions [1,2,3] and after pituitary TSH release stimulation with goitrogens[4,5,6]. Besides this, it is known that hypothyroidism is frequent in diabetic subjects[7]. To further characterize the alterations in the thyroid function of diabetic rats, we have studied different parameters of thyroid metabolism in rats which were made diabetic by treatment with a single dose of streptozotocin. This drug is an N-nitrosurea derivative of glucosamine that exerts its cytotoxic action on pancreatic «beta» cells[8,9], while the «alpha» cells and the exocrine pancrea-

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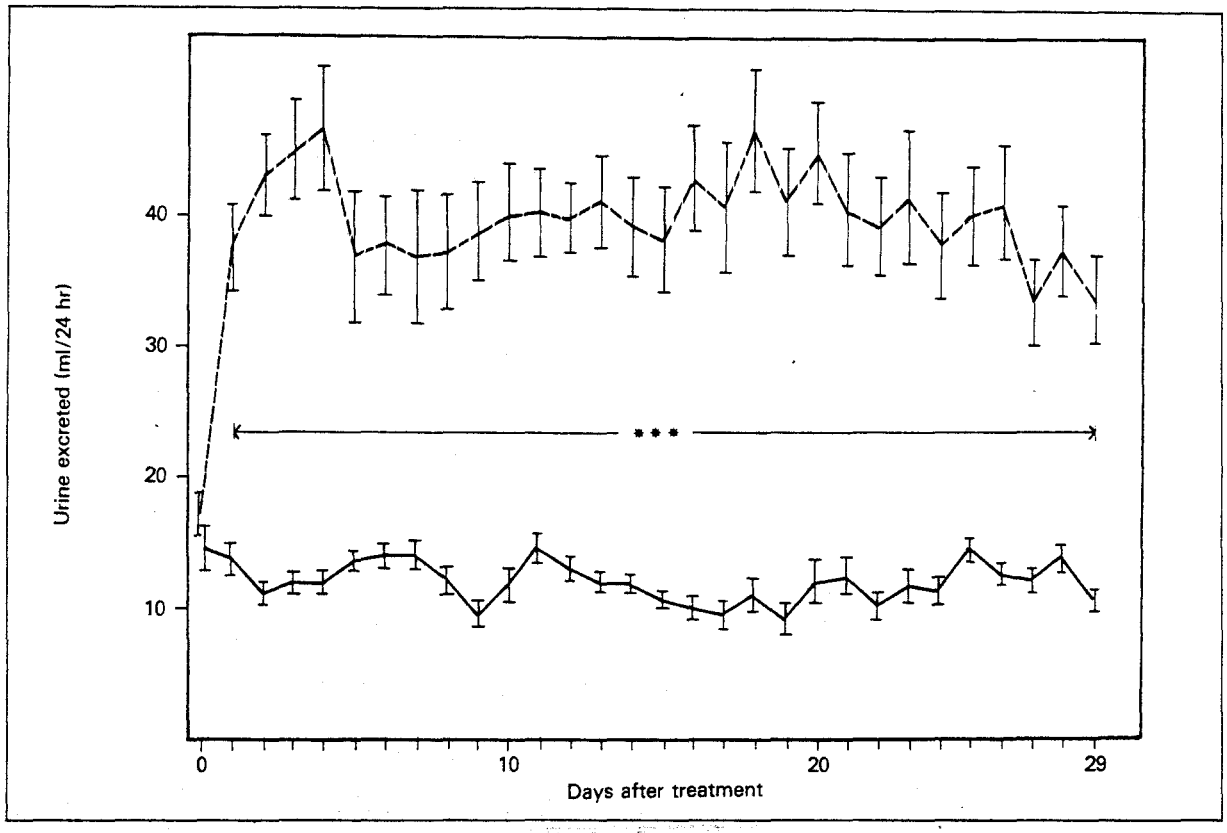

Fig. 1. Effect of a single i.p. injection of streptozotocin (70 mg/kg body weight) on the daily urine excretion in the rat. Mean ± SEM of 14 rats/group. Asterisks correspond to the statistical difference between the streptozotocin treated rats (– – – – –) and the controls (———), injected with placebo: ★ ★ ★ = $p < 0.001$.

tic tissue remain intact[10,11] and show no evidence of tissue damage.

MATERIAL AND METHOD

Female Wistar rats fed on a medium residue, low-iodine diet (0.07 μg of iodine/g)[12] were housed in individual cages and divided into two groups. One group was given a single intraperitoneal injection of streptozotocin (supplied by Dr. W.E. Dulin, The Upjohn Co., Mich., U.S.A.) (70 mg/kg) in 0.5 ml citrate buffer (0.01 M), pH 4.5 within 5 min. after it was put in solution, to produce an insulin deficiency. The other group was injected with 0.5 ml of the same buffer and used as controls. At different days after the treatment, blood specimens were collected in drops from the cut tip of the tail in porcelain plates which contained dried heparine. Protein-free supernatants were prepared with $Ba(OH)_2$-$ZnSO_4$[13] and analyzed for glucose[14]. On the 28th day after the treatment all the animals were injected intraperitoneally with 10 μCi of ^{131}I-iodide and the changes in thyroidal radioactivity were followed by «in vivo» counting of the neck. At the end of the experiment the animals were sacrificed by decapitation and blood samples were collected from the neck in heparinized beakers. The thyroids were rapidly dissected, weighed and digested at 37°C with pronase (Sigma Chemical Co.) for 8 hours[15] in 0.05 M Tris-HCL buffer, pH 8.6, containing 0.01 M methylmercapto imidazole. Aliquots were used for the evaluation of total iodine and for spotting on chromatographic strips along with stabe carrier T_4, T_3, DIT, MIT, I^- and methyl-mercapto imidazole. This procedure was followed to avoid artifactual deiodination during digestion and chromatography[16]. Chromatography in n-butanol-ethanol-1 N ammonia (5: 1:2:) and the identification of the spots were carried out as previously described[17]. Plasma protein-bound iodine was evaluated after double precipitation of aliquots of plasma with 5 % trichloroacetic acid. All the ^{127}I measurements were performed by a modified Zak procedure[18]. Statistical evaluation of the data was carried out following standard tests[19].

RESULTS

The treatment of normal rats with a single dose of streptozotocin (70 mg/kg) produces an intense diabetes that is visualized by an increase in the amount of daily urine excreted, which is observed from the 2nd. day after the treatment and maintained for the 29 days of the experiment (figure 1). As shown in figure 2, body weight decreases in the streptozotocin-treated animals despite the fact that the

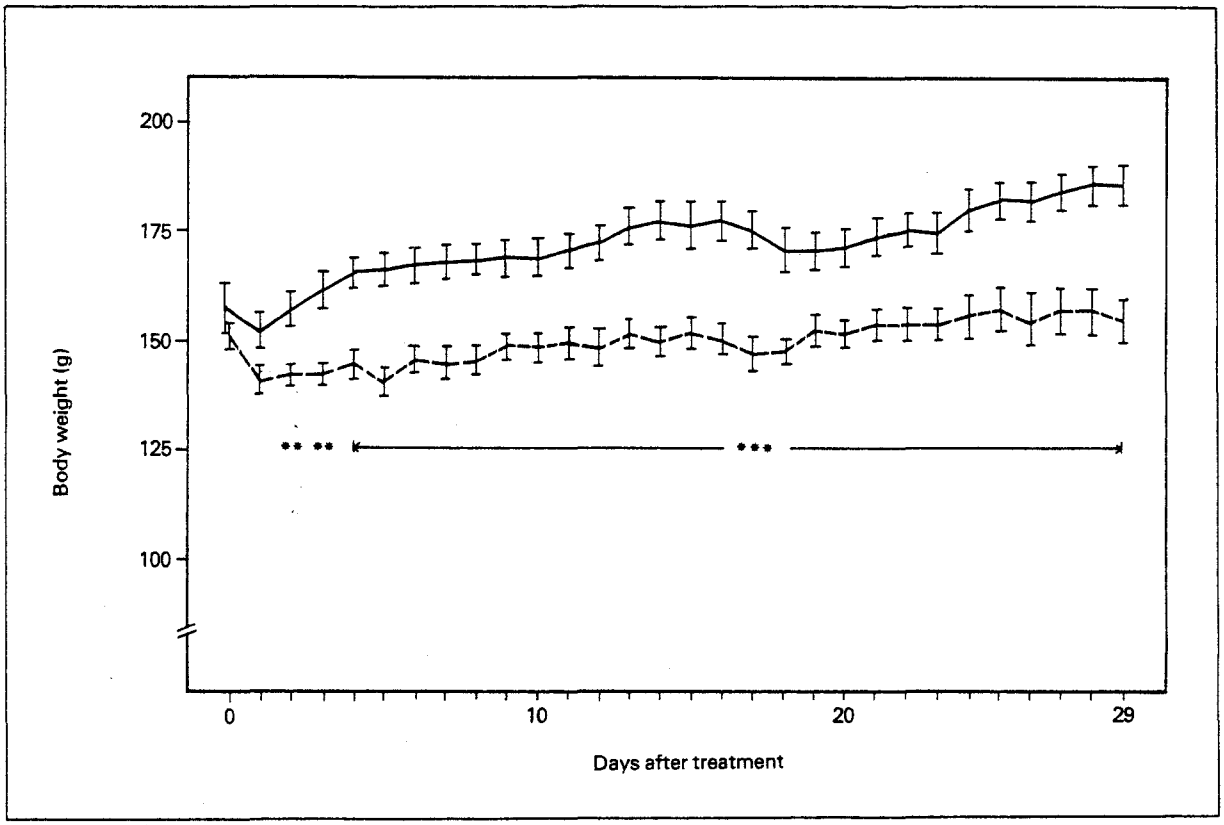

Fig. 2. Effect of a simple i.p. injection of streptozotocin (70 mg/kg body weight) on the body weight in the rat. Mean ± SEM of 15 rats/group. Asterisks correspond to the statistical difference between the streptozotocin treated rats (– – – –) and the controls (———), injected with placebo: ★★ = p<0.01, ★★★ = p<0.001.

daily food intake of these animals is increased, as observed during the last week of the experiment (mean daily food intake in the controls was 13.4 ± 0.2 and in the streptozotocin-treated animals was 16.7 ± 0.3, $p < 0.001$). Figure 3 summarizes the values of blood glucose levels. Mean glucose levels rose above 400 mg/100 ml in the streptozotocin-treated animals and these values were maintained up to the time the animals were sacrificed on the 29th day after treatment. As observed by other authors[3], thyroid weight is reduced in the streptozotocin-treated animals as compared with the values observed in the controls (table I), and the difference is significant both in absolute values and when corrected per 100 g of body weight. The content of iodine in the thyroid is greater in the streptozotocin-treated animals than in the controls, while the plasma protein-bound iodine concentration does not differ between the two groups (table I). The change of radioactivity in the thyroid over different periods of time after the intraperitoneal administration of ^{131}I-iodide is shown in figure 4. The treatment with streptozotocin produces a significant reduction in the uptake and release of radioactivity by the thyroid: at short intervals after the injection of the tracer, a reduced amount of radioactivity is found in the thyroids of the streptozotocin-treated animals as compared to the controls; it disappears more slowly from the thyroid of rats of the first group. As a result, 24 hours after the administration of the ^{131}I-iodide, the difference between the amounts of radioactivity in the glands of the streptozotocin-treated animals and of the controls disappears. The reduced release of labelled iodinated compounds from the thyroid is also supported by the reduced amount of plasma protein-bound-labelled-iodine observed in the streptozotocin-treated animals at 24 hours after the administration of the tracer (table I). At this time, the distribution of radioactive idoaminoacids in the thyroid differs between both experimental groups (table II). The percentages of

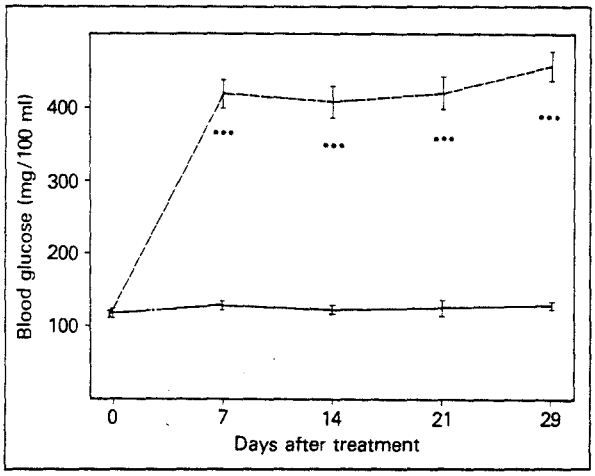

Fig. 3. Effect of a single i.p. injection of streptozotocin (70 mg/kg body weight) on blood glucose levels in the rat. Mean ± SEM of 4-20 rats/group. Asterisks correspond to the statistical difference between the streptozotocin treated rats (– – – – –) and the controls (————), injected with placebo: ★★★ = p<0.001

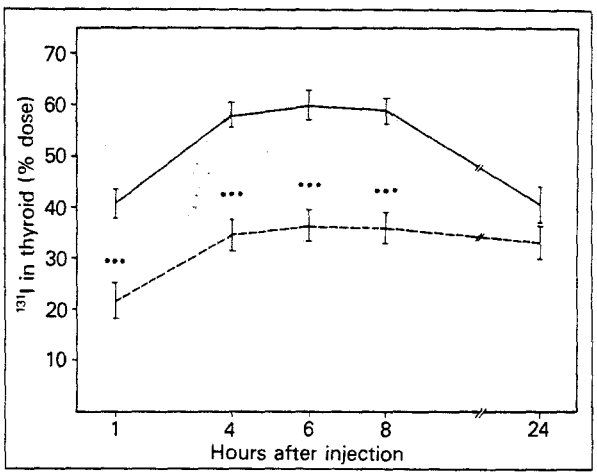

Fig. 4. Change of radioactivity in the thyroid after the i.p. injection of ^{131}I-iodide in rats 29 days after the treatment with a single dose of streptozotocin (70 mg/kg body weight) (– – – – –) and in controls (————). Mean ± SEM of 15 rats/group. Asterisks correspond to the statistical difference between the streptozotocin treated rats and the controls: ★★★ = p<0,001

labelled T_4, T_3 and iodide and the ratio MIT/DIT are reduced and the amount of DIT is augmented in the thyroids from the streptozotocin-treated rats as compared to the values in the controls (table II).

DISCUSSION

The present study shows a decreased rate of thyroid function in animals made diabetic by treatment with streptozotocin. The animals were fed a low iodine diet which is known to produce hyperfunctional goiter in normal animals[20]. The diabetic rats were unable to respond to this stimulus in spite of the fact that their availability of iodine is likely to be even smaller than in the controls: The increased excretion of urine probably produce a greater loss of iodide through the kidney. Despite this situation diabetic animals were able to maintain a normal circulating level of protein-bound iodine and even an increased amount of iodine in the thyroid. This later finding does not agree with the data of Pericás and Jolín[3] who observed a normal content of iodine in the thyroid of animals kept under similar experimental conditions. The disagreement can be the result of the different time the animals were kept under low iodine intake and to little disparities in the amount of iodine in the diet, since we have previously observed that very small variations in the daily iodine intake of the animals can produce great differences in the thyroid function of normal animals[17]. The net accumulation of iodine in the thyroid of the diabetic animals is probably the result of the juxtapositional effects of a reduced peripheral utilization of thyroid hormones and a decreased function of the thyroid gland where the reduction in the rate of release of the thyroid hormones might be

TABLE I. Changes in several parameters of thyroid function 24 hours after i.p. injection with ^{131}I-iodide in rats 29 days after treatment with a single dose of streptozotocin (70 mg/kg body weight) and in controls. Mean ± SEM of 15 rats/group. «p» corresponds to the statistical difference between both groups.

	Controls	Streptozotocin	p
thyroid weight (mg)	34.21 ± 1.05	23.93 ± 1.32	<0.001
thyroid weight (mg/100 g b.w.)	18.58 ± 0.66	15.67 ± 0.61	<0.01
^{127}I in thyroid (μg I)	1.29 ± 0.18	4.67 ± 0.57	<0.001
^{127}I in thyroid μg I/100 mg thyroid)	3.99 ± 0.55	21.67 ± 3.88	<0.001
plasma PBI^{127} (μg I/100 ml plasma)	2.87 ± 0.39	2.80 ± 0.39	NS
plasma PBI^{131} (% dose/ml plasma)	0.60 ± 0.04	0.28 ± 0.04	<0.001

TABLE II. Percentaje distribution of radioiodine-aminoacids in the thyroid 24 hours after i.p. injection of ^{131}I-iodide in rats 29 days after the treatment with a single dose of streptozotocin (70 mg/kg body weight) and in controls. Mean ± SEM of 4-14 rats/group. «p» corresponds to the statistical difference between both groups.

		Origin	DIT	MIT	I^-	T_4	T_3	MIT/DIT	T_3/T_4
Controls	Mean	9.04	29.20	38.12	5.41	13.69	4.55	1.32	0.33
	SEM	0.58	2.01	1.02	0.11	1.82	0.89	0.07	0.04
Streptozotocin	Mean	8.34	36.37	39.07	4.19	9.33	2.70	1.08	0.29
	SEM	0.69	1.12	0.53	0.31	0.62	0.28	0.03	0.02
	p	NS	<0.01	NS	NS	<0.01	<0.05	0.01	NS

greater than in the rate of their synthesis. Although it can be argued that a different isotopic dilution in the gland may be due to the augmented content of iodine in the thyroid of diabetic animals, our data on the uptake of iodide-I^{131} by the gland agree with those of Serif and Sihotang in other diabetogenic conditions[2] and support the first theory. It is also supported by the facts that the reductions in the early thyroidal uptake of the tracer and in the percentaje of labelled T_4 and T_3 content after 24 hours are smaller than the decrease in the plasma after ^{131}PBI concentration in the diabetic animals at the same time, when the amount of radioactivity in the gland did not differ from that of the controls. In any event, the net accumulation of iodine in the thyroid of the diabetic animals and the reduced weight of their glands demonstrates that the thyroid function of these animals is considerably reduced. The cause of this effect is probably multiple: a) the absence of adequate amounts of insulin and/or the alterations on carbohydrate, lipid and protein metabolism in the gland of these animals could produce a reduced response to TSH in their thyroids.

Tong [21] has recently shown that the stimulating action of TSH on the protein synthesis in thyroid cells is secondary to its effect on the glucose metabolism. b) The great urinary loss of fluid and electrolites in the diabetic animals could produce a change in the half life of plasma TSH and thus alter its effect on the thyroid function. c) In streptozotocin-treated animals there is a reduced concentration of plasma GH[22] and great metabolic alterations[23] which in the absence of insulin and the other endocrine changes observed by other authors[24] could produce direct or indirect inhibitory effects on the release of TSH by the pituitary, which in the last instance, could be responsable for the reduced thyroid function of these animals. The reduced stimulation of the thyroid by a decreased release of TSH by the pituitary is supported by the low concentration of TSH in both plasma and pituitary observed by Pericás and Jolín in similar conditions[3], and by the slightly reduced ratio of labelled T_3/T_4 in the gland found in the present study, as the opposite finding is observed wherever the thyroid is activated by an augmented release of TSH by the pituitary[20]. Probably each one of these mechanisms is implicated in the thyroid alterations observed in diabetic animals, although their comparative potencies should be very different and remain to be established.

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