Effects of Thyroid Function upon Insulin Secretion

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SUMMARY

Insulin secretion provoked by glucose in the pancreas of the rat is not modified by addition of thyroxine to the incubation media. Secretion of insulin is reduced (by 34 per cent) by prior thyroidectomy and restored to normal after administration of thyroxine (15 μg./kg. body weight) in vivo to thyroidectomized rats. These changes occur despite normal insulin content of the pancreatic tissue. Treatment of normal rats by higher doses of thyroxine (2,000 μg./kg.) causes marked reduction (ca 40 per cent) of both pancreatic insulin content and secretion. These alterations suggest that thyroid hormones may exert a delicately balanced influence upon islet function in a chronic process. DIABETES *16*:643-46, September, 1967.

The relationship between thyroid function and glucose metabolism is not clearly understood. It has been claimed that diabetes may occur in man and in animals as a result of hyperthyroidism, and that, conversely, thyroidectomy may improve pre-existing diabetes.[1-3] In attempting to elucidate the mechanisms of these changes, tolerance to glucose has been studied in clinical and experimental hyper- and hypothyroidism. These investigations, reviewed by Elrick et al.,[4] have led to conflicting results, especially in the case of the oral glucose tolerance tests where they are influenced by the rate at which glucose is absorbed from the intestinal tract. With use of the intravenous glucose tolerance test, a reduced rate of glucose utilization has generally been found in hypothyroidism,[4-7] and an accelerated or normal rate in hyperthyroidism.[4,5,8] These changes are the opposite of what one might expect from the effects of the thyroid hormone in diabetic patients and do not appear to be compatible with a tendency towards hypoglycemia after thyroidectomy and hyperglycemia in cases of hyperthyroidism.[4,5] Other factors than the rate of glucose utilization have, therefore, to be considered. In the case of hyperthyroidism, attention has been focussed upon the possible roles of increased gluconeogenesis[9] and increased degradation of insulin in the liver and kidney.[10] Apart from histological studies showing ultimate degeneration of the beta cells during prolonged hyperthyroidism,[1,11] little has been done to assess the effects of thyroid hormones upon islet function. This problem was investigated in the present study of the effect of glucose upon insulin secretion in vitro from pancreatic tissue of normal, thyroidectomized, and thyroxine-treated rats.

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MATERIALS AND METHODS

Rats: All animals came from the same batch of Albino rats (initial body weight 240 to 260 gm.; Holtzman, Wisconsin). Thyroidectomized animals (n = 5) were examined four weeks after surgery together with a group of five normal rats. A week later (i.e., five weeks after surgery), four thyroidectomized animals received daily intraperitoneal injections of l-thyroxine (15 μg./kg. body weight/day; l-thyroxine, Sigma Chemical Co., St. Louis, Mo.) dissolved in sodium hydroxide (4 mg./ml.; 0.1N) and further diluted (1/80; v/v) in saline. They were killed after four days of treatment. Finally, five normal rats received daily intraperitoneal injections of higher doses of thyroxine (2,000 μg./kg. body weight/day) for four days and were examined together with five normal animals. All experiments were carried out thirty to forty days after arrival of the animals in the laboratory. All animals had free access to food (Lab Chow, Ralston Purina Co., St. Louis, Mo.) and water, a daily check of body weight being kept during the period of treatment.

Experimental procedure: After decapitation, blood was collected and the plasma separated for sugar estimation with the AutoAnalyzer (Technicon Instruments Co., Chauncey, N.Y.) using a method based on that of Hoffman.[12] The pancreas was removed and divided into small pieces (ca 10 mg. each). These were placed in

groups of four into bicarbonate-buffered media (2 ml.) containing glucose (150 mg./100 ml.), bovine serum albumin (0.5 per cent, w/v; bovine albumin, Fraction V; Sigma Chemical Co., St. Louis, Mo.) and guinea pig anti-insulin serum. Sufficient anti-insulin serum was added to bind about twice the expected amount of secreted hormone, insulin secretion over ninety minutes of incubation at 36° C. being equated to the fall in insulin antibody content of the medium. After incubation, all pieces of pancreatic tissue from the same animal were homogenized and extracted with acid-alcohol. The methods for assay of insulin secretion in vitro and the insulin content of the pancreas are described elsewhere.[13-14] Mean rates of insulin secretion and the mean insulin contents of pancreatic tissue are here reported in relation to the amounts of incubated (μU./mg. wet wt./ 90 min.) or extracted (U./gm.) tissue.

RESULTS

Insulin secretion evoked by glucose (150 and 200 mg./100 ml.) in pancreatic tissue from normal and thyroidectomized rats was unaffected by addition of l-thyroxide (8 to 200 μM./l.) to the incubation medium (table 1).

Thyroidectomized animals gained less weight (3.1 ± 0.2 gm./day) than control animals (4.2 ± 0.2 gm. per day) during the four weeks following surgery. At death, there was a minor decrease in plasma-sugar concentration (table 2). Their mean body weight, pancreatic weight and total pancreatic insulin content were each reduced to the same extent (ca 18 per cent), so that insulin content relative to pancreatic weight (U./gm.) remained constant (table 2). Thyroidectomy caused a reduction (ca 34 per cent) in secretion of insulin evoked by glucose in vitro, an effect which was abolished by prior treatment of thyroidectomized rats with thyroxine. In these latter animals, the mean plasma sugar concentration was also restored to its normal value.

When normal rats were treated with high doses of l-thyroxine, there was progressive loss of body weight (—2.7 ± 0.3 gm./day) over four days, a slight but significant increase in plasma-sugar concentration, and a marked decrease both in the insulin content of the pancreas, and in the rate of insulin secretion which could be evoked by glucose in vitro (table 2).

DISCUSSION

From the results of the present experiments, it can be concluded that when thyroxine is added to the incubation medium it has no effect upon glucose-induced insulin secretion over ninety minutes by pancreatic tissue of normal or thyroidectomized rats. On the other hand, removal of the thyroid gland or administration of thyroxine in large doses to normal rats for four days in vivo reduces insulin secretion which can be evoked in vitro with glucose, an effect which was not observed with tissue from thyroidectomized rats treated with small doses of the hormone.

The reduced response of tissue from thyroidectomized rats was not associated with any reduction in the insulin content of that tissue and normal responsiveness was restored by administration in vivo of small doses of the hormone. After normal rats had been treated with large doses of hormone, however, both the secretory ability of the pancreatic tissue and its insulin content fell. Thus, relative to the insulin contents of these tissues, responsiveness to glucose was reduced by thyroidectomy but was unaffected by induction of hyperthyroidism. On this basis, the effect of the thyroid hormone upon insulin secreting beta cells is comparable to the so-called "biphasic" effect which it has on other tissues due to its action upon the normal delicate

TABLE 1

Effect of thyroxine upon insulin secretion. Mean insulin secretion (μU./mg./90 min. ± S.E.M.) by pancreatic tissue of normal and thyroidectomized rats incubated in media containing glucose alone (control output); effect of thyroxine is shown as mean change in insulin secretion rate (± μU./mg./90 min. + S.E.M.) with the number of observations in parenthesis.

Pancreatic tissue	Glucose concentration (mg./100 ml.)	Control output (μU./mg./90 min.)	Thyroxine concentration	Thyroxine effect (μM./l.) (± μU./mg./90 min.)
Normal	150	38.0 ± 3.1 (23)	8	—2.5 ± 3.9 (23)
Thyroidectomized	150	20.5 ± 1.6 (9)	8	+2.2 ± 3.2 (9)
Normal	200	60.8 ± 2.3 (28)	8	—0.8 ± 3.6 (14)
			200	+1.0 ± 7.0 (15)

TABLE 2

Effect of thyroidectomy and thyroxine administration on insulin secretion. For each experimental condition, the table indicates the body weight, the level of plasma sugar, the weight and insulin content of the pancreas at death; the insulin output provoked by glucose (150 mg./100 ml.) in vitro; and the ratio of insulin output/content. The mean values (± S.E.M.) are shown together with the number of determinations (in parenthesis) and the statistical significance (*$p < 0.05$; **$p < 0.02$; ***$p < 0.005$) of mean differences with corresponding normal values.

	Normal	Thyroidectomized	Thyroidectomized + thyroxine (15 γ/kg./d × 4)	Normal	Normal + thyroxine (2 mg./kg./d × 4d)
Body weight (gm.)	380±4 (5)	308±3*** (5)	347±16* (4)	387±9 (5)	344±9** (5)
Plasma-sugar (mg. per 100 ml.)	148±4 (5)	139±1* (5)	151±1 (4)	151±4 (5)	172±3*** (5)
Pancreas —weight (gm.)	1.26±0.03(5)	1.04±0.04***(5)	1.07±0.06**(4)	1.22±0.04(5)	1.29±0.06 (5)
—insulin content (U./gm.)	1.99±0.15(5)	1.97±0.09 (5)	2.03±0.12 (4)	2.00±0.15(5)	1.39±0.12** (5)
—insulin output (μU./mg./90 min.)	35.7 ±2.0 (54)	23.5 ±1.6 ***(54)	35.5 ±1.5 (72)	33.5 ±1.5 (63)	19.9 ±1.4*** (62)
—output/content (μU./mU.)	16.9 ±1.0 (5)	11.7 ±1.7 * (5)	17.6 ±1.4 (4)	16.4 ±0.7 (5)	15.6 ±1.5 (5)

balance between synthetic and degradative processes.[15] Deficiency of the hormone results in slowing of metabolic processes in general, the pancreas becoming less sensitive to glucose. After a latent period, too long for detection of any action of the hormone in vitro in the present system, thyroxine in large dosage could alter insulin synthesis, the islets becoming depleted of insulin and their ability to secrete this hormone being reduced. In the light of current knowledge, this concept provides a possible explanation for the present observations, but further investigation will be required for substantiation.

The findings are compatible with some results reported by Hales and Hyans[16] in man. In cases of thyrotoxicosis they found that glucose induces marked and prolonged hyperglycemia but, as judged from their stated levels, the degree of induced insulinemia was less than would normally be expected. Such a reduced response by the islets is also compatible with progressive failure of these cells leading to meta-thyroid diabetes.[1-17]

The part played by abnormal islet function in the metabolic derangements seen in thyroid diseases has yet to be investigated. In hypothyroidism it could be speculated that decreased utilization and metabolism of glucose is due to generalized reduction of all metabolic processes including that of the insulin secretory process. In hyperthyroidism, however, a reduced secretion of insulin would tend to oppose the direct stimulant effect of thyroxine upon glucose uptake[8] by the peripheral tissue, these opposite influences probably accounting for the variable rates of glucose utilization[4] observed in this condition. Moreover, an increase in gluconeogenesis, secondary to the relative lack of insulin,[18] may be favored in hyperthyroidism by a more rapid hepatic degradation of insulin.[10]

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"Moldy" Peanut Meal

A few years ago, large numbers of turkey poults and ducklings died in England. The death of these animals was finally attributed to the presence of peanut meal in their rations. It was subsequently shown that the most toxic meals were contaminated with a variety of molds. One of the molds, *Aspergillus flavus,* was shown to be present in all batches of the toxic meal (See Nutrition Reviews 20:174, 1962).

A crystalline material was isolated from one batch of the toxic meal. When 20 μg. of this was given to day old ducklings, death occurred within twenty-four hours. This material also produced characteristic liver changes which were similar to those seen in animals fed the moldy peanut meal. Rats fed low levels of the moldy meal developed hepatomas with metastases to the lungs and kidneys (See Nutrition Reviews, loc. cit.).

The first reports indicated that the moldy meal came primarily from Uganda, Brazil, and, to a lesser extent, from India. A recent report suggests that the same mold may contaminate some of the peanut meals commercially available in the United States. This is suggested by the finding of a high incidence of tumors in Charles River CD strain rats fed rations containing peanut meal as the primary source of protein (W. D. Salmon and P. M. Newberne, Cancer Research 23:571, 1963). As early as 1959, Salmon and Newberne (Ibid.) noted in their rats a low but consistent incidence of tumors that were not of spontaneous origin. These appeared in rats fed rations containing peanut meal and dried beef.

The rations used contained 7.9 per cent dried beef, 33.3 per cent extracted peanut meal, 19 per cent beef fat, vitamins and minerals in adequate amounts, and corn starch or sucrose to make 100 per cent. The commercial peanut meal used in this study was "high quality solvent-process meal" prepared from peanuts grown in the United States. Most of the peanuts were from government surplus stocks. This grade of peanuts is of "reasonably good quality, but some are 'blanched' as a result of improper storage conditions and are not suitable for the edible trade."

The meal was prepared by n-hexane extraction of the ground nuts which had been pressed to remove most of the oil. The extracted meal containing less than 1 per cent of oil showed no visible sign of mold growth when it was received in the laboratory. This material was then extracted for seventy-two hours with methanol in a steam jacketed continuous type extractor.

When four- to five-week old rats were fed the ration containing the dried beef and peanut meal, they gained weight at almost the same rate as those fed a similar ration containing 31.7 per cent dried beef. After about one year, fifteen out of eighty-eight animals fed the peanut meal ration showed hepatomas. There were no hepatomas in any of the rats receiving the dried beef as the sole source of protein. The incidence of hepatomas was not reduced by the addition of 0.3 per cent choline or of 0.9 per cent methionine to the diet. These results suggested that the hepatomas were different from those previously reported in rats fed a choline deficient diet over long periods of time (Salmon, D. H. Copeland, and M. J. Burns, J. National Cancer Inst. 15:1549, 1955).

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