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ACUTE EFFECT OF THYROID HORMONE ON INSULIN SECRETION IN RATS

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Abstract—To elucidate the mechanism of thyroid hormone-induced hyperinsulinemia, the acute and direct effect of thyroid hormone administration on insulin secretion was investigated in rats *in vivo* and *in vitro*. In the perfused rat pancreas, the addition of thyroxine (10 μg/dL) or 3,5,3′-triiodothyronine (150 ng/dL) to the perfusing medium did not affect insulin secretion. The administration of thyroxine (40 μg/kg, s.c.) *in vivo* increased the plasma insulin level from 11 ± 2 μUnits/mL (mean ± SD) to 30 ± 7 μUnits/mL, while blood glucose and plasma glucagon were unchanged. This phenomenon was inhibited completely by the preadministration of oxprenolol hydrochloride (2 mg/kg, s.c.), and inhibited partly by the preadministration of metoprolol tartrate (35 mg/kg, s.c.). These results suggest that thyroid hormone induces hyperinsulinemia via β-adrenergic stimulation in the rat.

Several investigators [1–4] have reported that elevated plasma insulin concentrations are observed in hyperthyroid patients and experimental hyperthyroid animals. Although the precise mechanism of this phenomenon remains to be elucidated fully, the hyperinsulinemia may be caused by insulin resistance and/or associated hyperglycemia in chronic thyroid hormone excess. The acute and direct effect of thyroid hormone administration on insulin release has not been examined. In the present study, to elucidate the mechanism of thyroid hormone-induced hyperinsulinemia, the acute and direct effect of thyroid hormone administration on insulin release was investigated in rats *in vivo* and *in vitro*.

MATERIALS AND METHODS

Animals. Male Wistar albino rats weighing approximately 200 g were used in the present study. After an overnight fast, the rats were anesthetized with intraperitoneal pentobarbital sodium (40 mg/kg) and used for the following experiments.

In vivo *experiment*. L-Thyroxine (T_4, Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in a small volume of 0.01 N NaOH and brought to a concentration of 10 μg/mL with saline. The experimental rats were given T_4 (40 μg/kg) subcutaneously, and control rats received vehicle alone. Oxprenolol hydrochloride (2 mg/kg; Ciba Geigy, Takarazuka, Japan) or metoprolol tartrate (35 mg/kg; Ciba Geigy) was administered subcutaneously 30 min before T_4 injection. Blood was drawn from the femoral vein at 0, 30, 40, 50, 60, and 105 min after injection of the β-blocker.

In vitro *experiment*. The pancreas of the rat was isolated and perfused by a method described elsewhere [5]. The celiac artery and the portal vein were cannulated, and the pancreas was perfused without recirculation with a synthetic medium at a flow rate of 3.5 mL/min. The perfusion medium consisted of a Krebs–Ringer bicarbonate buffer containing 0.5% bovine serum albumin (fraction V, Sigma Chemical Co.) and 4.6% Dextran T-70 (Green Cross Co., Tokyo, Japan). The pancreas was perfused for 15 min with 5.5 mM glucose in the perfusate; then the glucose level was raised to 16.7 mM by infusing glucose into the arterial tubing from a sidearm syringe. This glucose level was maintained for 30 min. The pancreas was perfused with the medium containing T_4 (10 μg/dL) or 3,5,3′-triiodothyronine (T_3, 150 ng/dL, Sigma Chemical Co.) in the same manner. The portal effluent was collected every 1 min in a chilled tube. During perfusion, the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4. The effluent was stored at −20° until the time of insulin assay.

Measurements. Blood glucose was measured by the glucose oxidase method [6]. Plasma T_4, T_3, insulin, and glucagon were measured by respective radioimmunoassays. The sensitivity of these assays was 25 ng/dL for T_3, 0.7 μg/dL for T_4, 0.1 ng/mL for insulin, and 20 pg/mL for glucagon. Intra- and interassay coefficients of variation were 5 and 10% in T_3, 6 and 11% in T_4, 5 and 10% in insulin, and 7 and 15% in glucagon respectively.

Analysis of data. Data are expressed as means ± SD. Differences in blood glucose and hormone levels were compared using analysis of variance and a two-tailed non-paired Student's *t*-test.

RESULTS

Changes in blood glucose, plasma insulin, glucagon, T_3 *and* T_4 *levels after thyroxine injection*. As shown in Fig. 1, no significant changes in blood glucose levels were observed by thyroxine administration in all groups. Plasma insulin levels increased significantly from 11 ± 2 to 30 ± 7 μUnits/mL in

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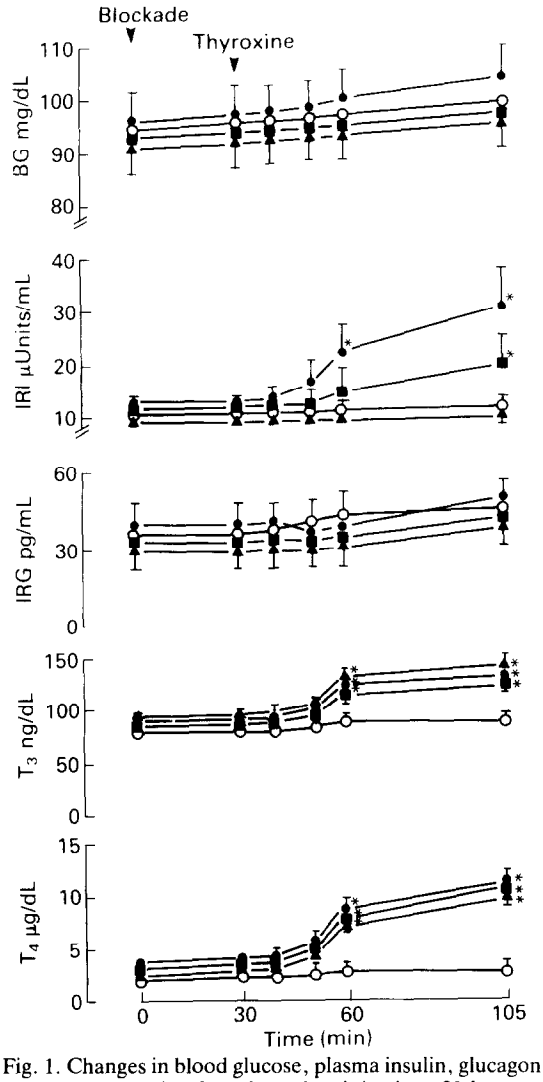

Fig. 1. Changes in blood glucose, plasma insulin, glucagon, T_3, and T_4 levels after thyroxine injection. Values are means ± SD; N = 7 for each treatment group. Abbreviations: BG, blood glucose; IRI, plasma insulin; and IRG, plasma glucagon; "blockade" indicates the injection of oxprenolol or metoprolol. Key: (○) control rats, (●) thyroxine-treated rats, (■) metoprolol-pretreated rats, and (▲) oxprenolol-pretreated rats. An asterisk (*) indicates a significant difference from controls ($P < 0.02$).

thyroxine-treated rats (T-rats), and from 11 ± 2 to 19 ± 5 μUnits/mL in metoprolol-pretreated rats (Met-rats). The insulin level was not changed in control or oxprenolol-pretreated rats (Ox-rats). Plasma insulin levels in T-rats and Met-rats were significantly higher than those in control and Ox-rats at 105 min. Plasma glucagon levels were not changed significantly by T_4 administration and were almost the same in all groups. Plasma T_3 levels were increased by T_4 administration, and plasma T_3 levels in T-rats, Ox-rats, and Met-rats were significantly higher than those in controls at 60 and 105 min. The T_4 level increased from 2 to 11 μg/dL in T-rats, Ox-rats, and Met-rats.

Insulin release from perfused pancreas. As shown in Fig. 2, basal insulin secretion was 2.5 ± 0.8 ng/min, and typical biphasic insulin release was observed by infusion of 16.7 mM glucose. These insulin responses were not altered by the addition of T_4 or T_3 to the perfusing medium.

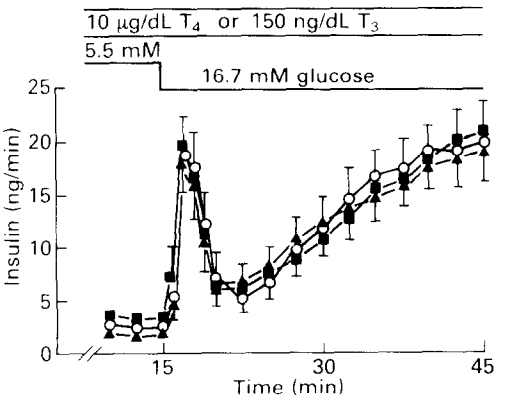

Fig. 2. Insulin release from perfused pancreas. Values are means ± SD; N = 5 for each treatment group. Key: (○) control, (■) pancreas perfused with 10 μg/dL T_4 and (▲) pancreas perfused with 150 ng/dL T_3.

DISCUSSION

Pentobarbital anesthesia had no effect on blood glucose, plasma insulin, glucagon, T_4, or T_3 concentrations. These results are consistent with the report of Lang *et al.* [7] that pentobarbital anesthesia (30–40 mg/kg) has no effect on blood glucose, plasma insulin, or glucagon levels in rats. In the present study, thyroid hormone administration did not alter insulin secretion from perfused pancreas; however, thyroxine administration rapidly increased the plasma insulin level (without hyperglycemia and hyperglucagonemia) in rats. These results indicate that thyroid hormone has no direct effect on insulin release from the pancreas and that thyroxine-induced hyperinsulinemia is not caused by hyperglycemia or hyperglucagonemia. Other studies [8, 9] have indicated that β-adrenergic blockers inhibit the peripheral conversion of T_4 to T_3. Because plasma T_4 and T_3 levels were similar in T-rats, Ox-rats, and Met-rats, β-blockers used in the present study did not affect the peripheral metabolism of T_4 or T_3. Thyroxine-induced hyperinsulinemia was abolished in part by metoprolol, and completely by oxprenolol, suggesting that thyroid hormone-induced hyperinsulinemia is induced by β-adrenergic stimulation. This result supports the suggestion that thyroid hormone may influence β-adrenergic responsiveness of the pancreas, because insulin release in response to epinephrine or isoproterenol is increased in hyperthyroid patients and animals [4, 10]. Although β-adrenergic stimulation (isoproterenol) is reported to induce hyperglycemia, hyperglucagonemia, and hyperinsulinemia in humans [11], glucagon and blood glucose levels were not altered in the present study. This difference may be due to the difference of species and/or experimental methods.

The precise mechanism by which thyroid hormone influences β-adrenergic responsiveness of the pancreas remains to be clarified. Because the phenomenon was not observed in perfused pancreas, some *in vivo* alterations induced by thyroxine treatment may enhance the β-adrenergic mechanism. It is also unclear why the glucose level does not fall in response to hyperinsulinemia. Insulin resistance may be responsible for the hyperinsulinemia. Further studies are needed.

In summary, we conclude that thyroid hormone induces hyperinsulinemia via β-adrenergic stimulation in the rat *in vivo*, and that the phenomenon may be responsible, in part, for the hyperinsulinemia in hyperthyroid patients and animals.

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