Acute changes in biliary excretion of reverse triiodothyronine in rats after insulin-induced hypoglycemia: effect of glucose, verapamil, cycloheximide and actinomycin D

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Biliary excretion of reverse triiodothyronine (rT₃) was estimated in rats during hypoglycemia induced by a 10-min infusion of 1 U of insulin (INS) and for the following 5 h. During that period an increase in biliary rT₃ was found. This was seen also during the infusion of exogenous glucagon (10 µg in 1.2 ml of saline per 1 h for 5 h) given independently of INS. The infusion of glucose (1 g/kg per 50 min or 2 g/kg per 110 min) following INS infusion delayed the increase in rT₃. The increase in rT₃ was prevented by actinomycin D (1 mg/kg) when injected before (90 min), but not after (30 min) INS, and also by cycloheximide (2.5 mg/kg) injected immediately before INS. The same dose of cycloheximide also prevented a similar increase of rT₃ during the infusion of exogenous glucagon. Verapamil (5 mg/kg divided into five doses per 4 h) blunted the increase of rT₃. These data indicate that following INS injection counter-regulatory hormones may be responsible for the increased production of rT₃; this altered metabolic activity apparently is dependent on protein synthesis.

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It is well known that the conversion of thyroxine (T₄) to triiodothyronine (T₃) or the inactive metabolite reverse triiodothyronine (rT₃) is influenced by several factors, such as drugs, nutrients or non-thyroidal illness (for a review, see Ref. 1).

Biliary excretion of rT₃ increases after a single iv injection of salicylate (2) as well as after short-term (4–5 h) intravenous infusion of linoleic acid (3), propylthiouracil (4), adrenaline and methoxamine (5), glucagon, vasopressin, angiotensin II (6) and neurotensin (7). As certain variations in biliary excretion of iodothyronines in rats after various interventions occur earlier than those in serum, monitoring of biliary iodothyronines is a useful technique for the in vivo study of acute changes of liver iodothyronine metabolism.

The concentration of T₄ in rat bile is approximately the same as in plasma, although the concentration of other iodothyronines is four to eight times higher than that in plasma (8). Biliary excretion of tri- and diiodothyronines thus predominantly reflects the rate of their metabolic production in the liver, their hepatic clearance from plasma being negligible ordinarily. In addition, only a few per cent of near-physiological doses of intravenously administered exogenous T₄ and T₃ appear in bile (9), indicating that biliary excretion of iodothyronines is influenced only negligibly by their plasma levels within the physiological range.

Exogenous administration of hormones that counter-regulate hypoglycemia acutely increases biliary rT₃ excretion (5–7). In the present study we have evaluated the effect of exogenous counter-regulatory hormones whose secretion was stimulated by acute insulin-induced hypoglycemia to expand these studies.

Materials and methods

Animals

Male Wistar Olac rats weighing about 300 g were purchased from Velaz (Prague), fed normal pelleted diet and maintained in a temperature-controlled (22 ± 1°C) and light-controlled (lights on from 6.00 to 18.00 h) room.

Experimental procedure

Groups of eight animals each (occasionally six or seven animals) were subjected to the following experimental procedure on the same day. After anesthesia with pentobarbital (initial dose 40 mg/kg ip; maintainance dose about 15 mg/kg every 60–90 min), thin polyethylene tubing was inserted into the bile duct and femoral vein. Such a procedure can be completed in eight animals within about 90 min. The bile was collected continuously into preweighed glass vials containing methimazole, which were changed every hour for 6 h.

The animals were heated externally to maintain body temperature. Following collection of the initial control
sample, intravenous infusions or pulse injections of insulin (INS; Novo, Copenhagen, Denmark), glucagon (GLG; Novo, Copenhagen, Denmark), glucose, verapamil (VER; Isoptin®, Lek, Ljubljana, Slovenia), actinomycin D (ACT D; Dactinomycin®, Merck, Sharp & Dohme, Rahway, NJ) or cycloheximide (CHEX; Sigma, St Louis, MS) were started using a microinfusion pump (Unita II: Braun, Melsungen, Germany). The details on doses of drugs and infusion rates are described in the legends to the figures. Control animals were infused with saline (S).

Radioimmunoassay of rT3 in bile

The aliquots of bile were treated with β-glucuronidase/arylsulfatase (Boehringer, Mannheim, Germany) and the concentration of total (i.e. conjugated plus unconjugated) rT3 was estimated by an in-house RIA (8) that later was modified slightly by using smaller aliquots of bile for individual estimations (6). The sensitivity of the assay is less than 0.5 µg/ml. The results were expressed as ng/time and the volume of bile was estimated by weighing the previously tared collection vials.

As the experiments were carried out over more than 2 years, from each new batch of animals some groups were taken that were subjected to the same treatment as those from previous batches. In this way some large pools of two to five groups of six to eight animals each were obtained. The samples from three to five groups usually were analyzed in the same assay. However, in some cases of pooled groups the data obtained in two or three different assays were pooled, the details being indicated in the legends to the figures. The between-assay variation was less than 20%.

Statistical evaluation

In each animal the first sample of bile was the control. The differences in the excretion of rT3 and bile volume at individual time intervals within individual or pooled groups were evaluated by analysis of variance followed by Duncan’s multiple range test. The main criterion used was whether or not there was any significant difference between the first sample and the following samples within the same group and whether there was a statistical difference (p < 0.05) from the reference pooled control group or the pooled group with acute insulin-induced hypoglycemia shown in Fig. 1.

Results

As shown in Fig. 1A (N = 34), the excretion of rT3 in the control group did not change within the first 4 h, while it decreased slightly but significantly (p < 0.05) during the last 2 h of observation. In contrast, insulin-induced hypoglycemia (Fig. 1B; N = 26) caused a significant increase (p < 0.05 to <0.01) 2–5 h after INS infusion as compared with the first sample.

In a majority of groups the volume of bile was increased significantly (p < 0.05 to <0.01) within 1–2 h after INS infusion (Fig. 1B and Fig. 2A,B,E,F).

The increase of rT3 induced by INS (Fig. 1B) was delayed by the infusion of glucose either for 50 min (Fig. 2A; N = 37) or for 110 min (Fig. 2B; N = 15), which was applied subsequently to the infusion of 1 U of INS in 0.5 ml of 30% glucose for 10 min. the differences in the latter case being less significant as compared with the control interval. After repeated doses of VER (Fig. 2C) the excretion of rT3 did not differ significantly from the first sample, the differences being significant (p < 0.05 to <0.01) only when the excretion of rT3 was compared with the second collected sample.

The excretion of rT3 was not influenced by the administration of ACT D alone (Fig. 2D). However, this drug administered 90 min before INS prevented the increase of rT3 (Fig. 2E), while the administration of ACT D 30 min after INS was without any effect in that respect (Fig. 2F).

The administration of 2.5 mg/kg CHEX alone did not affect the volume of bile and rT3 excretion (Fig. 3A), but completely prevented the increase of rT3 excretion after INS-induced hypoglycemia (Fig. 3B). The increase of rT3 induced by glucagon infusion (Fig. 3D) was completely prevented by 2.5 mg/kg CHEX (Fig. 3C).

Discussion

Insulin-induced hypoglycemia triggers the release of counter-regulatory hormones within a few minutes.
increased biliary rT₃ after the infusion of exogenous glucocorticogenic hormones and with the acute increase of rT₃ and decrease of T₃ in serum after glucagon administration in human subjects and dogs (11, 12). The effect of glucose infusion (Fig. 2A,B) on the delay of biliary rT₃ increase may be explained by its ameliorating effect on INS-induced hypoglycemia, thus delaying the release of counter-regulatory hormones, while the blunting effect of VER is in agreement with the known inhibitory effect of calcium flux-blockers on the hormonal stimulation of glucocogenesis (13).

The increase of rT₃ excretion after iv INS administration presumably is due to inhibition of liver 5'-deiodinase. Because the activity of type I deiodinase depends on the presence of SH groups (14), the decreased activity of that enzyme might result from a decreased availability of such a thiol co-factor. A decrease of non-protein SH groups (represented mainly by reduced glutathione) in the liver is produced by increased activity of counter-regulatory hormones such

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**Fig. 2.** Bile volume (left panels) and biliary excretion of reverse triiodothyronine (rT₃; right panels) in various groups of animals (numbers of animals in parentheses). Values are means ± sm. Black triangles indicate 10-min infusion of 1 U of insulin (INS) in 0.5 ml of 30% glucose (G) (A and B) or in 0.5 ml of saline (C, E and F) during the first 10 min of the second hour. (A) Five pooled groups (N = 37) infused with INS followed by infusion of 1.0 ml of 30% G per 50 min; the data were obtained in three different assays. (B) Two pooled groups (N = 15) infused the same dose of INS followed by infusion of 2.2 ml of 30% G per 110 min. The results show that the increase in rT₃ excretion after INS could be prevented partly by glucose infusion. (C) Three pooled groups (N = 20) infused with INS and then injected with 5 mg/kg verapamil in five doses of 0.5 ml each (arrows), the first injection consisting of a double dose (the data were obtained in two different assays). The results show a partial prevention of rT₃ increase by verapamil. (D) Actinomycin D injected iv in a dose of 1 mg/kg 30 min before the start of bile collection (arrow). (E) Two pooled groups subjects excepted to the same treatment with actinomycin D as in (D), but infused with INS; the data were obtained in two different assays. (F) One group infused with INS and injected with the same dose of actinomycin D as in (D) and (E) 30 min after the start of INS infusion (arrow). The results show that the decrease of rT₃ after INS could be prevented by actinomycin D when injected 90 min before INS (E) but not 30 min after INS (F), while actinomycin D itself could not influence the excretion of rT₃ (D). Statistical significance vs the second interval in (C): ×, increase (p < 0.05); ××, p < 0.01. For other symbols, see legend to Fig. 1.

(10), suggesting that there may be a relation between the stimulated formation of rT₃ and their glucocorticogenic action. This view is in agreement with our previous (5-7) and present (Fig. 3D) findings of
as in fasted (15, 16) or diabetic rats (17) and after the administration of vasopressin and adrenaline (16, 18–20). However, non-protein SH groups in the liver of fasted rats increased after glucose refeeding and concomitant increase of insulin/glucagon ratio (15) and were found to be related to the serum insulin level in short-term experiments (16).

The inhibitory effect of CHEX and ACT D on the increase of rT3 suggests that these effects depend on protein synthesis. However, under the in vivo conditions of our experiments some side effects of those drugs cannot be ruled out definitely. The dose of CHEX that we used was similar to that used in one study (21), while it was ten times lower than that used in another (22) that failed to demonstrate the inhibitory effect of this drug on the recovery of liver deiodinase by glucose refeeding after fasting.

Recently, an approximately 50% decrease of type I deiodinase mRNA in the liver was found in diabetic or 48-h fasted rats (23). As the level of insulin is decreased and that of glucagon (and other glucoagonetic hormones) is increased in both uncontrolled diabetes (24) and fasting (25), under both the above conditions the counter-regulatory action of glucagon should prevail. From this it might be hypothesized that the decrease of deiodinase activity may be related to the prevailing glucoagonetic type of glucose production. Although decreased deiodinase mRNA was found after 48 h (23), a 60% decrease of Spot 14 mRNA has been found as early as 45 min after glucagon injection (26), indicating that even rapid effects of glucagon on protein synthesis in the liver can occur. However, these questions remain to be solved in experiments using molecular methods.

References

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