RAPID TRANSIENT INCREASE OF BILIARY TRIIODOTHYRONINE EXCRETION DURING SHORT-TERM INFUSION OF GLUCOSE AND ARGININE IN RATS

P. LANGER, K. GSCHWENDTOVÁ

Institute of Experimental Endocrinology, Slovak Academy of Sciences, 833 06 Bratislava, Slovakia

Objective. To estimate the excretion of triiodothyronine by bile in groups of rats infused with glucose, arginine or glucose combined with various doses of insulin.

Materials and Methods. Groups of about eight male Wistar Olac rats were anesthetized by pentobarbital and thin polyethylene tubings were inserted into bile duct and femoral vein. The bile was collected into pre-weighed glass vials which were changed every hour. The first one hour interval was considered as control and during the second hour the following i.v. infusions were applied: 1. 2.4 ml 30 % glucose; 2. arginine (80 mg/2.4 ml saline); 3. 2.4 ml 30 % glucose containing 62.5, 125, 250 or 500 mU insulin. In some groups cycloheximide (2.5 mg/kg) or somatostatin (20 μg/kg) were used. The aliquots of bile were treated with ß-glucuronidase/arylsulfatase and the concentration of total (i.e. conjugated plus unconjugated) triiodothyronine was estimated by specific in-house radioimmunoassay. The results were expressed as ng/hr and the volume of bile was estimated by weighing the previously tared collection vials.

Results. Significant increase of biliary T_3 excretion was found during the 60 min infusion of glucose or arginine. However, in fed rats such increase did not continue after the termination of infusion, while in fasted rats the increase was observed still for next 60 min after the infusion. The attempts to further stimulate the excretion of T_3 by the addition of small insulin doses (62.5, 125, 250 and 500 mU) to the infused glucose showed inversed effect: by such intervention the increase of T_3 was blunted by higher doses, while at lower doses unsignificant increase appeared. The increase of biliary T_3 excretion was also blunted by cycloheximide (translation inhibitor) and somatostatin (insulin release inhibitor) both in normal fed and 24 hr fasted rats.

Conclusions. Short-term i.v. infusion of glucose and arginine resulted in immediate and transient increase of biliary T_3 excretion which was inhibited by both the cycloheximide and somatostatin. Taken together with our previous findings, this supports the view on rapid fluctuation of hepatic iodothyronine metabolism as related to preprandial (prevailing effect of gluconeogenetic hormones resulting in preferential formation of rT_3) and postprandial period (predominant effect of insulin resulting in preferential formation of T_3).

Key words: Biliary triiodothyronine – Glucose – Arginine – Insulin – Iodothyronine metabolism – Liver – Cycloheximide – Somatostatin

It is well known that the conversion of thyroxine (T_4) to metabolicaly active triiodothyronine (T_3) or inactive reverse triiodothyronine (rT_3) is influenced by several factors (for review see DOCTER et al. 1993; KAPTEIN 1996; MCIVER and GORMAN 1997).

During past several years we developed an experimental model suitable to study acute changes in iodothyronine metabolism in rat liver by the estimation of biliary excretion of individual iodothyronines in samples of bile obtained in subsequent short time

intervals. The concentration of T_4 in rat bile was previously found approximately the same as in plasma, although that of T_3, rT_3 and several diiodothyronines was about four to eight times higher than in plasma (Foeldes et al. 1982). Thus, biliary excretion of tri- and diiodothyronines predominantly reflects the rate of their metabolic production in the liver, while their hepatic clearance from plasma appears negligible. In addition, only a few per cent of near-physiological doses of intravenously administered exogenous T_4 and T_3 appear in bile (Langer et al. 1987) thus indicating that biliary excretion of iodothyronines is influenced only negligibly even by their physiological plasma levels. From this follows that certain variations in biliary excretion of iodothyronines after various interventions occur earlier than those in serum and thus the monitoring of biliary iodothyronines is a useful technique for the *in vivo* study of acute changes in liver iodothyronine metabolism.

We previously found increased biliary rT_3 after salicylate (Foeldes et al. 1983), linoleic acid (Langer et al. 1988) and after the inhibitor of liver type I deiodinase propylthiouracil (Langer and Gschwendtova 1992).

In addition, we repeatedly found increased biliary rT_3 after the infusion of several hormones that counterregulate hypoglycemia such as adrenaline (Langer and Foeldes 1988), glucagon, vasopressin and angiotensin II (Langer et al. 1989) and neurotensin (Langer and Gschwendtova 1991). Recently, we reported even the effect of insulin-induced hypoglycemia apparently resulting in the increased secretion of endogenous counterregulatory hormones on the increase in biliary rT_3 in rats which was prevented by actinomycim D and cycloheximide and blunted by verapamil (Langer and Gschwendtova 1995). In addition, cycloheximide also prevented the increase of biliary rT_3 after the infusion of glucagon. Recently the increase of biliary rT_3 after insulin induced hypoglycemia in rats was prevented by previous adrenal medullectomy, inhibition of glucagon secretion by somatostatin and prevention of adrenaline and glucagon effect on the liver cells by phentolamine and verapamil, respectively (Langer and Gschwendtova 1997).

Taken together, our previous studies showed that the production of rT_3 in the liver resulting in increased biliary excretion is stimulated not only by the administration of exogenous gluconeogenetic hormones, but also by the increase of their endogenous production due to insulin induced hypoglycemia. Since, vice versa, the decrease of rT_3 with simultaneous increase of T_3 production has been repeatedly shown by estimation the blood levels after refeeding of previously fasted chicks (Nockels et al. 1988), rats (Burman et al. 1979; Gavin et al. 1981, 1988; Nishida and Pittman 1990; reviewed by Visser 1981 and by Engler and Burger 1984) and man (Scriba et al. 1979; Davidson et al. 1979; Johannessen et al. 1981), we attempted to evaluate possible acute effect of glucose and arginin as important nutrients stimulating the release of insulin on biliary excretion of T_3 using our experimental model.

Materials and Methods

Animals. Male Wistar Olac rats weighing about 300 g were purchased from Velaz (Prague, Czech Republic), 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 (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 i.p.; maintenance 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 six hours. The animals were heated externally to maintain body temperature. Following collection of the initial control samples (1st hour), intravenous infusions of glucose (G), glucose with insulin (I) or arginin (AR) were started using a microinfusion pump (Unita II; Braun, Melsungen, Germany). The details on doses and infusion rates are described below and in the legends to the figures. In some cases, two or even more groups of eight animals each subjected to the same treatment were pooled.

Six groups of 3 to 10 animals each were subjected to the same type of surgery as described above. Thin polyethylene tubings were inserted into their

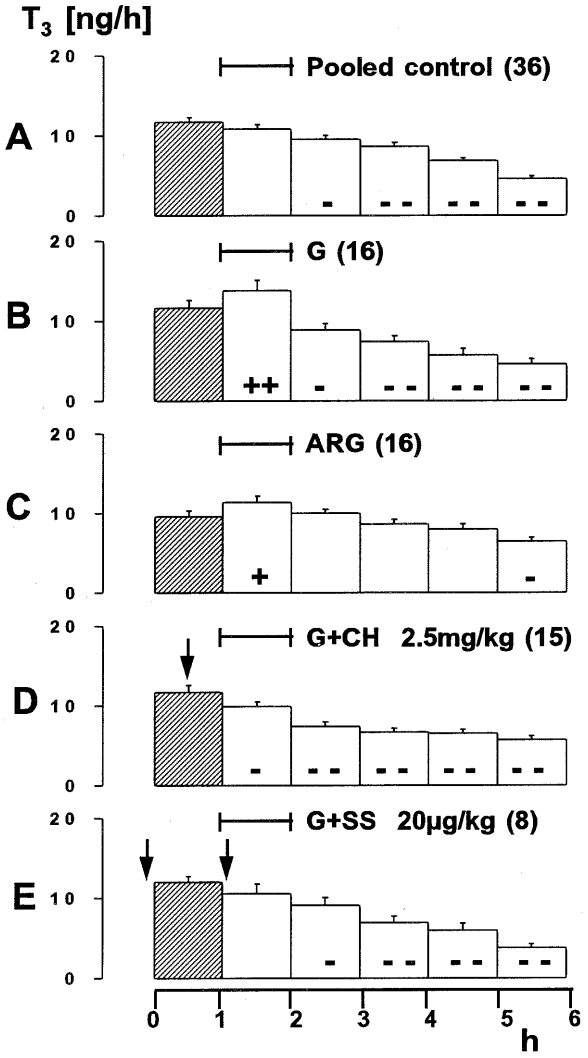

Fig. 1
Biliary excretion of 3,5,3'-triidothyronine (T_3) in: 1. panel A – pooled control group (N=36) infused saline (1.5 ml/60 min); 2. panel B – pooled groups infused 30 % glucose (1.5 ml/60 min; N=16); 3. panel C ´ pooled groups infused arginine (80 mg in 1.5 ml saline per 60 min; N=16); 4. panel D - pooled groups infused glucose (as above) and injected cycloheximide i.v. (2.5 mg/kg, CHEX, Sigma, St. Louis, MS; N=15) 30 min before glucose infusion (arrow); 5. panel E – one group injected glucose (as above), injected somatostatin (SS; Stilamin^R, Serono, Freiburg, Germany) twice 10 µg/kg as indicated by arrows and then infused with 12 µg SS/kg in 3.0 ml saline for 5 hours (0.6 ml/hr), N=8). In each case the infusion of glucose was administered during the second hour as indicated by the horizontal abscissa.
Striated columns = control interval. The columns show means and S.E. + or ++ = increase (P<0.05 or <0.01, respectively) vs. control interval; – or — = decrease (P<0.05 or <0.01, respectively) vs. control interval.

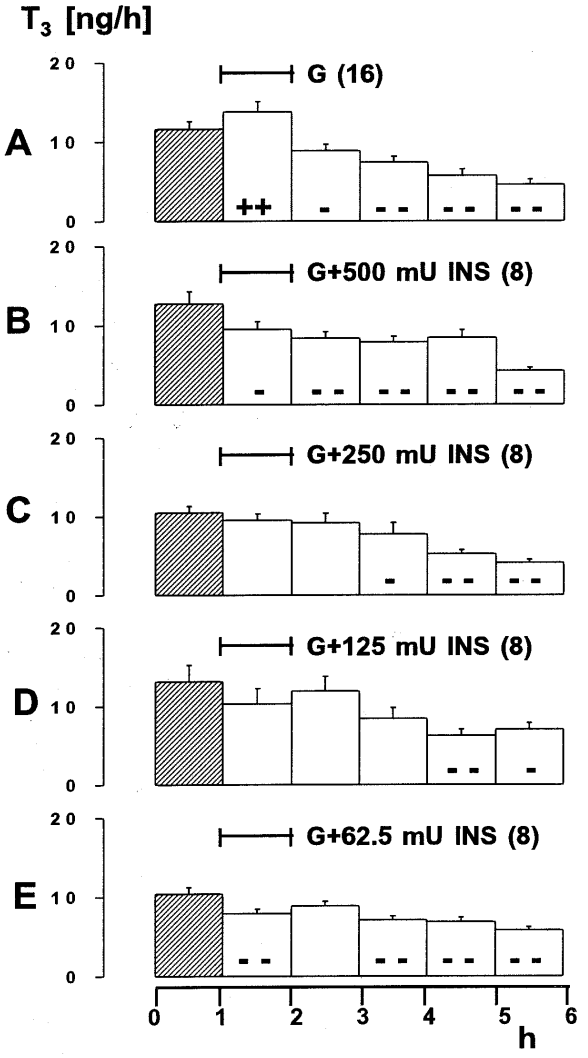

Fig. 2
Biliary excretion of 3,5,3'-triidothyronine (T_3) in: 1. panel A – pooled groups infused 30 % glucose (1.5 ml/60 min; N=16); 2. panel B – one group infused 30 % glucose (as above) together with 500 mU insulin (INS, N=8); 3. panel C – one group infused 30 % glucose (as above) together with 250 mU INS (N=8); 4. panel D – one group infused 30 % glucose (as above) together with 125 mU INS (N=8); 5. panel E – one group infused glucose (as above) together with 62.5 mU INS (N=8). In each case the infusion of glucose was administered during the second hour as indicated by the horizontal abscissa.
Striated columns = control interval. The columns show means and S.E. + or ++ = increase (P<0.05 or <0.01, respectively) vs. control interval; – or — = decrease (P<0.05 or <0.01, respectively) vs. control interval.

femoral artery and both femoral veins and heparin 750 U/kg was injected into one of femoral veins. One vein was used for the infusions of glucose (400 and 800 mg/hr), insulin (1000 mU/1 hr) or 400 mg glucose with various doses of insulin for one hour (for details see legend to Fig. 4). The other femoral vein was used for external arterio-venous shunt, the arterial and venous tubings being connected with small piece of tightly bound larger tubing which enabled to disconnect the shunt. In 15-60 min intervals (see Fig. 4) about 0.5 ml blood was obtained from the arterial tubing, centrifuged and about 100-150 µl plasma aliquot was transfered to small tube and frozen for glucose estimation. This plasma was replaced by the same volume of previously obtained plasma from donor rats, the blood being thus reconstituted and injected into the venous tubing. In the plasma aliquots the level of glucose was estimated with the aid of glucose-oxidase method using commercial kit (Lachema, Brno, Czech Republic).

Radioimmunoassay of T_3 in bile. The aliquots of 25 µl bile were treated with 25 µl ß-glucuronidase/arylsulfatase (Boehringer, Mannheim, Germany) and the concentration of total (i.e. conjugated plus unconjugated) T_3 was then estimated in 50 µl aliquots of supernatant corresponding to 25 µl bile obtained by centrifugation of inicial mixture by an in-house radioimmunoassay (Foeldes et al. 1982) that later was modified slightly by using smaller aliquots of bile for individual estimations (Langer et al. 1989). The sensitivity of the assay is less than 0.5 ng/ml. The results were espressed as ng/hr and the volume of bile was estimated by weighing the previously tared collection vials.

Statistical evaluation. In each animal the first sample of bile was control. The differences in the excretion of T_3 in 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 ($P<0.05$) either from the reference pooled control group or from some other similarly treated group as indicated in the elegends to figures.

Results

Fig. 1 shows the reference pooled control group of 36 rats (panel A) in which biliary excretion of T_3 was estimated in several previous experiments using the same experimental procedure as in this study. In this group, as usual, the amount of T_3 in bile gradually decreased, the values from the 3rd to 6th hour being significantly lower that during the first (control) interval. In contrast, during the infusion of glucose (panel B; N=16) or arginine (panel C; N=16) the biliary excretion of T_3 significantly increased, while the gradual decrease of this hormone followed after the termination of infusion which was similar to the control group shown in the panel A.

Moreover, the increase of T_3 excretion was supressed by the administration of cycloheximide (panel D; N=15) or somatostatin (panel E; N=8), both being administered before the infusion of glucose.

Panel A (Fig. 2) is the same as panel B (Fig. 1) and it should be compared with the panels B-E (Fig. 3). The panels B and C (Fig. 3) show that no increase of biliary T_3 was found after the higher doses of insulin (500 and 250 mU, respectively) were infused together with glucose, while after lower doses (125 and 62.5 mU, panel D and E, respectively) unsignificant increase of T_3 was observed in the next 60 min after the termination of infusion. However, it should be added that even such unsignificant increase is at least valid to be noted, since two decades of our experience showed that even such small increase of T_3 is unusual and apparently results only from some experimental manipulation such as glucose plus insulin infusion.

Fig. 3 shows, first, that in the animals fasted for 24 hrs before the experiment (panel F; N=14) the biliary excretion of T_3 was lower than that in fed animals (compare the values in all panels, Fig. 1). In addition, in this group the increased T_3 excretion continued even for one hour after the termination of glucose infusion. As also shown, even in fasted rats the increase of T_3 excretion was supressed by cycloheximide (panel G; N=15).

Fig. 4 (upper panel) shows the rapid and striking increase of glycemia in groups of rats after the infusion of various amounts of glucose. In contrast, there was a clear decrease of glycemia after the infusion of 1000 mU insulin. The lower panel (Fig. 4) shows

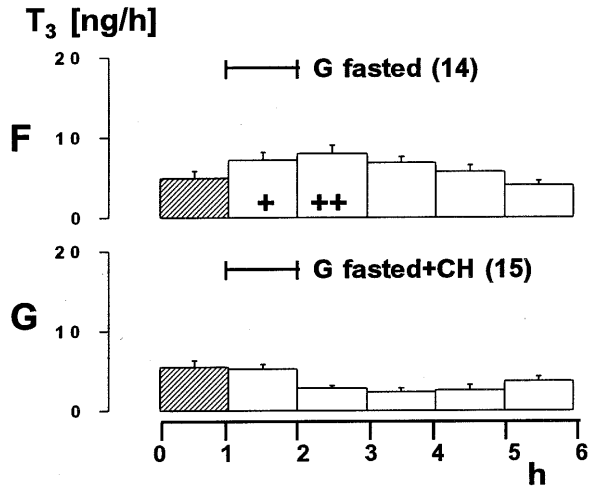

Fig. 3

Biliary excretion of 3,5,3'-triidothyronine (T_3) in: 1. panel F - pooled groups of rats fasted for 24 hrs and infused glucose (1.5 ml 30 % glucose per 60 min; N=14); 2. panel G - pooled groups fasted for 24 hrs, infused glucose (as above) and injected cycloheximide i.v. (2.5 mg/kg, CHEX, Sigma, St. Louis, MS; N=15) 30 min before glucose infusion (arrow). In each case the infusion of glucose was administered during the second hour as indicated by the horizontal abscissa.

Striated columns = control interval. The columns show means and S.E. + or ++ = increase (P<0.05 or <0.01, respectively) vs. control interval; – or — = decrease (P<0.05 or <0.01, res[pectively) vs. control interval.

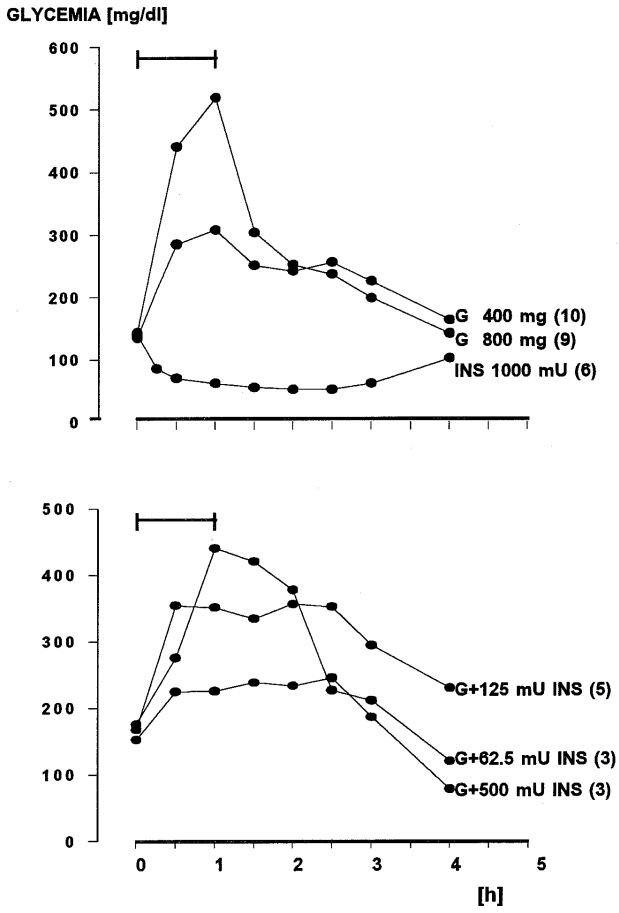

Fig. 4

Glycemia in groups of rats (the number of rats in each group is indicated in brackets) infused various doses of glucose (G), insulin (INS) or glucose with insulin for 60 min (as indicated by the horizontal abscissa.

the interplay between simultaneously infused glucose and insulin resulting in the changes of glycemia. It may be seen that the infusion of highest dose (500 mU) of insulin resulted in the smallest increase of glycemia, while the difference between the effect of lower doses (62.5 mU and 125 mU) of insulin infused with the same amount of glucose did not remarkably differ.

Discussion

It was demonstrated that rapid increase of T_3 biliary excretion occurs in rats during the infusion of glucose and arginine, both being recognized as stimulators of insulin secretion which results in the stimulation of numerous anabolic steps related to the utilization of those nutrients. This finding is in aggreement with the observations of increased T_3 production under various conditions related to either fed or postprandial state. Thus, Koh et al. (1986) found rapid increase of serum T_3 level after glucose ingestion which was blunted in glucose intolerant subjects (Koh et al. 1994). Similarly, Burger et al. (1980) found increased serum T_3 level in rats after carbohydrate and protein refeeding following 4 days starvation. Mariash and Oppenheimer (1985) reviewed the interaction of thyroid hormone and nutritional signals and suggested the hepatic protein called S_{14}, the mRNA of which increased after T_3 or high carbohydrate diet, to be involved in the molecular mechanism by which T_3 and glucose interact to influence gene expression. In addition, Nimalasuriya et al. (1986) demonstrated that the diurnal T_3 rhythm is

not TSH dependent and supposed some unidentified dietary signal which alters the efficiency of the peripheral tissue T_4 to T_3 conversion.

Such views seem to be indirectly supported by the inverse effect of fasting and refeeding on reverse T_3 levels in man which increase during fasting, but normalize during refeeding (LoPresti et al. 1991). This finding is in aggreement with the series of our observations on the effects of exogenous and endogenous counterregulatory hormones on biliary excretion of rT_3 in rats as mentioned in the Introduction.

It was also observed that the administratiom of cycloheximide or somatostatin results in the inhibition of increased T3 production during the infusion of glucose. Such effect of translation inhibitor cycloheximide supports the possible role of translation processes in the mechanism of increased T_3 production. Similar effect of somatostatin may result from the inhibition of insulin release which supports the view on the participation of endogenous insulin in the increased T_3 prodsuction during the glucose infusion.

Taken together with our previous findings, this supports the view on rapid fluctuation of hepatic iodothyronine metabolism as related to preprandial (prevailing effect of gluconeogenetic hormones resulting in preferential formation of rT_3) and postprandial period (predominant effect of insulin resulting in preferential formation of T_3).

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Corresponding author: Pavel Langer, M.D.
Institute of Experimental Endocrinology
Vlarska 3
833 06 Bratislava
Slovakia
Phone; 00421-7-54772800,
Fax: 00421-7-54774247
E-mail: ueenlang@savba.savba.sk

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