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0014-4754/90/010063-05$1.50 + 0.20/0

Glucose-evoked recovery of hepatic thyroxine 5'-deiodinase independent of de novo protein synthesis in fasted rat

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Received 7 April 1989; accepted 28 August 1989

Summary. The glucose-evoked recovery of Type I thyroxine 5'-deiodinase activity in the hepatic microsomes of fasted rat was not inhibited by either cycloheximide, puromycin or actinomycin D during 3 h after glucose feeding; however, [^{3}H]-leucine uptake by the liver or the hepatic microsomal fraction was significantly inhibited by cycloheximide and puromycin but not by actinomycin D. These results indicate that the glucose-evoked recovery of deiodinase activity may be independent of de novo protein synthesis.
Key words. Thyroxine 5'-deiodinase; starvation; caloric ingestion; cycloheximide; puromycin; actionomycin D.

Dietary intake is one of the modulatory factors of thyroid hormone metabolism in the peripheral tissues[1]. Starvation reduces the conversion of hepatic thyroxine (T_4) to 3,5,3'-triiodothyronine (T_3) and results in a decrease of serum T_3 level[2], whereas refeeding a starved subject with a simple carbohydrate such as glucose returns both the enzyme activity and hormonal concentration to normal levels[3]. Since serum insulin level is also elevated by refeeding, one explanation for the glucose-evoked increase of 5'-deiodinase activity can be a de novo synthesis of the enzyme protein via an insulin-mediated pathway of energy supply in the tissue[4]. To examine this hypothetical explanation, the present study was designed to determine whether inhibitors of protein synthesis also suppress the elevation of glucose-dependent activity of thyroxine 5'-deiodinase in fasted rats.

Materials and methods

125-I labeled T_3 (> 1200 μCi/ng) and T_4 (> 1200 μCi/ng) were purchased from Amersham Chemical Co. (Arlington Heights, IL, USA). Tritiated leucine ([^{3}H]-Leu, 146.5 Ci/nM) was acquired from New England Nuclear (Boston, MA, USA). Dithioerythritol (DTE), T_3, T_4, bovine serum albumin (BSA), cycloheximide (CH) and actinomycin D (AD) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Puromycin (PM) was purchased from Nutritional Chemical Co. (Cleveland, OH, USA).

Male Sprague-Dawley rats, weighing 220 g, were maintained on Purina chow pellets (Wayne Lab. USA) and tap water ad libitum. The dosages of protein inhibitors were chosen as the highest dose which the test animals survived 48 h after injection. The animals received a daily subcutaneous injection of T_4 at 0.75 μg/100 g b.wt for five consecutive days before fasting[5]. The T_4 supplement was continued during fasting until the day before sacrifice. The animals were divided into three groups and each fasted for 48 h before experiment. The control group was given a bolus of water through an intragastric tube. The second and third groups were refed with a bolus of 50% glucose (1 ml/100 g b.wt) in the same way. The second group was given a protein synthesis inhibitor by intraperitoneal injection (i.p.) as described in the legends while the third group was given an i.p. injection containing 0.9% NaCl solution only. All animals were sacrificed 3 h after refeeding. Under anesthesia, blood was withdrawn from the abdominal vein; then the liver was perfused with 0.9% NaCl solution containing 10 μ/ml heparin, excised and minced. Homogenization took place with 0.05 M Tris-HCL containing 5 mM DTE, 10 mM EDTA, 0.25 M sucrose at pH 7.2. The microsomal fraction was harvested according to the method of Hogeboom[6]. The resulting pellet after centrifugation at

105,000 × g was gently resuspended in buffer with a glass homogenizer in one half volume of the original homogenate. This suspension served as the source of hepatic 5'-deiodinase. The enzyme activity was determined by measuring the conversion of added T_4 to T_3 as described previously [7]. T_4 and T_3 in sera and in the reaction media were assayed by double antibody radioimmunoassay [8]. Serum insulin was measured by a kit obtained from Cambridge Medical Diagnostics, Inc. (Billerica, MA, USA). When [^{3}H]-Leucine was given (20 μCi in 0.9% NaCl per animal) it was administered i.p. at the time of refeeding. Aliquots of tissues or microsomes were immersed in two volumes (v/w) of NCS tissue solubilizer (Amersham, Arlington Heights, IL, USA) and incubated overnight at 37 °C. The radioactivity was counted by Beckmann liquid scintillation counter.

Protein was determined by the method of Lowry et al.[9]. Statistical significance was calculated by either paired or unpaired Student's t-test.

Results

Figure 1 shows the influence of fasting, refeeding and the effect of cycloheximide on the hepatic deiodinase activity. The enzyme activity was low in the fasted group and significantly raised in the refed groups. The administration of 14 mg/kg of CH demonstrated no significant suppression in the elevated enzyme activity in refed animals. When PM (200 mg/kg) or AD (2.1 mg/kg) replaced CH, the glucose-evoked deiodinase activity was likewise unaffected. In the puromycin study, the 5'-deiodinase activity was 0.90 ± 0.08 pmole T_3 produced/min/100 mg protein in the fasted animals, 1.47 ± 0.05 in the refed animals treated with PM, and 1.63 ± 0.12 in the refed animals treated with saline (3 animals in each group). (Fasted vs Refed + PM: $p < 0.01$; Fasted vs Refed: $p < 0.01$; Refed + PM vs Refed: p NS) In the actinomycin study the 5'-deiodinase activity was 0.74 ± 0.09 p mole T_3 produced/min/100 mg protein in the fasted animals, 1.43 ± 0.20 in the refed plus AD animals, and 1.34 ± 0.17 in the refed plus saline group (4 animals in each group). (Fasted vs Refed + AD: $p < 0.05$; Fasted vs Refed: $p < 0.05$; Refed + AD vs Refed: p NS). Statistical analysis of data from these two studies demonstrated that neither PM nor AD significantly altered the glucose-induced increase in 5'-deiodinase activity.

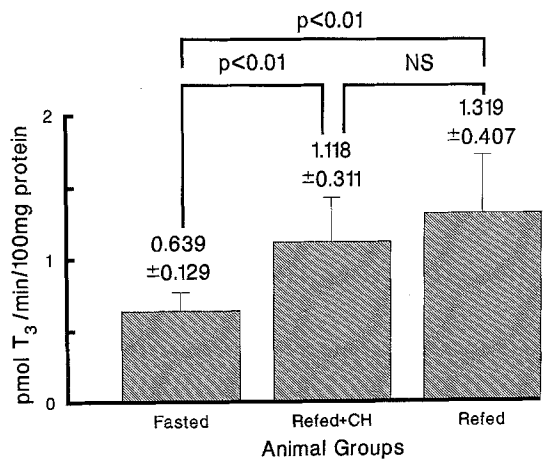

Figure 1. Cycloheximide effect on the glucose-evoked recovery of thyroxine 5'-deiodinase activity in the livers of fasted rats. Each animal was given CH by i.p. injection, 14 mg/kg in 4 equally divided doses during refeeding over 3 h. The enzyme activity was expressed in pmole T_3 produced /min/100 mg protein. The values represent the mean ± SD for nine animals in each group. Statistical evaluation by paired and unpaired t-tests gave the same results and are indicated in the figure.

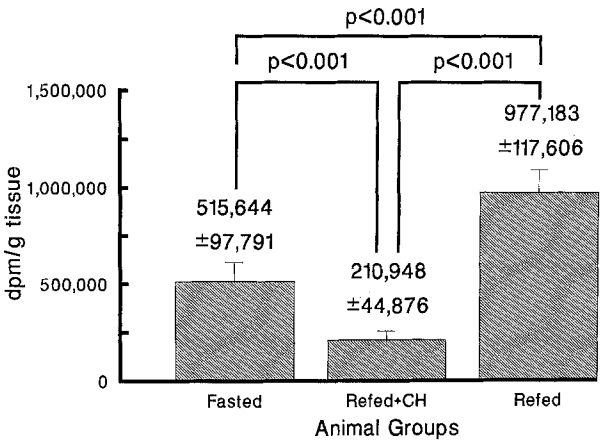

Figure 2. Cycloheximide effect on the glucose-evoked recovery of [^{3}H]-labeled leucine uptake in the livers of fasted rats. The dosage and the administration of CH were the same as that described in the legend of figure 1. The values represent the mean SD for six animals in each group.

To monitor whether the administered amount of CH indeed inhibited protein synthesis during refeeding, the hepatic uptake and microsomal uptake of [^{3}H]-Leu were measured. Figure 2 shows the tissue uptake of radioactivity in the refed plus CH group was only 21% of the refed group and 41% of the fasted group. The radioactivity in the serum obtained from each group was not changed by fasting, refeeding or CH administration. The microsomal uptake of [^{3}H]Leu in the refed plus CH group showed similar suppression in the radioactivity uptake as that by the whole liver. The inhibition of [^{3}H]-Leu uptake was also observed after treatment of PM; however, as expected no inhibition was observed after AD treatment.

The responses of serum T_4 and insulin levels to CH were measured. The T_4 levels were not significantly changed perhaps because the animals had received daily T_4 supplement. The T_4 level was 2.90 ± 0.21 μg/dl in the fasted, 3.03 ± 0.54 in the refed plus CH, 2.89 ± 0.46 in the refed plus saline group and 3.67 ± 0.87 μg/dl in the intact animals (n = 10 for each group). Serum insulin level was lowered by fasting but it returned to 59% of the normal level 3 h after refeeding. CH treatment did not affect the recovery of insulin level by refeeding. It was 15.9 ± 0.67 μU/ml in the fasted, 54.9 ± 6.28 in the refed plus CH, 36.9 ± 5.85 in the refed and 62.8 ± 7.62 μU/ml in the intact animals (9 animals in each group).

Discussion

It has been shown [10] that caloric intake is one of the most influential modulators on hepatic 5'-deiodinase activity. The calories can be composed of a single carbohydrate such as glucose without protein or lipid [3]. The mechanism by which glucose enhances the recovery of 5'-deiodinase activity in fasted animals remains unclear. Since glucose ingestion elevates blood glucose and insulin levels in addition to the enzyme activity, one can expect that the glucose-evoked deiodinase activity might be mediated through a new protein synthesis via insulin action [4]. The recovery of 5'-deiodinase activity seems to be in parallel to the raised insulin levels. Recently Nishida et al. [11] observed a similarity in responses of thyroxine 5'-deiodinase and of a hepatic stearoyl CoA desaturase to starvation and refeeding. The desaturase enzyme activity was obligatorily mediated by insulin and only secondarily by thyroid hormone [12]. Grau et al. [4] had reported that the T_3 production in hepatic microsomes was elevated when insulin was infused in vivo and the addition of CH to the infusion did not affect T_3 production. Gavin and colleagues [13] studied the effect of glucose on type II 5'-deiodinase in cultured mouse neuroblastoma cells. They found both the type II 5'-deiodinase activity and the uptake of ^3H labeled amino acid reduced after incubating the cells with puromycin for 8 h. Their study did not include the effect of actinomycin on the uptake of ^3H labeled amino acid for comparison. Therefore the differences in our observations and conclusions may be accounted for by differences in our methods and the different enzyme sources used, as the type I and type II 5'-deiodinase have very different characteristics.

In our study, CH was used as an inhibitor of protein synthesis at the translational stage. Puromycin, being another inhibitor at the translational stage of nuclear protein, and actinomycin, being an inhibitor at the transcriptional stage, were also tested. We had hoped that comparing the CH effect with those of PM and AD may help to indicate the specific stage where these inhibitors block the 5'-deiodinase enzyme recovery. Both CH and PM inhibited the hepatic [^3H]-Leu uptake in the refed group but not the type I 5'-deiodinase activity. AD did not suppress the leucine uptake nor the hepatic 5'-deiodinase activity in the refed group. From these results the specific stage where glucose exerts its influence on the enzyme activity is not identified, but these findings have provided strong evidence that the glucose-dependent recovery of type I 5'-deiodinase activity does not require new protein synthesis in vivo.

Acknowledgments. The study was supported in part by the U.S.V.A. Medical Research Service. The study was performed during Dr. Nishida's sabbatical from University of Tokushima. His current address is: Faculty of Pharmaceutical Sciences, University of Tokushima, Tokushima, Japan.

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0014-4754/90/010067-03$1.50 + 0.20/0

Influence of sodium balance on atrial natriuretic factor in rats with one-kidney, one-clip renal hypertension

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Received 9 January 1989; accepted 22 August 1989

Summary. The influence of sodium intake on the gene expression and circulating levels of atrial natriuretic factor (ANF) was investigated in unanesthetized rats with one-kidney, one-clip renal hypertension. After clipping, the rats were maintained for 3 weeks either on a salt-deficient (n = 11) or a regular-sodium diet (n = 10). Animals which had received the regular-sodium diet exhibited significantly higher ANF mRNA levels in their right and left atria than salt-restricted animals, whereas there was no significant difference in plasma ANF levels.
Key words. Renal hypertension; sodium; atrial natriuretic factor; messenger RNA.