

# Somatostatin Inhibits Rat Hepatic T<sub>4</sub>-5'-deiodinase

## THE EFFECT IS INDEPENDENT OF THE ASSOCIATED HYPOINSULINEMIA

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**ABSTRACT** Somatostatin decreases the serum 3,5,3'-triiodothyronine (T<sub>3</sub>) concentration in athyreotic subjects treated with L-thyroxine (T<sub>4</sub>). The present study was performed to determine the effect of somatostatin on T<sub>4</sub>-5'-deiodinase activity in rat tissue homogenate preparations. This enzyme is an important regulator of T<sub>3</sub> production. Continuous somatostatin infusion at high dose (4 µg/kg per min subcutaneously) and low dose (0.8 µg/kg per min subcutaneously) for 48-72 h significantly increased ( $P < 0.001$ ) the mean aorta plasma somatostatin-like immunoreactivity concentration to 786±65 and 448±58 pg/ml, respectively compared with the normal mean of 69±17 pg/ml in the carbohydrate-fed rat (20% glucose in water ad lib.). The mean hepatic T<sub>4</sub>-5'-deiodinase activity at both 48 h (100±5 pmol/min per 100 mg protein) and 72 h (90±7 pmol/min per 100 mg protein) was significantly reduced in the high-dose group ( $P < 0.005$ ), compared with the mean enzyme activity in the glucose-fed control group (138±6 pmol/min per 100 mg protein). There was a negative correlation ( $r = -0.9$ ,  $P < 0.01$ ) between the alterations in the peripheral plasma somatostatin-like immunoreactivity concentration and hepatic T<sub>4</sub>-5'-deiodinase activity. High-dose somatostatin did not consistently lower the serum T<sub>3</sub> concentration in the glucose-fed rat. Somatostatin had no effect on pituitary T<sub>4</sub>-5'-deiodinase activity in the glucose-fed rat. High-dose somatostatin also significantly inhibited ( $P < 0.01$ )

the glucose-refeeding reactivation of hepatic T<sub>4</sub>-5'-deiodinase in the 72-h-fasted rat. The mean enzyme activity after 96 h was 96±8 pmol/min per 100 mg protein compared with 127±4 pmol/min per 100 mg protein in the refed control group. Somatostatin had a similar inhibitory effect on serum T<sub>3</sub>. There was a positive correlation ( $r = 0.5$ ,  $P < 0.01$ ) between the somatostatin-induced alterations in serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase during refeeding. A significant positive correlation ( $r = 0.7$ ,  $P < 0.005$ ) was noted between the somatostatin effect on hepatic T<sub>4</sub>-5'-deiodinase activity and the induced hypoinsulinemia in the fed group. In addition, a significant negative correlation ( $r = -0.9$ ,  $P < 0.001$ ) was noted between the suppressed enzyme activity and the serum glucose/insulin ratio in the refed group. However, although low-dose somatostatin also induced the same degree of hypoinsulinemia ( $P < 0.05$ ) in the fed and refed groups it had no effect on hepatic T<sub>4</sub>-5'-deiodinase activity. Furthermore, despite the induction of hyperinsulinemia during refeeding, the high-dose somatostatin inhibitory effect on enzyme activity persisted.

Thus, somatostatin inhibited hepatic T<sub>4</sub>-5'-deiodinase activity in the carbohydrate-fed rat and prevented the carbohydrate-refeeding normalization of enzyme activity in the 72-h-fasted rat. The effect of somatostatin on enzyme activity was independent of the associated hypoinsulinemia. In the carbohydrate-fed animal the somatostatin effect was selective, as the hormone had no effect on pituitary T<sub>4</sub>-5'-deiodinase activity. These data suggest that somatostatin could play a role in the peripheral metabolism of thyroid hormones.

## INTRODUCTION

A number of studies have demonstrated that somatostatin modulates the hypothalamic-pituitary-thyroid axis. These effects are inhibitory and on a chronic basis

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could eventuate in hypothyroidism (1-5). A number of reports also suggest that somatostatin may modulate the extrathyroidal or peripheral metabolism of thyroid hormones (6, 7). Weeke et al. (6) demonstrated that a prolonged somatostatin infusion (24 h) significantly decreased the serum  $T_3/T_4$  ratio and increased serum reverse  $T_3$  in patients treated for myxedema (6). In addition, Loos et al. (7) found that an 8-h somatostatin infusion decreased serum 3,5,3'-triiodothyronine ( $T_3$ )<sup>1</sup> levels in athyreotic subjects treated with L-thyroxine ( $T_4$ ) (7). These somatostatin effects on  $T_3$  metabolism may have been mediated through a reduction in the activity of tissue  $T_4$ -5'-deiodinase, the enzyme that regulates extrathyroidal production of  $T_3$  from  $T_4$  (8). Such a somatostatin effect seems possible as dietary modulation and diabetes significantly alter both  $T_3$  metabolism and somatostatin kinetics (9-11). Fasting and diabetes are associated with an increased pancreatic content of somatostatin and impaired  $T_3$  production from  $T_4$  (9-11). The impaired  $T_3$  metabolism is consequent to a reduction in the activity of the tissue (liver) enzyme  $T_4$ -5'-deiodinase (9, 10). Thus, it is possible that somatostatin could have an extraislet inhibitory effect, via the hepatic portal system on this liver enzyme.

The present study was performed to determine if somatostatin has an effect on hepatic  $T_4$ -5'-deiodinase activity in the rat. We found that somatostatin inhibited hepatic  $T_4$ -5'-deiodinase in the glucose-fed rat, and that it also prevented the glucose-refeeding reactivation of this enzyme in the fasted animal.

## METHODS

$T_4$ ,  $T_3$ , and dithiothreitol (DTT) were obtained from Sigma Chemical Co., St. Louis, MO. [<sup>125</sup>I] $T_3$ , labeled in the phenolic ring position of sp act 500-900  $\mu\text{Ci}/\mu\text{g}$  was purchased from New England Nuclear, Boston MA. Goat anti-rabbit  $\gamma$ -globulin serum was obtained from Antibodies, Inc., Davis, CA. Innovar (fentanyl 0.05 mg and droperidol 2.5 mg/ml) was purchased from McNeill Laboratories, Inc., Fort Washington, PA and Alzet osmotic minipumps (No. 2001) from Alza Corp., Palo Alto, CA. Rat insulin was obtained from Novo Research Institute, DI-2880 Bagsvaerd, Denmark, <sup>125</sup>I-insulin, from New England Nuclear, and antiporcine insulin serum from Miles Laboratories, Inc., Elkhart, IN. Pork insulin (Iletin II, U-500) was purchased from Eli Lilly & Co., Indianapolis, IN. Somatostatin was purchased from Sigma Chemical Co. Other chemicals were reagent grade and were purchased from commercial suppliers.

**Animals and diets.** Incubations were performed on hepatic and pituitary preparations obtained from male Sprague-Dawley rats. Within each experiment the animals (groups,  $n = 4$ ) were closely matched for weight and age. For 1 wk before each study period the animals were maintained on

an ad lib. intake of  $H_2O$  and Purina rodent laboratory chow (No. 5001, Ralston Purina Co., St. Louis, MO). This standard rodent chow contains complex carbohydrates (49.8%), protein (23.4%), fat (4.5%), fiber (5.0%), minerals (7.3%), and a standard multivitamin content. Two animal models were used; glucose-fed rats (20% glucose in water) not exposed to prior fasting and 72-h-fasted rats refed with glucose ad lib. (20% glucose in water). The glucose-fed rats consumed on average of 60 kcal/d, whereas the refed rats drank an average of 80 kcal/d. In the feeding experiments, somatostatin pumps were implanted subcutaneously after 72 h of ad lib. glucose feeding and compared with an equivalent 20% glucose-fed group, each group fed for a further 72 h. The 72-h-fasted rats (water ad lib. only) were refed for variable intervals with 20% glucose in water before killing. In the refeeding experiments, somatostatin-treated groups (somatostatin: 4  $\mu\text{g}/\text{kg}$  per min, subcutaneously, delivered from Alzet osmotic minipumps) were compared with glucose-refed control groups. All animals were treated with  $T_4$  ( $T_4$ : 1.5  $\mu\text{g}/100$  g per d subcutaneously, delivered by osmotic minipump) for 1 d before and throughout the experimental period. This was necessary in order to maintain euthyroidism, especially in the fasted and somatostatin-exposed animals. The Alzet osmotic minipumps were implanted subcutaneously under Innovar anesthesia.

**Hepatic  $T_4$ -5'-deiodinase analysis.** Enzyme analysis was performed in liver homogenate preparations as previously described (12). The conversion of  $T_4$  (1  $\mu\text{M}$ ) to  $T_3$  was studied in 2% homogenate preparations (pH 7.2) enriched with 5 mM DTT and 10 mM EDTA. The initial rate of the reaction was studied; samples for  $T_3$  analysis (100  $\mu\text{l}$ ) were removed from incubations (37°C) at 15 min and added to 0.9 ml of ice-cold, iodothyronine-free, normal human serum (serum extracts).  $T_3$  was measured in the serum extracts by the previously described radioimmunoassay (12). In each experiment the amount of product was corrected by the appropriate recovery and the amount of iodothyronine present in unincubated, control tubes.

**Pituitary  $T_4$ -5'-deiodinase analysis.** Pituitaries were weighed, placed in 20 vol (wt/vol) 0.05 M iced Tris HCl, enriched with 0.25 M sucrose and 100 mM DTT and homogenized in a hand-held glass homogenizer with a ground glass pestle for 60-90 s until no discrete fragments of tissue were visible (13). Pituitaries from four rats per group were pooled to prepare the homogenates for in vitro studies according to weight and the volume of homogenate required. Before incubation, homogenates (100  $\mu\text{l}$ ) were prewarmed to 37°C for 10 min in a water bath.  $T_4$  (10  $\mu\text{l}$ ) was added to a final concentration of 1.3  $\mu\text{M}$  and incubations were carried out at 37°C under nitrogen. At 30 and 60 min 25- $\mu\text{l}$  samples of the incubation mixtures were removed and added to 300  $\mu\text{l}$  of ice-cold normal human serum, which had been treated with activated charcoal to remove the iodothyronines, and which contained 1 mM propylthiouracil (PTU). These mixtures of serum and incubation media (serum extracts) were then analyzed for  $T_3$  (12). Control experiments consisted of (a) incubation of substrate ( $T_4$ ) in buffer without homogenate, (b) pituitary preparations without added substrate, and (c) added  $T_4$  to homogenate without incubation (time-zero tubes). The final  $T_3$  concentration was corrected for  $T_4$  cross-reactivity and  $T_3$  recovery. All incubations were performed in triplicate.

**Analysis of serum  $T_4$ ,  $T_3$ , glucose, insulin, and somatostatin.** Rat serum  $T_4$  and  $T_3$  samples were analyzed by the specific radioimmunoassays previously described (12). The cross-reactivity of  $T_4$  in the  $T_3$  assay was 0.13% (12). Neither somatostatin or insulin cross-reacted in either the  $T_4$  or  $T_3$  assays. Serum glucose was measured by the glucose

<sup>1</sup> Abbreviations used in this paper: BW, body weight; DTT, dithiothreitol; PTU, propylthiouracil;  $T_3$ , 3,5,3'-triiodothyronine;  $T_4$ , L-thyroxine; TSH, thyrotropin.

oxidase method, using an autoanalyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Serum insulin was measured by a modification (14) of the method of Grodsky and Forsham using specific anti-insulin antibodies and charcoal for the separation of free <sup>125</sup>I-insulin from bound (15). Plasma somatostatin-like immunoreactivity was measured by Dr. M. Berelowitz, University of Cincinnati Medical Center, Cincinnati, OH (16). Blood samples taken from the rat aorta (under Innovar anesthesia) were placed in iced polypropylene tubes containing 500 kallikrein inhibitory units, aprotinin (Trasylol, FBA Pharmaceuticals, Inc., New York), and 12 mg EDTA/ml of blood. The samples were mixed, immediately centrifuged at 3,000 rpm at 4°C for 20 min, plasma separated and stored at -20°C until use. Plasma samples were extracted (acid/ethanol) before somatostatin analysis.

**Hepatic glycogen and protein.** The percentage of hepatic glycogen was determined by Cardell's modification (17) of the method of Seifter et al. (18). Liver specimens were frozen between blocks of dry ice, and glycogen was extracted from a known amount of tissue by boiling the samples in 30% KOH, cooling, and diluting with distilled H<sub>2</sub>O and ethyl alcohol to a final concentration of 60%. Glycogen was precipitated in the cold in the presence of lithium bromide crystals. It was then sedimented by centrifugation, resuspended in cold 95% ethyl alcohol containing LiBr, and reprecipitated. The glycogen was then resuspended in water and analyzed for glucose by the phenolsulfuric acid procedure. The percentage of liver weight glycogen was determined from the percentage of glucose by multiplying by 0.9, which takes into account the differences in molecular weight. The homogenate's protein content was measured by the method of Lowry et al. (19).

**Statistical methods.** Mean values (mean ± SE) from each experimental group were compared with controls using Student's *t* test for unpaired data. Correlation coefficients were derived by standard methods (20).

## RESULTS

The effect of a continuous somatostatin infusion (somatostatin: 4 µg/kg per min subcutaneously) on body

weight (BW), serum T<sub>4</sub>, T<sub>3</sub>, glucose, and insulin concentration, hepatic glycogen content and T<sub>4</sub>-5'-deiodinase activity in the glucose-fed rat is demonstrated in Table I. These data are representative of a number (3) of similar studies on these parameters in the somatostatin-treated glucose-fed rat. The somatostatin treated groups had a higher BW after both 48 h (*P* < 0.025) and 72 h (*P* < 0.025) of hormone infusion than the mean BW in the glucose-fed control group. Somatostatin had no effect on the mean serum T<sub>4</sub> concentration after 48–72 h compared with the mean value in the control group. The mean serum T<sub>3</sub> concentration was significantly less (*P* < 0.025) in the 48 h somatostatin group compared with the control mean. However, the mean serum T<sub>3</sub> level was normal in the 72-h group. Table I also shows that fasting for 48 h had no effect on the mean serum T<sub>4</sub> concentration but that it significantly reduced the mean serum T<sub>3</sub> concentration (*P* < 0.025) compared with the respective mean in the glucose-fed control group.

The somatostatin infusion significantly reduced the mean hepatic T<sub>4</sub>-5'-deiodinase activity at 48 h and 72-h compared with the enzyme activity in the control group (*P* < 0.005) (Table I). The degree of enzyme reduction was equivalent to the effect of fasting for 48-h. Table I shows that the mean enzyme activity in the 48-h-fasted group at 110±4 pmol/min per 100 mg protein was significantly less (*P* < 0.025) than that of the control group, and overlapped the mean enzyme activity in the 48-h and 72-h somatostatin-treated groups. There was no correlation between the alterations induced by somatostatin on serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase activity in the glucose-fed animals (*r* = 0.5, *P* = 0.1).

TABLE I  
Effects of Continuous Somatostatin (SS) on BW, Serum T<sub>4</sub>, T<sub>3</sub>, Glucose and Insulin, Liver Glycogen Content, and T<sub>4</sub>-5'-deiodinase Activity (T<sub>4</sub>-5'-D) in the Glucose-fed Rat\*

	Serum					Liver	
	BW	T <sub>4</sub>	T <sub>3</sub>	Glucose	Insulin	Glycogen	T <sub>4</sub> -5'-D
	g	µg/dl	ng/dl	mg/dl	ng/dl	mg/g	pmol/min/100 mg protein
Glucose-fed (G)	178±2	2.8±0.1	61±4	126±2	120±10	7.1±0.1	138±6
G plus SS 48 h (4 µg/Kg/min)	190±3†	2.5±0.3	47±3‡	152±5§	80±14	7.8±1.4	100±5§
G plus SS 72 h (4 µg/Kg/min)	197±4†	2.2±0.3	58±4	156±7§	90±16	—	90±7§
Fast 48 h (F)	162±2	3.0±0.4	40±5‡	103±13	70±10	0.5±0.2¶	110±4‡

\* The animals in each group were fed glucose (20% glucose in water) ad. lib. for 72 h before each experiment. Each rat was treated with T<sub>4</sub> (1.5 µg/100 g/d subcutaneously), for 72 h before and during the experimental period, delivered from an Alzet osmotic minipump implanted subcutaneously. SS was delivered subcutaneously from a separate osmotic minipump at (4 µg/kg per min) for 48–72 h. The data represent the mean (±SE) from four rats per group, each analyzed separately.

† *P* < 0.025, G plus SS or F vs. G.

§ *P* < 0.005, G plus SS vs. G.

|| *P* < 0.05, G plus SS or F vs. G.

¶ *P* < 0.001, F vs. G.

Further studies were then performed to elucidate the mechanism of the somatostatin effect on hepatic  $T_4$ -5'-deiodinase activity. Because we recently showed that insulin normalized the low hepatic  $T_4$ -5'-deiodinase activity in the diabetic rat (10), it seemed possible that the somatostatin effect could be due to a perturbation of carbohydrate metabolism. Table I demonstrates that the mean serum glucose concentration was significantly higher in both the 48-h ( $P < 0.005$ ) and 72-h ( $P < 0.005$ ) somatostatin-treated groups, compared with the mean in the glucose-fed control group. These alterations in serum glucose consequent to somatostatin were associated with a reduction in the mean serum insulin levels in the 48-h ( $P < 0.05$ ) and 72-h ( $P < 0.05$ ) somatostatin-treated groups, compared with the mean insulin level in the control group. An equivalent reduction in serum insulin to that induced by somatostatin, was noted in the group fasted for 48 h. There was a positive correlation between the somatostatin-induced alterations in serum insulin (Table I) and hepatic  $T_4$ -5'-deiodinase activity ( $r = 0.7$ ,  $P < 0.005$ ). Somatostatin had no effect on the hepatic glycogen content after 48 h of infusion. Fasting for 48 h did not significantly reduce the mean serum glucose concentration. However, the hepatic glycogen content was significantly less ( $P < 0.001$ ) in the fasted group compared with the mean in the glucose-fed control group. There was no relationship between the changes in serum glucose or the hepatic glycogen content and hepatic  $T_4$ -5'-deiodinase activity. Thus, it seemed possible that the reduced hepatic  $T_4$ -5'-deiodinase activity in the glucose-fed groups treated with somatostatin could be consequent to the associated hypoinsulinemia.

The effect of a continuous somatostatin infusion at a lower dose (0.8  $\mu\text{g}/\text{kg}$  per min subcutaneously) on serum  $T_3$  and hepatic  $T_4$ -5'-deiodinase activity and

serum insulin concentration was then elucidated. It is evident from Table II that the continuous low-dose somatostatin infusion significantly lowered the mean serum insulin levels at 48 h ( $P < 0.05$ ) and 72 h ( $P < 0.05$ ) compared with the mean in the glucose-fed control group. However, despite the relative hypoinsulinemia, somatostatin at this dose had no effect on either the mean serum  $T_3$  concentration or hepatic  $T_4$ -5'-deiodinase activity after either 48 or 72 h of continuous infusion. There was no correlation between the somatostatin-induced alterations in serum insulin and hepatic  $T_4$ -5'-deiodinase activity.

The effect of a continuous (72 h) somatostatin infusion (4  $\mu\text{g}/\text{kg}$  per min) on pituitary  $T_4$ -5'-deiodinase activity in the glucose-fed rat was also determined. Somatostatin had no effect on pituitary enzyme activity. The mean enzyme activity in the 72-h somatostatin-treated group was  $28 \pm 0.2$  fmol/min per mg protein compared with a mean of  $27 \pm 0.3$  fmol/min per mg protein, in the glucose-fed control group. In addition, fasting for 72 h did not alter pituitary  $T_4$ -5'-deiodinase activity; the mean enzyme activity was  $25 \pm 0.2$  fmol/min per mg protein.

The mean peripheral plasma somatostatin-like immunoreactivity concentration was  $69 \pm 17$  pg/ml in the glucose-fed control group. Fasting for 48 h did not affect the plasma somatostatin level: the mean concentration at the termination of the fasting period was  $41 \pm 10$  pg/ml. Low dose somatostatin infusion for 48 h (0.8  $\mu\text{g}/\text{kg}$  per min subcutaneously) significantly increased ( $P < 0.001$ ) the mean somatostatin level ( $448 \pm 58$  pg/ml) compared with the mean somatostatin concentration in the glucose-fed control group. Moreover, high-dose somatostatin infusion for 48 h (4.0  $\mu\text{g}/\text{kg}$  per min subcutaneously) significantly increased ( $P < 0.001$ ) the mean somatostatin concentration ( $786 \pm 65$

TABLE II  
Alterations in Serum  $T_3$  and Insulin and Hepatic  $T_4$ -5'-Deiodinase ( $T_4$ -5'-D) Activity during Low-Dose Somatostatin (SS) Infusion in the Glucose-fed Rat\*

	Glucose-fed (G)	G plus SS (48 h)	G plus SS (72 h)	Fast (72 h)
Serum $T_3$ (ng/dl)	$86 \pm 4$	$69 \pm 8$	$71 \pm 11$	$45 \pm 9$ †
Hepatic $T_4$ -5'-D (pmol/min/100 mg protein)	$118 \pm 4$	$106 \pm 9$	$116 \pm 4$	$50 \pm 3$ †
Serum insulin (ng/dl)	$68 \pm 8$	$48 \pm 2$ §	$44 \pm 3$ §	$44 \pm 6$ §

\* The animals in each group were fed glucose (20% glucose in water) ad lib. for 72 h before each experiment. Each rat was treated with  $T_4$  (1.5  $\mu\text{g}/100$  g/d subcutaneously), for 72 h before and during each experimental period, delivered from an Alzet osmotic minipump implanted subcutaneously. Somatostatin (SS) was delivered from a separate subcutaneously implanted minipump for 72 h at 0.8  $\mu\text{g}/\text{kg}$  per min. The data represent the mean ( $\pm$ SE) from four rats per group, each analyzed separately.

†  $P < 0.005$ , F vs. G.

§  $P < 0.05$ , G plus SS or F vs. G.

pg/ml) compared with the low-dose somatostatin mean. There was a negative correlation between the alterations in hepatic T<sub>4</sub>-5'-deiodinase activity and plasma somatostatin levels during high dose somatostatin infusion in the glucose-fed rat, ( $r = -0.9$ ,  $P < 0.01$ ).

This inhibitory effect of high dose somatostatin on hepatic T<sub>4</sub>-5'-deiodinase was studied further in the glucose-refed rat. We have recently demonstrated that glucose-refeeding reactivated the low hepatic T<sub>4</sub>-5'-deiodinase activity in the fasted rat (14). The effect of somatostatin on this process was examined. Fig. 1 demonstrates a representative time course of reactivation of hepatic T<sub>4</sub>-5'-deiodinase in the 72-h fasted rat. Partial enzyme reactivation occurred by 72 h of refeeding and the enzyme normalized compared with the glucose-fed control group by 96 h. It is evident from Fig. 1 that somatostatin infusion prevented this process. The mean hepatic enzyme activity in the somatostatin groups remained at the fasting level during the refeeding period, the mean enzyme activity at 72 h ( $P < 0.05$ ) and 96 h ( $P < 0.01$ ) was significantly less than the respective glucose-refed controls. In addition fasting for 72 h significantly lowered ( $P < 0.001$ ) the mean serum T<sub>3</sub> concentration ( $52 \pm 4$  ng/dl) compared with the mean in the glucose-fed control of  $92 \pm 5$  ng/dl. Refeeding with glucose normalized the mean serum T<sub>3</sub> concentration by 48 h. Somatostatin decreased the refeeding stimulation of serum T<sub>3</sub>. The mean serum T<sub>3</sub> in each somatostatin group was lower than the control group at the time points studied. At 48 h of refeeding the mean serum T<sub>3</sub> was  $62 \pm 3$  ng/dl in the somatostatin group compared with  $93 \pm 6$  ng/dl in the control group ( $P < 0.005$ ); after 72 h the T<sub>3</sub> values were  $74 \pm 8$  ng/dl vs.  $82 \pm 10$  ng/dl ( $P = 0.5$ ) and at 96 h the T<sub>3</sub> values were  $86 \pm 2$  ng/dl vs.  $109 \pm 8$  ng/dl ( $P < 0.025$ ). There was a positive correlation between the alterations in serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase activity consequent glucose refeeding and somatostatin ( $r = 0.5$ ,  $P < 0.01$ ).

The mean serum T<sub>4</sub> concentration was not altered by fasting for 72-h. Moreover, refeeding did not adjust the mean serum T<sub>4</sub> level nor did the exposure to somatostatin.

Studies were then performed to determine the mechanism of this somatostatin effect on hepatic T<sub>4</sub>-5'-deiodinase activity. Table III demonstrates the effects of somatostatin ( $4 \mu\text{g/kg}$  per min) infusion during glucose-refeeding on BW, serum T<sub>4</sub>, T<sub>3</sub>, glucose, and insulin, hepatic glycogen content and T<sub>4</sub>-5'-deiodinase. Fasting for 72-h induced a significant reduction in the mean BW ( $P < 0.005$ ), serum glucose level ( $P < 0.001$ ), serum T<sub>3</sub> concentration ( $P < 0.001$ ), hepatic glycogen content ( $P < 0.001$ ), and hepatic T<sub>4</sub>-5'-deiodinase activity ( $P < 0.01$ ) compared with the respective means in the glucose-fed control group. Fasting for 72-h had no effect on the mean serum T<sub>4</sub> or insulin concentration or the glucose-to-insulin ratio. Refeeding with glucose for 72-h did not normalize the mean BW or serum glucose. However, refeeding normalized the mean serum T<sub>3</sub> concentration, in fact, the mean was significantly higher ( $P < 0.005$ ) than the control value. The mean hepatic glycogen content and T<sub>4</sub>-5'-deiodinase activity were also restored to normal by refeeding. The somatostatin infusion (initial 30 h only) again reduced the refeeding stimulation of serum T<sub>3</sub>. The mean serum T<sub>3</sub> concentration in the somatostatin-refed group was significantly less ( $P < 0.05$ ) than the mean value in the equivalent refed control group. In addition, the somatostatin infusion prevented the normalization of hepatic T<sub>4</sub>-5'-deiodinase activity. The mean enzyme activity was significantly less in the glucose-refed group treated with somatostatin compared with the glucose-fed control group ( $P < 0.005$ ). There was a positive correlation between the alterations in serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase ( $r = 0.7$ ,  $P < 0.01$ ). Furthermore, despite the absence of significant changes in the mean serum insulin levels in the various groups, there

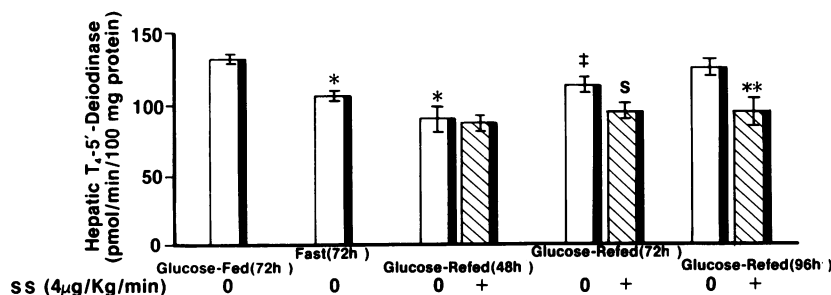


FIGURE 1 The effect of continuous somatostatin (SS) infusion ( $4 \mu\text{g/kg}$  per min subcutaneously) on hepatic T<sub>4</sub>-5'-deiodinase activity during glucose-refeeding (GR) in the 72-h-fasted rat (F). SS was infused only during the first 30 h of glucose-refeeding. \* $P < 0.005$ , F or GR (48 h) vs. G; † $P < 0.01$  GR (72 h) vs. G; ‡ $P < 0.05$ , GR 72 h plus SS vs. GR 72 h; § $P < 0.05$ , GR 72 h plus SS vs. GR 96 h.

TABLE III  
Somatostatin (SS) Effect on BW, Serum T<sub>4</sub>, T<sub>3</sub>, Glucose and Insulin, Hepatic Glycogen and T<sub>4</sub>-5'-deiodinase Activity (T<sub>4</sub>-5'-D) during Glucose-Refeeding in the Rat\*

	Glucose-fed (G)	Fast (F)	Glucose-refed (GR)	
	72 h	72 h	72 h	72 h plus SS
BW (g)	234±2	213±5‡	205±6§	199±4
Serum T <sub>4</sub> (µg/dl)	3.4±0.2	4.2±0.7	3.8±0.5	3.5±0.7
Serum T <sub>3</sub> (ng/dl)	93±7	47±4 <sup>  </sup>	140±8‡	108±9¶
Serum glucose (mg/dl)	137±2	109±3 <sup>  </sup>	111±6§	117±7§
Serum insulin (ng/dl)	61±6	46±5	50±11	46±6
Glucose/insulin (mg/ng)	2.2±0.3	2.4±0.2	2.2±0.5	2.5±0.4
Hepatic glycogen (mg/g)	3.8±0.9	0.9±0.2 <sup>  </sup>	4.9±0.8	3.0±0.5
Hepatic T <sub>4</sub> -5' D (pmol/min/100 mg protein)	139±18	75±5*°	141±12	87±6‡‡

\* Each animal was treated with T<sub>4</sub> (1.5 µg/100 g/d subcutaneously) delivered from an Alzet osmotic minipump during the experimental period. In the somatostatin group, SS (4 µg/Kg per min) was delivered (during the first 30 h of glucose-refeeding) from a separate subcutaneously implanted osmotic minipump. The data represents the mean (±SE) from four animals per group, each analyzed separately.

‡ P < 0.005, F or GR vs. G.

§ P < 0.05, GR or GR plus SS vs. G.

<sup>||</sup> P < 0.001, F vs. G.

¶ P < 0.05, GR plus SS vs. GR.

\*° P < 0.01, F vs. G.

‡‡ P < 0.005, GR plus SS vs. GR or G.

was a significant inverse correlation between enzyme activity and the glucose-to-insulin ratio, ( $r = -0.9$ ,  $P < 0.001$ ).

A detailed time course of the somatostatin effect on the serum insulin response to glucose-refeeding in the fasted rat is demonstrated in Fig. 2. Fasting (72 h) significantly reduced the mean serum insulin level compared with the glucose-fed control group ( $P < 0.05$ ). The somatostatin infusion during the initial 30 h of refeeding, significantly blunted the mean serum insulin response to the glucose intake at 8 h ( $P < 0.001$ ) and 24 h ( $P < 0.001$ ) compared with the response in the equivalent control groups. Thereafter (48–96 h), there was no difference between the somatostatin-treated groups and the controls with regards to the mean serum insulin levels. In addition, the mean serum insulin after glucose refeeding (24 h) in the fasted rats was significantly higher than the mean in glucose-fed rats ( $P < 0.001$ ). Thus, the somatostatin-induced inhibition of the glucose-refeeding reactivation of hepatic T<sub>4</sub>-5'-deiodinase could have been due to the suppression of the initial hyperinsulinemic response to nutrient replacement.

Studies were then performed to determine the effect of low-dose (0.8 µg/kg per min subcutaneously) somatostatin on serum T<sub>4</sub> and T<sub>3</sub> levels, carbohydrate metabolism, and hepatic T<sub>4</sub>-5'-deiodinase activity during glucose-refeeding. Table IV shows that fasting for

72 h induced the expected reduction in the mean serum T<sub>3</sub> ( $P < 0.01$ ), the mean hepatic glycogen content ( $P < 0.001$ ), and the mean enzyme activity ( $P < 0.01$ ) compared with the respective means in the glucose-fed control group. Refeeding with glucose normalized the liver glycogen content in the control and somatostatin groups. Continuous (72 h) low-dose somatostatin infusion (0.8 µg/kg per min) did not prevent the glucose-refeeding (72 h) normalization of the mean serum T<sub>3</sub> concentration or reactivation of hepatic T<sub>4</sub>-5'-deiodinase. The mean enzyme activity was similar in both refeeding groups after 72 h and equivalent to the mean in glucose-fed control group. The mean serum T<sub>4</sub> concentration was similar in all the groups. However, despite the lack of a somatostatin effect on hepatic T<sub>4</sub>-5'-deiodinase activity, during glucose-refeeding, the mean serum glucose concentration and mean serum insulin level were significantly higher ( $P < 0.001$ ) and lower ( $P < 0.05$ ) than the respective means in the refed control group (Table IV). There was no correlation between the glucose-to-insulin ratio and hepatic T<sub>4</sub>-5'-deiodinase activity. These data suggested that the somatostatin (4 µg/kg per min) inhibition of enzyme reactivation during refeeding was not due to the associated hypoinsulinemia.

The effect of a co-infusion of insulin and somatostatin during glucose refeeding was then studied (Fig. 3). In this experiment, as before, somatostatin was only infused

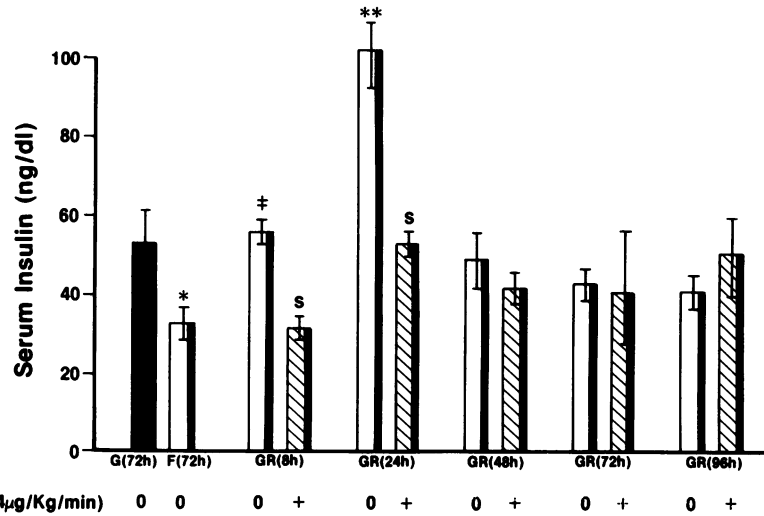


FIGURE 2 Normal time course of serum insulin concentration during glucose-refeeding (GR) in the 72-h-fasted (F) rat, (open columns) compared with the effect of somatostatin (SS) on serum insulin during refeeding, (hatched columns). SS was given by continuous infusion (4 µg/kg per min) for the initial 30 h of glucose-refeeding. A further group glucose-fed (G) for 72 h represent the basal control (solid column). \* $P < 0.05$ , F vs. G; † $P < 0.01$ , GR vs. F; § $P < 0.001$ , GR plus SS vs. GR; \*\* $P < 0.001$ , GR vs. G.

during the initial 30 h of glucose-refeeding. Insulin was infused for the entire duration (96 h) of the refeeding period. Incremental insulin infusions (1–3 U/100 g body wt per d) failed to prevent the inhibitory effect of somatostatin on hepatic  $T_4$ -5'-deiodinase activity during glucose-refeeding. The mean enzyme activity in each of the groups treated with somatostatin

plus insulin was equivalent to the mean in the somatostatin-treated group and significantly less ( $P < 0.005$ ) than the mean in the control group refed with glucose alone. This effect of somatostatin was evident despite the significant incremental increase in the mean serum insulin level consequent to insulin infusions at 1 U/100 g body wt per d ( $P < 0.05$ ), 2 U/100 g body wt

TABLE IV  
Effect of Low-Dose Somatostatin (SS) on Serum  $T_4$ ,  $T_3$ , Glucose and Insulin, Hepatic  $T_4$ -5'-deiodinase ( $T_4$ -5'-D) and Glycogen during Glucose-Refeeding (GR) in the rat\*

	Glucose-fed (G)	Fast (F)	Glucose-refed (GR)	
	72 h	72 h	72 h	72 h plus SS
Serum $T_4$ (µg/dl)	4.0±0.4	4.1±0.1	4.9±0.5	4.8±0.6
Serum $T_3$ (ng/dl)	62±4	44±2†	64±7	65±12
Serum glucose (mg/dl)	169±7	136±22	187±2	252±10§
Serum insulin (ng/dl)	53±11	38±2	52±10	28±2 <sup>  </sup>
Glucose/insulin (mg/ng)	4.2±0.7	4.9±0.6	3.8±0.9	9.0±0.6§
Hepatic glycogen (mg/g)	4.4±0.3	0.7±0.1¶	5.2±0.3	4.9±0.4
Hepatic $T_4$ -5'-D (pmol/min/100 mg protein)	75±3	45±7†	62±2	67±3

\* Each animal was treated with  $T_4$  (1.5 µg/100 g/d subcutaneously), before and during the experimental period, from an implanted osmotic minipump. SS was continuously infused (0.8 µg/kg per min subcutaneously) for the entire refeeding period (72 h) from a separate osmotic minipump. The data represent the mean (±SE) from four animals per group, each analyzed separately.

†  $P < 0.01$ , F vs. G.

§  $P < 0.001$ , GR plus SS vs. GR.

<sup>||</sup>  $P < 0.05$ , GR plus SS vs. GR.

¶  $P < 0.001$ , F vs. G.

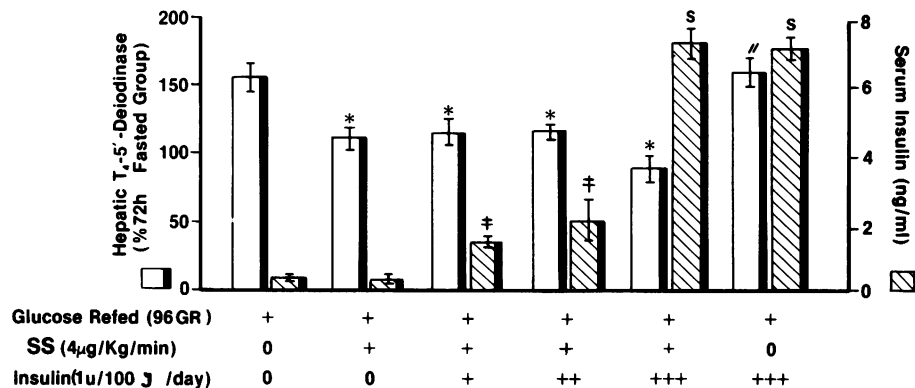


FIGURE 3 The effect of coinfusions of somatostatin (SS) and insulin on the glucose-refeeding (GR) reactivation of hepatic T<sub>4</sub>-5'-deiodinase activity (open columns) and serum insulin concentration (hatched columns) in the 72-h-fasted rat. SS was infused continuously during the initial 30 h of refeeding (4 µg/kg per min subcutaneously). Insulin was infused continuously throughout the refeeding period (1-3 U/100 g body wt per d subcutaneously). \*  $P < 0.005$ , GR plus SS or GR plus SS plus insulin vs. GR; †  $P < 0.05$ , GR plus insulin vs. GR; §  $P < 0.001$ , GR plus insulin vs. GR; ‡  $P < 0.005$ , GR plus insulin vs. GR plus SS plus insulin.

per d ( $P < 0.05$ ), and 3 U/100 g body wt per d ( $P < 0.001$ ), compared with the mean serum insulin in the control group. Furthermore, Fig. 3 demonstrates that insulin infusion per se (3 U/100 g body wt per d) did not affect, the glucose refeeding reactivation of hepatic T<sub>4</sub>-5'-deiodinase. The mean enzyme activity in this insulin-treated group was equivalent to the mean in the control glucose-refed group, and significantly higher than that in the somatostatin-treated groups ( $P < 0.005$ ). Fasting and refeeding with glucose had no effect on the mean serum T<sub>4</sub> concentration. Moreover, although fasting and refeeding did not significantly alter the mean serum glucose levels, there was a broad range of serum glucose levels due to the tendency towards hypoglycemia in the groups that received the higher insulin doses. Refeeding normalized the mean serum T<sub>3</sub> level ( $94 \pm 4$  ng/dl) compared with the mean in the fasted group at  $37 \pm 4$  ng/dl. Although somatostatin did not prevent an increase in the mean serum T<sub>3</sub> levels consequent to refeeding ( $67 \pm 3$  ng/dl), the incremental increase was significantly less ( $P < 0.005$ ) than in the control group. The co-infusion of insulin with somatostatin did not overcome this somatostatin effect. The mean serum T<sub>3</sub> was similar ( $62 \pm 5$  ng/dl) in the 4-U/d insulin group and significantly less in both the 2-U/d insulin group ( $40 \pm 3$  ng/dl) ( $P < 0.005$ ) and the 6-U/d insulin group ( $46 \pm 5$  ng/dl) ( $P < 0.025$ ) compared with the mean T<sub>3</sub> in the somatostatin control group at  $67 \pm 3$  ng/dl. There was a positive correlation between the alterations induced in serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase activity ( $r = 0.9$ ,  $P < 0.001$ ).

#### DISCUSSION

This study demonstrates that a continuous subcutaneous infusion (4 µg/kg per min) of somatostatin reduced

hepatic T<sub>4</sub>-5'-deiodinase activity in the glucose-fed rat and prevented the glucose-refeeding reactivation of this enzyme in the 72-h-fasted rat. These somatostatin effects have not been previously demonstrated. The data provide strong evidence that somatostatin may be a modulator of the peripheral metabolism of thyroid hormones. This deduction is supported by the somatostatin-induced alterations in the serum T<sub>3</sub> concentration. Somatostatin reduced the glucose-refeeding stimulation of serum T<sub>3</sub> in the fasted rat. The mean serum T<sub>3</sub> was lower in the somatostatin groups compared with the equivalent control groups during refeeding. In addition, a significant positive correlation was noted between the somatostatin-induced changes in serum T<sub>3</sub> and hepatic T<sub>4</sub>-5'-deiodinase activity during glucose-refeeding. In contrast, somatostatin did not consistently lower the serum T<sub>3</sub> concentration in the glucose-fed rat treated with somatostatin. However, under these conditions the hepatic enzyme may not be the only regulator of T<sub>3</sub> metabolism. T<sub>4</sub>-5'-deiodinase in kidney, muscle, or fat (8) may not be affected by somatostatin to the same degree as the hepatic enzyme in the glucose-fed rat. Furthermore, since a static serum T<sub>3</sub> is not a valid indicator of T<sub>3</sub> production, T<sub>3</sub> kinetic studies need to be performed to definitively determine whether somatostatin alters extrathyroidal T<sub>3</sub> neogenesis in addition to hepatic T<sub>4</sub>-5'-deiodinase activity and to elucidate the relationship between these parameters. However, the positive correlation noted between serum T<sub>3</sub> and hepatic enzyme activity during refeeding and somatostatin treatment suggests that somatostatin mediated its effect on T<sub>3</sub> metabolism through hepatic T<sub>4</sub>-5'-deiodinase activity.

The present study supports the previous reports that somatostatin does modulate the extrathyroidal metab-



olism of iodothyronines. The study of Loos et al. (5) demonstrated that a somatostatin infusion for 8 h significantly decreased serum  $T_3$  in athyreotic man treated with  $T_4$ . In addition, Weeke et al. (6) showed that a prolonged 24-h somatostatin infusion significantly decreased the serum  $T_3/T_4$  ratio and increased serum reverse  $T_3$  in patients treated for myxedema. Thus, somatostatin does probably affect extrathyroidal  $T_3$  neogenesis. However, as indicated, further studies are needed to elucidate whether the alterations in  $T_3$  metabolism consequent to somatostatin can be attributed to the effects of this hormone on hepatic  $T_4$ -5'-deiodinase.

In the glucose-fed rat the inhibitory action of somatostatin on hepatic  $T_4$ -5'-deiodinase activity was apparent within 48 h and persisted during a further 24 h of hormone infusion. Earlier time points were not examined as anesthesia and surgery (21, 22) are also known transient inhibitors of hepatic  $T_4$ -5'-deiodinase activity. The reduction in enzyme activity (25–30%) consequent to somatostatin during these short-term glucose feeding studies was equivalent to the effect of fasting for the same period, each group being compared with the enzyme activity in the glucose-fed control group. The inhibitory action of somatostatin on hepatic  $T_4$ -5'-deiodinase activity in the fasted refed rat became apparent at 48–72 h during glucose-refeeding. The effect persisted to 96 h, a surprising fact, since somatostatin was only given during the initial 30 h of glucose-refeeding. Studies were not performed beyond 96 h, so we did not determine the duration of the somatostatin effect or whether hepatic  $T_4$ -5'-deiodinase activity would eventually normalize.

The effect of somatostatin on hepatic  $T_4$ -5'-deiodinase activity was clearly dose related. The somatostatin infusion at the higher dose (4  $\mu\text{g}/\text{kg}$  per min) inhibited enzyme activity, whereas the lower dose (0.8  $\mu\text{g}/\text{kg}$  per min) was without effect. There was a significant negative correlation between the alterations in hepatic  $T_4$ -5'-deiodinase activity and the increases in plasma somatostatin concentration consequent to the infusion at the higher dose. The peripheral plasma levels achieved consequent to hormone infusion at the low dose were similar to the reported hepatic portal somatostatin levels in disorders such as diabetes (23). Although the mean peripheral plasma somatostatin concentration in the high dose group was ~75% higher than the mean in the low dose group, similar endogenous hepatic portal values have been reported in the diabetic rat consequent to arginine stimulation (23). Thus, while the plasma somatostatin concentration achieved in the high dose group was probably supra-physiological, equivalent portal values may be attained following specific stimulators (23). The known kinetics of somatostatin did predict much higher peripheral hormone levels (24). Much of the infused somatostatin

apparently underwent degradation at the subcutaneous infusion site. The relatively close relationship between the peripheral plasma somatostatin levels achieved in the high dose group and the reported portal plasma levels in other conditions such as diabetes (11, 23), which are associated by reduced hepatic  $T_4$ -5'-deiodinase activity, suggests that the demonstrated somatostatin effect could be physiological. The plasma levels achieved in the low dose group were similar to those required to induce other reported effects of somatostatin (11, 25, 26).

The mechanism by which somatostatin induced its effect on hepatic  $T_4$ -5'-deiodinase was not elucidated. However, this study did address a number of possibilities, and was controlled for other somatostatin actions. It is well known that somatostatin can induce secondary hypothyroidism by inhibiting the pituitary release of thyrotropin (TSH) (1, 2). In addition, recent reports suggest that somatostatin also decreases the thyroid secretion of  $T_4$  in response to TSH (5, 27). Consequently, at high dosage somatostatin could cause primary hypothyroidism. This may have physiological relevance, as a number of investigators have demonstrated somatostatin in the parafollicular cells of rat and human thyroid (28–30). To prevent the development of hypothyroidism and its associated reduction in hepatic  $T_4$ -5'-deiodinase activity (31), all animals were treated with exogenous  $T_4$  during the experimental period. Serum  $T_4$  levels were not affected by somatostatin at either dosage. Thus, hypothyroidism did not develop during this study in either the glucose-fed or glucose-refed rat models.

Somatostatin is known to influence nutriment absorption and carbohydrate metabolism (10, 25, 26). However, the demonstrated absence of weight loss and the normal hepatic glycogen content in the somatostatin-treated glucose-fed and -refed animals in the present study suggest that there was no impairment of glucose absorption. A recent study supports this view: somatostatin (10  $\mu\text{g}/\text{kg}$  per min) had no effect on the intestinal absorption of glucose in the rat (32). The normal hepatic glycogen content in the somatostatin-treated animals also indicates that hepatic glycogen metabolism was relatively normal. However, the mean serum glucose concentration was significantly higher in both the fed and refed somatostatin groups compared with the respective control groups. Thus, somatostatin did most likely impair the metabolism of glucose. Hyperglycemia consequent to somatostatin treatment is thought to reflect increased hepatic glucose output and decreased peripheral uptake of glucose (25). However, these actions of somatostatin have been attributed to its modulation of the endocrine system (insulin and glucagon) rather than to a direct effect on glucose metabolism (33). Although in the present study glucose absorption and hepatic glycogenesis were not impaired

by somatostatin, the associated hyperglycemia indicates a possible perturbation of carbohydrate metabolism that may have affected hepatic T<sub>4</sub>-5'-deiodinase activity. Both doses of somatostatin induced hypoglucagonemia (34). Currently it is believed that glucagon does not modulate T<sub>3</sub> metabolism (34, 35). A preliminary report had attributed some low serum T<sub>3</sub> states to the associated hyperglucagonemia (36).

Since in previous studies we had demonstrated that exogenous insulin normalized the low hepatic T<sub>4</sub>-5'-deiodinase activity in the diabetic rat (10) and that the glucose-refeeding reactivation of enzyme activity could be mediated by the endogenous insulin response (14), the effect of somatostatin on serum insulin was examined in detail. In the glucose-fed model there was a significant correlation between serum insulin and hepatic T<sub>4</sub>-5'-deiodinase activity during somatostatin treatment. This suggested that the effect could be mediated by the induced hypoinsulinemia. However, low dose somatostatin also induced an equivalent degree of hypoinsulinemia and had no effect on enzyme activity. This discordant response supported an action of somatostatin on rat hepatic T<sub>4</sub>-5'-deiodinase activity independent of insulin. Moreover, in the refeeding model, although the initial studies showed a significant correlation between the changes in the serum glucose to insulin ratio and hepatic T<sub>4</sub>-5'-deiodinase activity during somatostatin treatment, the cotreatment with insulin did not prevent the inhibition of the enzyme by somatostatin. The somatostatin inhibitory effect on enzyme activity was sustained despite the associated induced hyperinsulinemia. Insulin per se did not alter hepatic T<sub>4</sub>-5'-deiodinase in the 96-h refed rat. Furthermore, low dose somatostatin (72 h) did not prevent the glucose-refeeding reactivation of hepatic T<sub>4</sub>-5'-deiodinase, despite a sustained hypoinsulinemia during the refeeding period. Thus, the somatostatin effect was not mediated through the induced insulin deficiency. Studies using rat hepatocytes in monolayer culture are currently in progress to determine whether somatostatin has a direct effect on T<sub>4</sub>-5'-deiodinase.

The prolonged inhibitory effect of somatostatin during glucose-refeeding, suggests that the hormone inhibited hepatic T<sub>4</sub>-5'-deiodinase synthesis. The low enzyme activity cannot be attributed to a sulfhydryl cofactor depletion (9, 37) since the incubations were performed in DTT-enriched homogenate preparations (9, 37). In addition, if the low state of enzyme activity during fasting had been due to enzyme inactivation, a more rapid recovery would be expected. It thus seems most likely that somatostatin depleted the hepatic content of active T<sub>4</sub>-5'-deiodinase in the fed rat and prevented its reaccumulation during refeeding by blocking the hepatic synthesis of this enzyme.

The somatostatin effect on T<sub>4</sub>-5'-deiodinase did demonstrate tissue selectivity in that pituitary enzyme ac-

tivity remained normal in the fed rat during high dose somatostatin treatment. It is unlikely that this apparent pituitary resistance reflected inadequate tissue levels of somatostatin as even lower doses decrease the stimulated pituitary TSH and growth hormone release response (11, 25). Other inhibitors of hepatic T<sub>4</sub>-5'-deiodinase activity, such as PTU, also have a selective effect and do not inhibit pituitary enzyme activity (38). In addition, rat pituitary enzyme activity is not reduced by fasting, a modulation that significantly reduces liver enzyme activity (39). It is possibly that somatostatin only inhibits the "PTU-sensitive," T<sub>4</sub>-5'-deiodinase enzyme and that it has no effect on the "PTU-insensitive," T<sub>4</sub>-5'-deiodinase enzyme (40). The latter enzyme is the major regulator of T<sub>3</sub> neogenesis in the pituitary, whereas the former is predominantly found in peripheral tissues such as liver (40, 41). Further studies should elucidate the mechanism for this selective effect of somatostatin on T<sub>4</sub>-5'-deiodinase activity.

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