Glucagon Does Not Modulate the Alterations in T_3 Metabolism Consequent to Dietary Manipulation and Diabetes

LAURENCE A. GAVIN AND MARIE MOELLER

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024

SUMMARY

Low serum T_3 levels and hyperglucagonemia are characteristic features of a number of catabolic states such as fasting and uncontrolled diabetes. The present study was performed to elucidate the relationship between this hyperglucagonemia and T_3 metabolism. Serum glucagon and T_3 and hepatic T_4-5′-deiodinase activity ($T_4 \rightarrow T_3$) were examined in groups of rats (T_4-treated) fed (chow versus carbohydrate), fasted, or diabetic (streptozotocin 100 mg/kg i.p.) for 48–72 h.

In the carbohydrate-fed (20% glucose in H_2O ad libitum) group the mean serum T_3 concentration and mean hepatic T_4-5′-deiodinase activity were significantly higher ($P < 0.01$) and the mean serum glucagon level significantly lower ($P < 0.05$) than the respective means in the chow-fed control group. The mean serum T_3 concentration was significantly less ($P < 0.05$) in both the fasted (72 h) and diabetic (72 h) groups compared with the control mean, whereas the mean serum glucagon values were similar to the chow-fed group. The mean hepatic T_4-5′-deiodinase activity was low in the diabetic group ($P < 0.05$) but similar in the fasted group compared with the chow-fed control. A significant inverse correlation ($r = -0.9$; $P < 0.001$) was noted between these alterations in serum T_3, hepatic T_4-5′-deiodinase activity, and serum glucagon, suggesting that glucagon could be a modulator of T_3 metabolism.

Hyperglucagonemia was induced in the glucose-fed group with a continuous glucagon infusion for 48 h (0.15 μg/kg/min s.c.). Both the mean serum glucagon (395 ± 66 pg/ml) and glucose concentrations (152 ± 5 mg/dl) were significantly higher ($P < 0.01$) in the glucagon-treated group compared with the respective mean values (glucagon = 147 ± 8 pg/ml, glucose = 126 ± 2 mg/dl) in the control group. Despite this hyperglucagonemia there was no change in T_3 metabolism; serum T_3 values and enzyme activity were similar in both groups. Hypoglucagonemia was induced in both the fasted (72 h) and diabetic (72 h) groups by a continuous somatostatin infusion for 72 h (0.8 μg/kg/min s.c.). The mean serum glucagon was reduced from 84 ± 7 to 60 ± 7 pg/ml by somatostatin in the fasted group ($P < 0.05$) and from 137 ± 12 to 102 ± 5 pg/ml by somatostatin in the diabetic group ($P < 0.05$). Despite these significant reductions in serum glucagon in both the fasted and diabetic groups, no increase was noted in either serum T_3 or hepatic T_4-5′-deiodinase activity.

However, it was noted that the hyperglucagonemia consequent to the glucagon infusion in the glucose-fed group was associated with a relative excess of insulin. The mean serum molar ratio of insulin to glucagon was 6.9 ± 2.3 in the glucagon-treated group compared with 3.3 ± 0.3 in the glucose-fed control group. Thus, it seemed possible that the insulin excess could have masked a possible glucagon inhibitory effect on T_4-5′-deiodinase activity. A coinfusion (48 h) of glucagon and somatostatin was used to induce a significant reduction ($P < 0.05$) in the molar ratio of insulin to glucagon (1.5 ± 0.6) in the glucose-fed group. However, no reduction occurred in either serum T_3 or hepatic T_4-5′-deiodinase activity during this hyperglucagonemic state.

Thus, glucagon does not modulate T_3 metabolism and the low T_3 state of either fasting or diabetes is not related to the associated hyperglucagonemia. DIABETES *32*:798–803, September 1983.

From the Division of Endocrinology-Metabolism, Veterans Administration Medical Center and the Department of Medicine, University of California, San Francisco, California.

Address reprint requests to Dr. Laurence A. Gavin, Endocrine-Metabolic Service (115), Veterans Administration Medical Center, 4150 Clement Street, San Francisco, California 94121.

Received for publication 13 January 1983.

The transient low T_3* syndrome of acute illness is a ubiquitous but poorly understood finding in sick patients.[1] This may be a beneficial homeostatic response in catabolic states to preserve essential skeletal protein and vital energy reserves.[2] It has been dem-

*The following abbreviations are used throughout this article: T_4, L-thyroxine; T_3, 3,5,3′-triiodothyronine; DTT, dithiothreitol; SRIF, somatostatin.

onstrated that the low serum T_3 values associated with fasting and diabetes in the rat are consequent to reduced tissue (hepatic) T_4-5'-deiodinase activity ($T_4 \rightarrow T_3$).[3-6] Refeeding with glucose or treatment with insulin corrects the impaired T_3 metabolism in these respective states.[5,6] These studies and a further recent report suggest that the glucoregulatory hormones insulin and glucagon could mediate the effects of dietary manipulation and diabetes on hepatic T_4-5'-deiodinase activity.[5-7]

Hyperglucagonemia is characteristic of many of the catabolic states associated with the low T_3 syndrome.[8,9] Thus, it seemed possible that glucagon excess could be responsible for the alteration in T_3 metabolism consequent to the reduction in hepatic T_4-5'-deiodinase noted by us in the fasted and diabetic rat.[5,6] Indeed, a preliminary report has shown that glucagon does lower the serum T_3 concentration in the rat.[10] However, a recent detailed study showed that glucagon did not impair T_3 production in the intact rat or T_4-5'-deiodinase activity in rat liver homogenate preparations.[11] In contrast, it has been demonstrated that glucagon does inhibit the stimulatory effect of insulin on T_4-5'-deiodinase in rat hepatocytes in culture.[7]

The present study was undertaken to further elucidate the effects of glucagon on hepatic T_4-5'-deiodinase activity in the rat. The relationship of the glucagon effect to alterations in serum insulin was characterized.

METHODS

Materials. Thyroxine (T_4), dithiothreitol (DTT), and somatostatin (SRIF) were purchased from Sigma Chemical Co., St. Louis, Missouri. ^{125}I-T_3, labeled in the phenolic ring position (specific radioactivity: 500–900 μCi/μg) and ^{125}I-insulin (specific radioactivity: 95.5 μCi/μg) were purchased from New England Nuclear, Boston, Massachusetts. Goat anti-rabbit y-globulin serum was obtained from Antibodies, Inc., Davis, California, and EDTA from Eastman Kodak, Rochester, New York. Rat insulin was purchased from Novo Research Institute DK-2880, Bagsuaerd, Denmark, and anti-porcine insulin antibody from Miles Laboratories, Inc., Elkhart, Indiana. Glucagon was obtained from Eli Lilly and Co., Indianapolis, Indiana. Alzet osmotic minipumps (#2001) were purchased from Alza Corp., Palo Alto, California. Innovar (fentanyl 0.05 mg and droperidol 2.5 mg/ml) was purchased from McNeill Laboratories, Inc., Fort Washington, Pennsylvania. Other chemicals were reagent grade and were purchased from commercial suppliers.

Animals and diets. T_4-5'-deiodinase activity was determined in hepatic homogenate preparations obtained from male Sprague-Dawley rats. Within each experiment the animals (group = 4) were closely matched for weight (200–250 g) and age. Before each study period the animals were maintained on ad libitum intake of tap water and Purina Rodent Laboratory Chow, #5001 (25% protein content), from Ralston Purina Co., St. Louis, Missouri. All animals were treated with T_4 (1.5 μg/100 g/day s.c.) during the experimental period. The T_4 was delivered from Alzet osmotic minipumps, which were implanted s.c. (under Innovar anesthesia) at least 3 days before each experiment. The serum T_4 concentration was maintained within the normal range during the study periods by this delivery system. Apart from the Purina-fed control group (P), three other groups were studied. The various parameters were analyzed in serum and liver homogenate samples from rats glucose-fed ad libitum for 72 h (G) (20% glucose in H_2O), fasted for 72 h (F) (H_2O ad libitum), and diabetic for 72 h (D.M.), consequent to a single i.p. injection of streptozotocin (Upjohn Co., Kalamazoo, Michigan) at a dose of 100 mg/kg body wt. Animals were checked for glycosuria at 24 and 48 h after injection. We used only animals demonstrating 2% glycosuria at 24 h on ketodiastix (Ames Co., Elkhart, Indiana). During the study period the diabetic animals were fed Purina Chow and tap water ad libitum. At the end of each study period, all animals were killed, and blood was drawn for the analysis of serum glucose, T_4, T_3, glucagon, and insulin. In addition, the livers were removed, blotted dry on gauze mesh, and stored on ice for immediate processing.

Hormone modulation. Glucagon was delivered to the G group rats for 48–72 h by continuous infusion from Alzet osmotic minipumps implanted s.c. The dose delivered (0.15 μg/kg/min) rapidly increased the serum glucagon concentration 2–3-fold compared with that of the controls and sustained a steady state during the study period. Somatostatin (SRIF) was infused over 48–72 h to both F group and D.M. group rats. The dose rate was 0.8 μg/kg/min, delivered from Alzet osmotic minipumps implanted s.c. This dose significantly reduced the serum glucagon levels in both groups compared with the respective control groups. In addition, SRIF was given for 48 h at the same dose rate (0.8 μg/kg/min s.c.) to a G group also treated with glucagon (0.15 μg/kg/min s.c.). These hormones were delivered from separate Alzet osmotic minipumps implanted s.c. simultaneously.

Hepatic T_4-5'-deiodinase analysis. The enzyme analysis was performed in liver homogenate preparations as previously described.[5] The conversion of T_4 (1 μM) to T_3 (T_4-5'-deiodinase activity) was studied in 2% homogenate preparations (pH 7.2) enriched with 5 mM DTT and 10 mM EDTA at 37°C. Activity rates were calculated from initial reaction rates. Samples for T_3 analysis (100 μl) were removed from the 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 for T_3.[4,5] Control experiments consisted of (1) incubation of substrate (T_4) in buffer without homogenate, (2) liver preparations without added substrate, and (3) addition of T_4 to the liver preparations without incubation (time-zero tubes). In each experiment the amount of T_3 produced was corrected by the appropriate recovery, the T_4 cross-reactivity (0.13%) in the T_3 assay, and the amount of iodothyronine present in the incubated control tubes (time-zero tubes). The protein concentration of the incubation liver preparation was determined by the method of Lowry et al.[12] using bovine serum albumin as a standard.

Analysis of serum glucose, insulin, and glucagon. Rat serum glucose was measured by the glucose-oxidase method, using a Yellow Springs Autoanalyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Plasma glucagon was determined by Gerich's modification[13] of the method of Unger et al. using the 30K antiserum.[14] The samples for glucagon analysis were collected into glass tubes (B-D #4854, purchased from Becton Dickinson Co., Rutherford, New Jersey) at 4°C containing 0.3 cc of 0.5 M ben-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024

TABLE 1
The effect of dietary composition, fasting, and diabetes on the serum glucoregulatory hormones and T_3 metabolism (mean ± SEM)*

Dietary groups (72 h)	Body wt. change (g%)	Serum Glucose (mg/dl)	Serum Glucagon (pg/ml)	Serum Insulin (ng/dl)	Serum T_3 (ng/dl)	Hepatic T_4-5′-deiodinase (pmol/min/100 mg protein)
Purina-fed (P)	(+) 9 ± 2	147 ± 2	144 ± 7	84 ± 2	23 ± 3	30 ± 4
Glucose-fed (G)	(−)13 ± 1†	116 ± 4†	108 ± 9‡	53 ± 8§	41 ± 2§	76 ± 12§
Fasted (F)	(−)25 ± 2†	86 ± 10†	147 ± 4	33 ± 4†	14 ± 1‡	23 ± 3
Diabetes (D.M.) (Purina-fed)	(−) 6 ± 1†	458 ± 13†	137 ± 12	25 ± 7†	15 ± 3‡	17 ± 4‡

*The data represent the mean from at least four rats analyzed separately in each group. Each animal was treated with T_4 (3 μg/day) delivered from an osmotic minipump (implanted s.c.) throughout the study period. Hepatic T_4-5′-deiodinase was determined in 2% homogenate preparations (pH 7.2) enriched with 1 μM T_4, 5 mM DTT, and 10 mM EDTA.
†$P < 0.001$, G, F, or D.M. versus P; ‡$P < 0.05$, G, F, or D. M. versus P; §$P < 0.01$, G versus P.

zamidine. The samples were immediately processed, the plasma was decanted and stored frozen (−20°C) in plastic tubes until glucagon analysis. Serum insulin was measured by a modification of the method of Grodsky and Forsham[15] using specific anti-insulin antibody and charcoal for the separation of free ^{125}I-insulin from bound.[16]

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 linear regression analysis.[17]

RESULTS

The effects of dietary composition, fasting, and diabetes on the glucoregulatory hormones glucagon and insulin and on T_3 metabolism are outlined in Table 1. The P group gained weight during the 72-h study period, whereas the G group, the F group, and the D.M. group each lost a significant amount of weight ($P < 0.001$) compared with P. The mean serum glucose concentration in both G and F was significantly less than the mean in P ($P < 0.001$), whereas the mean value was significantly greater in D.M. ($P < 0.001$). Fasting for 72 h or diabetes did not alter the serum glucagon levels compared with the mean in P. The mean serum glucagon concentration in G was significantly less than in P ($P < 0.05$). The mean serum insulin level was significantly less in each of the three experimental groups [G ($P < 0.01$), F ($P < 0.001$), and D.M. ($P < 0.001$)] compared with the mean in P. The mean serum T_3 concentration was significantly less in both F ($P < 0.05$) and D.M. ($P < 0.05$) compared with P, whereas the mean value was significantly higher in G ($P < 0.01$) compared with the control group. Hepatic T_4-5′-deiodinase activity was the same in F compared with P, but was significantly higher in G ($P < 0.01$) and lower in D.M. ($P < 0.05$) compared with the control group P.

There was a significant correlation between the changes in serum T_3 concentration and hepatic T_4-5′-deiodinase activity in response to dietary manipulation and diabetes ($r = +0.98$; $P < 0.001$). There was no correlation between the alterations in serum insulin values or the glucose-to-insulin ratio and hepatic T_4-5′-deiodinase activity consequent to dietary modification and diabetes.

The relationship between the alterations in serum glucagon concentration and hepatic T_4-5′-deiodinase activity is outlined in Figure 1. This figure shows that there was a significant inverse correlation between the serum glucagon values and the hepatic enzyme activity as modified by feeding (Purina and glucose), fasting, and diabetes ($r = -0.92$; $P < 0.001$). There was no correlation between the insulin-to-glucagon ratio and hepatic enzyme activity.

The significant inverse relationship noted between serum glucagon and hepatic T_4-5′-deiodinase activity suggested that this hormone could be inhibitory with respect to the enzyme activity. Table 2 demonstrates the effect of altering serum glucagon levels on rat hepatic T_4-5′-deiodinase activity in three different situations. Experiment no. 1 (Table 2) shows that a continuous glucagon infusion (48 h) significantly increased both the mean serum glucose and glucagon concentrations in a glucose-fed group (G) compared with the respective means in the control group ($P < 0.01$). Despite this significant elevation in serum glucagon there

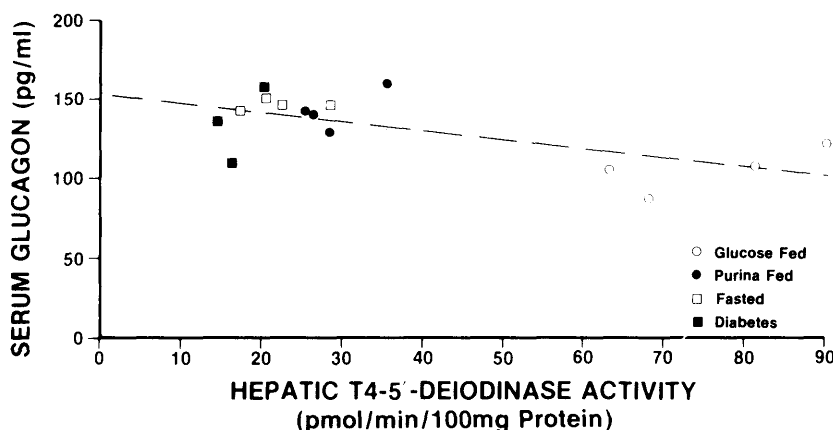

FIGURE 1. Relationship between the alterations in serum glucagon and hepatic T_4-5′-deiodinase activity in the fed (20% glucose in H_2O p.o., ad libitum 72 h versus Purina chow), fasted (72 h), and diabetic (72 h) rats. T_4-5′-deiodinase activity was determined in 2% liver homogenate preparations enriched with 1 μM T_4, 5 mM DTT, and 10 mM EDTA. A significant inverse correlation was found between serum glucagon and enzyme activity ($r = -0.9$; $P < 0.001$).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024

TABLE 2
The effect of serum glucagon modulation on hepatic T_4-5′-deiodinase activity (mean ± SEM)*

Experiment	Serum Glucose (mg/dl)	Serum Glucagon (pg/ml)	Serum T_3 (ng/dl)	Hepatic T_4-5′-deiodinase (pmol/min/100 mg protein)
1. Glucose-fed 48 h (G)	126 ± 2	147 ± 8	64 ± 7	137 ± 3
G + glucagon (0.15 μg/kg/min s.c.)	152 ± 5†	395 ± 66†	63 ± 4	139 ± 9
2. Fasted 72 h (F)	92 ± 2	84 ± 7	25 ± 3	50 ± 5
F + somatostatin (0.8 μg/kg/min s.c.)	86 ± 5	60 ± 7‡	30 ± 3	44 ± 3
3. Diabetes 72 h (D.M.)	595 ± 35	137 ± 12	35 ± 4	15 ± 1
D.M. + somatostatin (0.8 μg/kg/min s.c.)	512 ± 10	102 ± 5§	37 ± 2	18 ± 2

*The mean data were derived from at least four rats per group, each analyzed separately. All animals were treated with T_4 (3 μg/day) during the study period. T_4, glucagon, and somatostatin were delivered from separated osmotic minipumps implanted s.c. Hepatic T_4-5′-deiodinase activity was determined in 2% homogenate preparations (pH 7.2) enriched with 1 μM T_4, 5 mM DTT, and 10 mM EDTA.
†$P < 0.01$, G + glucagon versus G; ‡$P < 0.05$, F + somatostatin versus F; §$P < 0.05$, D.M. + somatostatin versus D.M.

was no change in T_3 metabolism as reflected by the similar mean serum T_3 values and mean hepatic T_4-5′-deiodinase activity in G and G treated with glucagon. Experiment no. 2 (Table 2) demonstrates that a 72-h somatostatin (SRIF) infusion significantly decreased the mean serum glucagon concentration in a fasted group (F) compared with the mean value in the control group F ($P < 0.05$). However, there was no alteration in the mean serum T_3 or hepatic T_4-5′-deiodinase; these parameters were similar in both groups. Moreover, despite a significant reduction in the mean serum glucagon concentration in the diabetic group consequent to SRIF compared with the glucagon concentration in D.M. ($P < 0.05$), no change occurred in the mean serum T_3 or mean hepatic T_4-5′-deiodinase activity (Table 2, experiment no. 3).

Although modulation of the serum glucagon did not modify hepatic T_4-5′-deiodinase activity, in the glucose-fed group treated with glucagon, the glucagon effect could have been masked by a counterregulatory response in serum insulin. Figure 2 demonstrates the effects of manipulating both glucagon and insulin on hepatic T_4-5′-deiodinase activity. The center panel in Figure 2 demonstrates that the glucagon infusion over 48 h significantly increased both the mean serum glucagon concentration (395 ± 66 pg/ml) and the mean serum insulin value (416 ± 80 ng/dl) in the glucose-fed group compared with the mean glucagon concentration of 147 ± 8 pg/ml ($P < 0.01$) and mean insulin value of 84 ± 14 ng/dl in the control group G ($P < 0.005$). The glucagon infusion did not alter the mean hepatic T_4-5′-deiodinase activity at 139 ± 9 pmol/min/100 mg protein compared with the enzyme activity in the control group G at 137 ± 3 pmol/min/100 mg protein. However, the glucagon infusion in G did induce a relative excess of insulin. The molar ratio of insulin to glucagon in G + G was 6.9 ± 2.3 compared with 3.3 ± 0.3 in G. The right-hand panel in Figure 2 shows that despite a suppression of this insulin response to glucagon with SRIF, the hyperglucagonemia did not inhibit hepatic T_4-5′-deiodinase activity. The mean hepatic T_4-5′-deiodinase activity at 130 ± 10 pmol/min/100 mg protein in this group was similar to that in G and G + G. The mean serum insulin concentration in the SRIF-treated group (99 ± 16 ng/dl) was significantly less than in G + G at 419 ± 80 ng/dl ($P < 0.005$). The molar ratio of insulin to glucagon in this SRIF group at 1.5 ± 0.6 was significantly less than the mean in G at 3.3 ± 0.3 ($P < 0.05$). SRIF infusion alone (0.8 μg/kg/min s.c.) in a further glucose-fed group had no effect on hepatic T_4-5′-deiodinase activity.

DISCUSSION

The present study demonstrated that there was a significant inverse relationship between the alterations in serum glucagon and T_3 metabolism during feeding (chow versus carbohydrate), fasting, and diabetes. Both the mean serum T_3 concentration and mean hepatic T_4-5′-deiodinase activity were highest in the glucose-fed group, whereas the mean serum glucagon concentration was lowest, compared with these parameters, in the other groups. The mean serum glucagon concentration was similar in the chow-fed, fasted, and diabetic groups. These differences in serum glucagon levels agree with many previous well-documented studies.[8,9,18] The present study confirms the previous reports, which demonstrated that both serum T_3 and hepatic T_4-5′-deiodinase activity are higher in the glucose-fed compared with the chow-fed rat.[19,20] Furthermore, it has been shown that there is no difference between the latter group and the fasted rat when enzyme activity is analyzed in a DTT-enriched homogenate preparation.[19,21] The finding that hepatic

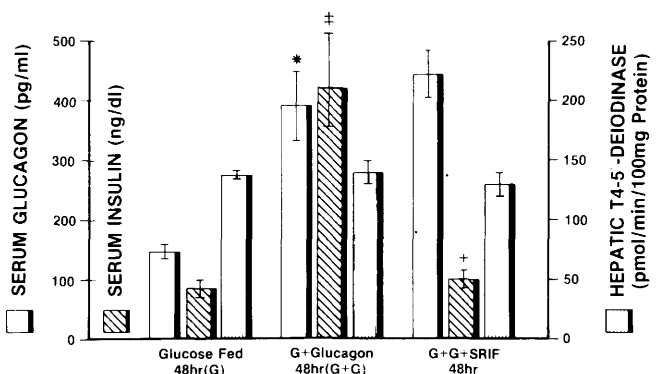

FIGURE 2. The effect of modulation of serum glucagon and insulin in the glucose-fed rat (G) on hepatic T_4-5′-deiodinase activity. The center panel demonstrates that glucagon infusion (0.15 μg/kg/min s.c.) for 48 h significantly increased both serum glucagon and insulin (G + G). The right panel shows the effect of a coinfusion of glucagon (0.15 μg/kg/min s.c.) and SRIF (0.8 μg/kg/min s.c.) on these glucoregulatory hormones, (G + G + SRIF). *$P < 0.01$, G + G versus G; ‡$P < 0.005$, G + G versus G; †$P < 0.005$, G + G + SRIF versus G + G.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024

T_4-5′-deiodinase activity was decreased in the diabetic group also supports the previous observations.[3-5] Thus, these initial studies suggested that glucagon might inhibit hepatic T_4-5′-deiodinase activity and that this hormone could consequently mediate the effects of dietary modulation and diabetes on T_3 metabolism.

Such an interaction of glucagon and T_3 has been suggested by a number of other studies. Glucagon reduces the number of nuclear T_3 receptors on rat hepatocytes,[22] an effect that should reduce the bioactivity of thyroid hormones.[23] In contrast, the plasma glucagon response to a protein meal is blunted,[24] and the glycemic response to a glucagon infusion is reduced in hyperthyroidism.[25] In addition, the low glucagon metabolic clearance rate associated with starvation is normalized by T_3.[26] Thus, glucagon and T_3 appear to inversely modify their respective metabolic activities.

However, manipulation of serum glucagon failed to alter T_3 metabolism. Glucagon infusion, over 48 h, significantly increased the mean serum glucagon concentration in the glucose-fed group. This degree of hyperglucagonemia induced a significant hyperglycemia but had no effect on T_3 metabolism. The serum T_3 and hepatic T_4-5′-deiodinase activity were similar in both groups. The dose of glucagon used has been shown to significantly alter enzyme activity in the urea cycle.[27] These results confirm the recent study on the Purina-fed normal rat, which also failed to show inhibition of hepatic T_4-5′-deiodinase activity consequent to prolonged glucagon infusion at 0.2 μg/kg/min (1 wk i.v.).[11] Furthermore, the SRIF-induced reduction in serum glucagon in the fasted and diabetic groups was not associated with any increase in either the serum T_3 or hepatic T_4-5′-deiodinase activity. It thus seemed unlikely that the alterations in T_3 metabolism noted in the fed (chow versus carbohydrate), fasted, and diabetic animals could be consequent to the changes in serum glucagon. Moreover, although we recently demonstrated that SRIF can inhibit hepatic T_4-5′-deiodinase in the fed rat, the dose used in the present study does not have that effect.[28] Thus, the absence of an increase in hepatic T_4-5′-deiodinase activity consequent to the reduction of serum glucagon in the fasted and diabetic groups could not be attributed to the SRIF. However, because this dose of SRIF lowers both serum glucagon and insulin[18,29] and since insulin therapy reverses the low hepatic T_4-5′-deiodinase activity in the diabetic rat, the effect of serum glucagon modulation on hepatic T_4-5′-deiodinase activity could have been masked by the associated changes in serum insulin.

The molar ratio of serum insulin to glucagon in the glucose-fed rat was 3.3 ± 0.3. This was increased to 6.9 ± 2.3 in the glucose-fed group treated with glucagon. Thus, although the ratio did not reach significance, the hyperglucagonemic state was associated with a relative excess of insulin. Thus, the absence of an inhibitory effect of glucagon on hepatic T_4-5′-deiodinase activity could have been counterbalanced by the stimulatory effect of insulin.[7] However, when the insulin-to-glucagon ratio was significantly decreased in the glucose-fed group by a coinfusion of glucagon and SRIF, no alteration was noted in enzyme activity. The mean hepatic T_4-5′-deiodinase activity in this group was similar to that in the glucose-fed control group. This state of relative insulin deficiency and glucagon excess should have magnified any inhibitory effect of glucagon on this hepatic enzyme. Although both hormones were measured in peripheral blood, a similar ratio should be present in the hepatic portal system. Thus, the peripheral blood levels should reflect the intrahepatic hormone changes.

Thus, glucagon does not inhibit hepatic T_4-5′-deiodinase activity in the fed, fasted, or diabetic rat. These findings are in agreement with the recent report by Senga et al., who demonstrated that an equivalent dose of glucagon did not inhibit hepatic T_4-5′-deiodinase activity in the chow-fed rat.[11] In addition, a study by Sato et al. failed to detect a direct inhibitory effect by glucagon on T_4-5′-deiodinase activity on rat hepatocytes in culture.[7] Therefore, glucagon does not appear to be an inhibitor of hepatic T_4-5′-deiodinase activity.

However, the present study was confined to a specific analysis for a selective glucagon effect. Thus, it remains possible that glucagon might inhibit T_4-5′-deiodinase activity in other tissues such as kidney or brain. However, we did not detect any alteration in the mean serum T_3 concentration during glucagon modulation and Senga et al. showed normal T_3 production rates in the hyperglucagonemic rat.[11] Thus, it is most unlikely that the alterations in T_3 metabolism characteristic of dietary modulation, fasting, and diabetes are consequent to the associated hyperglucagonemia.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Dr. John Karam (Department of Medicine, University of California San Francisco) for performing the serum glucagon assays; Dr. Gerold Grodsky (Department of Biochemistry and Biophysics, University of California San Francisco) for helping us set up the serum insulin assay, and the secretarial help of Peggy Mathews.

This study was supported by the Research Division of the Veterans Administration (L. A. Gavin, Clinical Investigator), grant AM24013 (L. A. Gavin) from the NIH, and Research funds from the Academic Senate, University of California San Francisco.

REFERENCES

1 Wartofsky, L., and Burman, K. D.: Alterations in thyroid function in patients with systemic illness: the euthyroid sick syndrome. Endocr. Rev. 1982; 3:164–217.

2 Schimmel, M., and Utiger, R. D.: Thyroidal and peripheral production of thyroid hormones. Ann. Intern. Med. 1977; 87:760–68.

3 Chopra, I. J., Wiersinga, W., and Frank, H.: Alterations in hepatic monodeiodination of iodothyronines in the diabetic rat. Life Sci. 1981; 28:1765–76.

4 Pittman, C. S., Lindsay, R. H., Senga, O., Chambers, J. B., and Hill, J. B.: The effects of diabetes mellitus on 3,5,3′-triiodothyronine production. Life Sci. 1981; 28:1677–81.

5 Gavin, L. A., McMahon, F., and Moeller, M.: The mechanism of impaired T_3 production from T_4 in diabetes. Diabetes 1981; 30:694–99.

6 Gavin, L. A., and Moeller, M.: The mechanism of recovery of hepatic T_4-5′-deiodinase during glucose-refeeding: role of glucagon and insulin. Metabolism 1983; 32:543–51.

7 Sato, K., and Robbins, J.: Thyroid hormone metabolism in primary cultured rat hepatocytes: effects of glucose, glucagon and insulin. J. Clin. Invest. 1981; 68:475–83.

8 Gerich, J. E., Langlois, M., Noacco, G., Lorenzi, M., Karam, J. H., and Forsham, P.: Comparison of suppressive effects of elevated plasma glucose and free fatty acid levels on glucagon secretion in normal and insulin-dependent diabetic subjects: evidence for selective alpha cell insensitivity to glucose in diabetes mellitus. J. Clin. Invest. 1976; 58:320–25.

9 Fisher, M., Sherwin, R. S., Hendler, R., and Felig, P.: Kinetics of glucagon in man: effects of starvation. Proc. Natl. Acad. Sci. USA 1976; 73:1735–39.

10 Clarke, C. C., Bode, H. H., and Meara, P.: The effect of glucagon on

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024

T_4 deiodination in vivo and in vitro. Proceedings Endocrine Society (Anaheim) 1979; 61:73.

[11] Senga, O., Pittman, C. S., Lindsay, R. H., Chambers, J. B., and Hill, J. B.: Comparison of peripheral thyroid hormone metabolism in normal rats and in rats receiving prolonged glucagon infusion. Endocrinology 1982; 110:2011–17.

[12] Lowry, O. H., Rosebrough, N. J., and Farr, A. L.: Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 193:265–75.

[13] Gerich, J. E., Karam, J. H., and Forsham, P. H.: Stimulation of glucagon secretion by epinephrine in man. J. Clin. Endocrinol. Metab. 1973; 37:479–81.

[14] Unger, R., Eisentraut, A. M., and McCall, M. S.: Measurement of endogenous glucogen in plasma and the influence of blood glucose concentration on its secretion. J. Clin. Invest. 1962; 41:682–89.

[15] Grodsky, G. M., and Forsham, P. H.: Comparative binding of beef and human insulin to insulin antibodies produced in man and guinea pig. J. Clin. Invest. 1961; 40:799–804.

[16] Trankel, B. J., and Grodsky, G. M.: Effects of continuous low dose insulin treatment on subsequent incidences of diabetes in genetically prediabetic Chinese hamsters. Diabetes 1979; 28:544–47.

[17] Snedecor, G. W., and Cochran, W. G.: Statistical Methods. 6th edit. Ames, Iowa State University Press, 1967:91–119.

[18] Jaspan, J. B., and Rubenstein, A. H.: Circulating glucagon: plasma profiles and metabolism in health and disease. Diabetes 1977; 26:887–902.

[19] Gavin, L. A., McMahon, F., and Moeller, M.: Carbohydrate in contrast to protein feeding increases the hepatic content of active thyroxine-5′-deiodinase in the rat. Endocrinology 1981; 109:530–36.

[20] Harris, A. R., Fang, S. L., Vagenakis, A. G., and Braverman, L. E.: Effects of starvation, nutriment replacement and hypothyroidism on in vitro hepatic T_4 to T_3 conversion in the rat. Metabolism 1980; 27:1680–90.

[21] Chopra, I. J.: Alterations in monodeiodination of iodothyronines in the fasted rat: effects of reduced nonprotein sulfhydryl groups and hypothyroidism. Metabolism 1980; 29:161–67.

[22] Dillman, W. H., Bonner, R. A., and Oppenheimer, J. H.: Glucagon administration decreases hepatic nuclear triiodothyronine binding capacity. Endocrinology 1978; 102:1633–36.

[23] Oppenheimer, J. H.: Thyroid hormone action at the cellular level. Science 1979; 203:971–79.

[24] Kabadi, U. M., and Eisenstein, A. B.: Impaired α-cell response in hyperthyroidism. J. Clin. Endocrinol. Metab. 1980; 51:478–82.

[25] Perez, G., Ungaro, B., Covelli, A., Morrone, G., Lombardi, G., Seopacasa, F., and Rossi, R.: Altered glucoregulatory response to physiological infusions of epinephrine and glucagon in hyperthyroidism. J. Clin. Endocrinol. Metab. 1980; 51:972–77.

[26] Burman, K. D., Smallridge, R. C., Jones, L., Ramos, E., O'Brian, J., Wright, F., and Wartofsky, L.: Glucagon kinetics in fasting: physiological elevations in serum 3,5,3′-triiodothyronine increase the metabolic clearance rate of glucagon. J. Clin. Endocrinol. Metab. 1980; 51:1158–65.

[27] Snodgrass, P. J., Lin, R. C., Muller, W., and Aoki, T.: Induction of urea cycle enzymes of rat liver by glucagon. J. Biol. Chem. 1978; 253:2748–53.

[28] Gavin, L. A., and Moeller, M.: Somatostatin inhibits rat hepatic T_4-5′-deiodinase: the effect is independent of the associated hypoinsulinemia. In press. J. Clin. Invest. 1983.

[29] Lins, P. E., Efendic, S., Meyers, C., Coy, D., Schally, A., and Luft, R.: Selective effects of some somatostatin analogs on glucagon as opposed to insulin release in rats in vivo. Metabolism 1980; 29:728–31.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/32/9/798/352852/32-9-798.pdf by guest on 11 October 2024