The role of somatostatin and/or dopamine in basal and TRH-stimulated TSH release in food-restricted rats

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Abstract. The present study was carried out to examine the role of endogenous dopamine and somatostatin in the mechanisms involved in the restricted feeding-induced inhibition of TSH secretion in rats. GH secretion was examined in parallel. Restricted feeding by 50% or 75% was associated with a decrease in the pituitary and circulating levels of TSH and GH in both untreated and TRH-treated groups (p<0.001), the changes being proportional to the feeding level. Intravenous injections of the dopamine antagonists, domperidone or haloperidol, failed to affect the magnitude of the differences in plasma TSH and GH levels among control and food-restricted groups, indicating that dopaminergic mechanisms had little effect on the regulation of TSH and GH secretion during restricted feeding in rats. Cerebroventricular injection of somatostatin anti-serum resulted in a marked increase in plasma TSH and GH levels in all the experimental groups (p<0.001). The increase in plasma GH and TSH induced by somatostatin anti-serum was greater in rats fed a 25% diet than in either controls or rats fed 50% of the diet; the values for the latter two groups were also different (p<0.001). The decreased TSH and GH values in somatostatin anti-serum-treated food restricted rats as compared with those in control animals on somatostatin anti-serum or normal rabbit serum can probably be attributed to the decreased available pituitary TSH and GH pools. The data indicate that long-term restricted feeding affects anterior pituitary function in rats, presumably reflecting alterations in the secretion of an inhibiting hormone, somatostatin.

Anterior pituitary release of TSH is regulated by a balance of stimulatory and inhibitory effects of at least three hypothalamic factors, thyrotropin-releasing hormone, somatostatin and dopamine (1); functional TRH, SRIH and dopamine receptors are present in thyrotropes (2-4). In addition, thyroid hormone (T₃) feeds back at the level of thyrotropes to suppress synthesis and secretion of TSH (5). Thus, at the pituitary thyro trope, the stimulatory effect of TRH competes with the inhibitory effect of T₃, SRIH and dopamine. However, the endocrine feedback control mechanisms of hypothalamic-pituitary-thyroid function during restricted feeding differ from those operating in normal animals. In this model basal and TRH-induced TSH secretion are low in spite of very low plasma thyroid hormone levels (6). These alterations could result from changes in thyrotropic function of the inhibitory effects of T₃, SRIH and dopamine, or the stimulatory TRH action.

The present study was performed to evaluate the role of endogenous SRIH and dopamine potentially responsible for abnormal TSH secretion in food-restricted rats by determining the effects of SRIH anti-serum and dopamine antagonists on basal and TRH-induced TSH release. The GH secretion was examined in parallel.

Materials and Methods

Animals and experimental design
Male Wistar rats, bred in our colony, weighing 160-180 g, were maintained three or four per cage with free access to laboratory rat chow and water, on a 14-h light, 10-h dark cycle (lights on at 07.00 h) at 22±2°C. After an adaptation period of a week, groups of rats were fed ad libitum (control) or limited to 50% (Group 1) or 25% (Group 2) of the food consumed by the control group.
Experiments were performed after a period of 18-days of food restriction.

In the first experiment, the effect of dopamine antagonists was tested on basal and TRH-induced TSH release. On the day of the experiment, ether anesthetized rats received a dose of either haloperidol (200 μg/kg, 100 μl, iv) or domperidone (150 μg/kg, 100 μl, iv) between 11.00 and 12.00 h. Blood samples were withdrawn just before and 30 min after injections. Animals injected with the vehicle only served as control. Then, TRH (50 μg/kg, 100 μl) or saline were injected iv; blood samples were collected 5 and 15 min after injections. The second experiment was carried out to examine the role of endogenous SRIH on basal and TRH-stimulated TSH secretion. On the test day, pentobarbital anesthetized rats were placed in a stereotaxic instrument (David Kopf Co; Tujunga, CA) and received a dose of either SRIH anti-serum or normal rabbit serum (5 μl intraventricularly). The stereotaxis coordinates of Paxinos & Watson (7) were used as reference to place the tip of a Hamilton syringe on the right lateral ventricle. Owing to the long time of manipulation in this experiment pentobarbital instead of ether was used as an anesthetic. The protocol for obtaining blood samples was the same as that used in the first experiment. After centrifugation, plasma was stored at −20°C until hormonal assays were performed. The rats were killed by decapitation and the anterior pituitary was rapidly removed, frozen, and stored at −70°C until assayed. The brains were immersed in 10% formalin, and sectioned on a freezing microtome; 50 μm slices were stained with hematoxylin and eosin. The site of injection was determined microscopically.

Reagents
Haloperidol (Sigma Chemical Co, St. Louis, MO), was dissolved in 0.3% tartaric acid. Domperidone, kindly provided by Dr S. Erill (Esteve Laboratories, Spain), was dissolved in saline. Both dopamine antagonists were prepared at the time of the experiments. TRH (Sigma) was prepared in saline. The rabbit antiserum to SRIH was kindly provided by Dr Fernandez-Durango (Department of Physiology, Faculty of Medicine, University Complutense, Madrid, Spain). It has been characterized and is highly specific (8).

Assays
Plasma and pituitary TSH and GH were measured by RIAs with the materials and protocols kindly provided by the NIDDK Rat Pituitary Agency. Results are expressed in terms of reference standards (NIDDK-rTSH-RP-2 and NIDDK-rGH-RP-2). The sensitivities for TSH and GH assays were approximately 0.4 and 2.2 μg/l, respectively. Intra- and inter-assay coefficients of variation for each RIA were below 4 and 6.6%, respectively. Plasma T₃ and T₄ concentrations were measured by specific RIAs (9,10). All samples from a single experiment were run in the same RIA.

Results
The data in Fig. 1 indicate that 18 days of food restriction in rats resulted in a significant reduction in body and pituitary weights, accompanied by a decrease in pituitary TSH and GH contents. Plasma TSH and GH concentrations were also significantly decreased in both food-restricted groups, as were plasma T₃ levels. These changes were more marked for Group 2 than for Group 1 rats. A decrease in plasma T₄ was also observed in Group 2 rats, whereas normal values were found in Group 1.

As previously reported (6,11), food restriction decreased basal TSH levels as well as TSH released in response to TRH administration (Fig. 2); thus, the increase over basal levels of plasma TSH at 5 and 15 min after TRH administration was, respectively, 324±29 and 748±69% for control rats, 282±31 and 582±51% for Group 1, and 280±21 and 468±56% for Group 2 rats. Pretreatment of the rats with domperidone or haloperidol induced a comparable slight, but significant (p<0.05-0.01) increase in basal and TRH-stimulated TSH levels in all experimental groups. The mean basal TSH levels in domperidone-treated control, Group 1 and Group 2 rats were increased by 16, 15, and 14%, respectively; the increase at 5 and 15 min after TRH injection was 20 and 21% for control rats, 16 and 17% for Group 1 rats, and 17 and 18% for Group 2 rats, respectively. Comparable changes were observed in haloperidol-treated groups. Consequently, the differences in TSH values among domperidone- or haloperidol-treated control and food-restricted rats were almost identical to those found between control rats and each food-restricted group treated with vehicle for domperidone or haloperidol.

Intraventricular administration of SRIH anti-serum induced a marked increase in plasma TSH levels above the values in normal rabbit serum-injected group (Fig. 3), the changes being more intense for Group 2 than for Group 1 rats, and greater for Group 1 than for control rats. Thus, compared with the corresponding normal rabbit serum-treated groups, the increase in TSH values,
Fig. 1.
Effects of food restriction on body and anterior pituitary weights, pituitary TSH and GH contents, and plasma T₄, T₃ and TSH levels. (FR₅₀) and (FR₂₅) rats received 50% and 25%, respectively, of the daily food consumption of the control group during 18 days. Data are means ± s.d from 18-20 rats per group. *: p<0.05 or less relative to control group.

basal or 5 and 15 min after TRH for the three groups were, 42, 55 and 63% for control rats; 71, 82 and 116% for Group 1 rats, and 73, 130 and 160% for Group 2 rats, respectively. However, after SRIH anti-serum administration, plasma TSH levels were lower in both food-restricted groups than in control rats. Plasma TSH in Group 2 on SRIH anti-serum was also lower than in normal rabbit serum-treated control animals. When compared with control values a decrease (p<0.001) in the pituitary TSH concentration was found in both food-restricted groups. The mean of pooled values from control, Group 1 and Group 2 rats of both experiments were, respectively,

Fig. 2.
Effects of domperidone (A) and haloperidol (B) on plasma TSH levels in untreated and TRH-treated control Group 1, (FR₅₀) and Group 2 (FR₂₅) rats. Domperidone (150 µg/kg, 100 µl, iv) or haloperidol (200 µg/k, 100 µl, iv), or their vehicles (100 µl, iv) were administered 30 min prior to TRH (50 µg/kg, 100 µl, iv) or saline (100 µl, iv) injections. Blood samples were withdrawn just prior each treatment and 5 and 15 min after TRH (TRH₇) and saline injections. Data are mean ± s.d for 5-7 rats per group. □ vehicle, □ domperidone or haloperidol, □ TRH₇, □ domperidone or haloperidol + TRH₇, □ domperidone or haloperidol + 1 TRH₇. *: p<0.05 or less relative to corresponding control values; *: p<0.05 or less relative to corresponding Group 1 values; ○: p<0.05 or less relative to corresponding domperidone- or haloperidol-untreated groups. For more details see the legend to Fig. 1.
2.86±0.31, 1.96±0.23 and 1.58±0.20 μg/mg protein. When pituitary TSH concentrations were plotted against the increase in plasma TSH (ΔTSH) values after TRH administration, a positive linear relationship (r=0.960, p<0.001, N=65) was found in rats from the first experiment. This relationship was not observed in rats treated with SRIH anti-serum.

As shown in Fig. 4, the mean plasma GH levels within an experimental group were indistinguishable when comparing the values in vehicle-, normal rabbit serum-, domperidone-, TRH-, and domperidone- plus TRH-treated animals. Within each treatment, plasma GH levels in control rats were 1.8-fold and 3.5-fold higher than those in Group 1 and 2, respectively. In contrast, SRIH anti-serum increased plasma GH differently from all other treatments (p<0.001) within each group. SRIH anti-serum induced a 7-fold increase in plasma GH in Group 1 rats and a 12-fold one in Group 2 rats, which were significantly (p<0.001) higher than the 5-fold elevation observed in control rats. However, after SRIH anti-serum injection plasma GH concentrations were higher in control rats than in food-restricted animals.

Discussion

In the present study we provide evidence already available (6,11,12) that the pituitary TSH and GH
content and secretion can be depressed by the peripheral metabolic status resulting from 18-days of food restriction. The decreased pituitary TSH responsiveness to exogenous TRH in food-restricted rats indicated that the reduction in plasma TSH levels after reduced feeding probably is not only mediated by TRH. These results differ from previously reported findings of normal or increased TSH responsiveness to TRH in fasted rats (13,14). The metabolic conditions resulting from the 18-days of food restriction in this study, probably are not comparable to those resulting from 2- to 4-day complete food withdrawal, as reported in other studies.

Although the pituitary TSH content certainly influences the response to TRH (15), other factors, such as dopamine and somatostatin, may also play a role. We tried solving this point by using dopamine antagonist and SRIH anti-serum. Neither diphenidol nor haloperidol decreased the differences in the circulating levels of TSH and GH in response to TRH administration between control and food-restricted groups, suggesting that the decreased secretion of both pituitary hormones during restricted feeding could not be explained by an increase in the dopaminergic influence. The possibility that endogeneous SRIH was inactivated by the anti-serum could account for the results in Figs. 3 and 4. Administration of SRIH anti-serum induced a marked increase in circulating TSH and GH levels and potentiated the effect of TRH on TSH secretion, the magnitude of the changes being proportional to the feeding level. These results are consistent with early findings indicating that antiserum to somatostatin reverses the starvation-induced inhibition of GH secretion (16) and basal, cold- and TRH-stimulated TSH release (17,18). Increased SRIH influence on pituitary somatotropes and thyrotropes in food-restricted rats could also explain the changes in the pulsatile GH and TSH secretion previously reported in these animals (11). The decreased GH and TSH values found in SRIH anti-serum-treated food-restricted rats as compared with those in control animals on normal rabbit serum can probably be attributed to the decreased available pituitary TSH and GH pools (15,19). Thus, these results not only provide evidence that in food-restricted rats SRIH plays a relevant role in the regulation of TSH and GH secretion, but also suggest that the decrease of both TSH and GH secretion in this situation could not be explained by altered dopaminergic influence.

While the present results provide evidence for changes in SRIH action induced by food-restriction on the pituitary TSH and GH secretion, important issues regarding how these metabolic conditions affect the TSH and GH contents in the pituitary remain to be explored.

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