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Chronic Ethanol Drinking and Food Deprivation Affect Rat Hypothalamic–Pituitary–Thyroid Axis and TRH in Septum

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Because chronic ethanol ingestion may perturb thyroid function, we evaluated the effect of 4-wk of oral 10% ethanol ingestion on the hypothalamic–pituitary–thyroid (HPT) axis and septal thyrotropin-releasing hormone (TRH) in 200-g male Wistar rats. Animals were divided into three groups: absolute control receiving tap water and food *ad libitum*; ethanol group receiving food *ad libitum* and 10% ethanol as the sole source of drinking fluid; pair-fed group receiving tap water and an amount of food corresponding to the consumption of ethanol group. After 4-wk of treatment, the body weight of the ethanol group was 7% and of the pair-fed rats 19% lower than that of the absolute controls. Both chronic ethanol treatment and food deprivation produced a decrease in plasma thyroid-stimulating hormone (TSH). Pair-fed rats also had a lower plasma T_3. Type I iodothyronine 5'-deiodinase activity in the liver was increased in the pair-fed and even more in the ethanol-treated group. The content and secretion in vitro of TRH from the hypothalamic paraventricular nucleus and median eminence were unchanged. TRH content in the septum was increased in both the ethanol and pair-fed groups. TRH secretion from the septum in vitro was lower in the pair-fed, but unchanged in the ethanol group. These data suggest that 4-wk of peroral ethanol intake affects thyroid function mostly at the extrahypothalamic level and that there is a contribution of concomitant food deprivation. Both ethanol treatment and food deprivation increased TRH content in the septum.

Key Words: Ethanol; food deprivation; TRH; septum; hypothalamus.

Introduction

Chronic alcohol ingestion causes various endocrine dysfunctions, including those of the hypothalamic–pituitary–thyroid (HPT) axis *(1,2)*, through mechanisms that are not yet clear. Various investigators have described decreased *(3)*, normal *(1,4,5)*, or increased *(6)* blood levels of thyroid hormones after ethanol exposure. After long-term administration of ethanol, thyroid and adrenal hyperplasia *(7)* or a reduction *(8)* of thyroid volume have been reported. Ethanol may alter intracellular thyroid hormone metabolism in both rat central nervous system (CNS) and liver *(9,10)*. However, the various investigators have used different doses of ethanol, durations of treatment, and methods of administration, making it difficult to draw a general conclusion. Moreover, ethanol consumption decreases food intake *(11)*, which makes the interpretation of results even more complicated.

Recently, we have shown that in vitro ethanol stimulates the release of thyrotropin releasing hormone (TRH) from the hypothalamic paraventricular nucleus (PVN) and median eminence (ME) *(12)*. In the present study, we examined the in vivo effect of long-term ethanol administration on the HPT axis in rats, with special attention to TRH in specific brain regions. TRH subserves numerous biologic functions both within the CNS and in extraneural tissue *(13)*. Hypophysiotropic TRH is synthesized in the parvocellular neurons of the hypothalamic paraventricular nucleus. Terminals of paraventricular neurons project mainly to the ME where this neurohormone is released into the portal circulation. In addition to its endocrine function in controlling pituitary secretion, TRH can reduce CNS sensitivity to alcohol and attenuate the narcotic and hypothermic actions of ethanol. A specific site for analeptic TRH actions has been localized particularly to the medial septum *(14,15)*. We were therefore interested to see how ethanol affects these two different TRH systems: in the septum and in the hypothalamus. This study was designed to determine the effects of long-term oral ethanol intake, including the role of associated changes in food and caloric intake. Experimental rats that drank 10% alcohol (E group) for

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Table 1
Body Weight (Body wt) and Liquid Intake During 4-wk of Treatment[a]

	Starting body wt, g	Final body wt, g	Liquid intake, mL/d/rat
AC	200.4 ± 2.6	366.5 ± 6.3	42.7 ± 1.4
E	200.7 ± 2.6	340.3 ± 4.6[b]	36.3 ± 1.6[b]
PF	200.7 ± 2.5	297.2 ± 5.0[b,c]	42.9 ± 2.0[c]

[a]AC—absolute control, E—ethanol-treated, PF—pair-fed rats.
[b]$p < 0.05$ compared to AC.
[c]$p < 0.05$ compared to E.

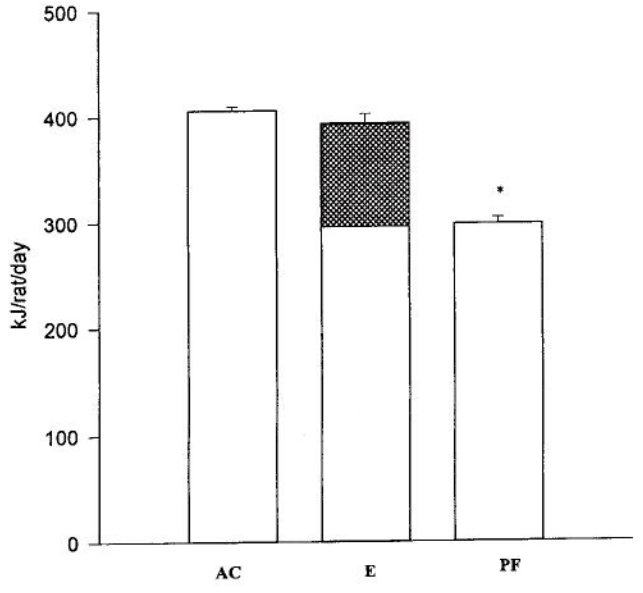

Fig. 1. Caloric intake during 4-wk of treatment expressed as an average of the daily intake during the experiment. AC—absolute control, E—ethanol-treated, PF—pair-fed rats. *$p < 0.05$ compared to A57 and E. ▭, energy from solid diet; ▦, energy from ethanol.

4 wk were compared to both untreated controls (AC, absolute control) and pair-fed animals (PF).

Results

Body Weight, and Caloric and Liquid Intake

Animals in each experimental group had similar initial body weights (Table 1). After 4 wk of treatment, the E group body wt was 7% and the PF group, 19% lower than in AC. Differences in body weight from AC were observed from the third day of treatment in both E and PF animals. Liquid intake (calculated as an average of the daily intake during the experiment) was lower in E than in AC and PF. The energy intake of the E group (an average of the daily intake), despite a 26% food deprivation, was similar to that of the AC group owing to the caloric contribution of ethanol (Fig. 1). The PF group had a 26% lower food and caloric intake than AC.

Table 2
Plasma Concentration of Thyroid Hormones and TSH after 4-wk of Treatment[a]

	T_3, nmol/L	T_4, nmol/L	TSH, ng/mL
AC	1.11 ± 0.04	41.63 ± 2.78	4.41 ± 0.56
E	1.05 ± 0.03	46.87 ± 3.80	3.14 ± 0.32[b]
PF	0.97 ± 0.04[b]	42.07 ± 3.10	2.44 ± 0.35[b]

[a]Differences between E and PF were not significant.
[b]$p < 0.05$ compared to AC.

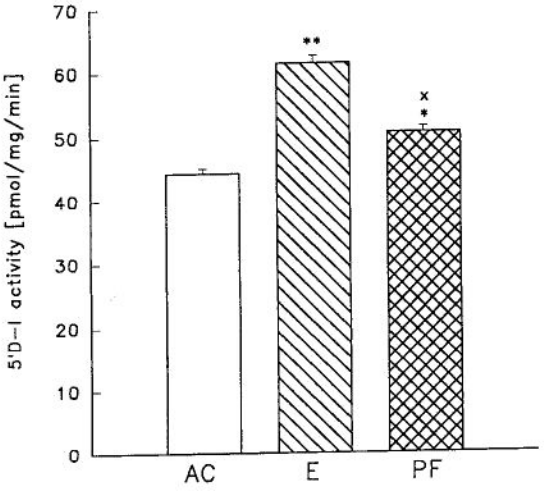

Fig. 2. Activity of type I iodothyronine 5'-DI in the liver microsomal fraction after 4-wk of treatment. *$p < 0.05$, **$p < 0.001$ compared to AC, $^{x}p < 0.05$ compared to E.

Plasma T_4, T_3, and Thyroid-Stimulating Hormone (TSH) Concentration

There were no significant differences in plasma total T_3 or T_4 concentration between E and AC (Table 2). PF animals had a lower T_3 level, but unchanged T_4. Both chronic alcohol treatment and food deprivation produced a decrease in plasma TSH compared to AC.

Activity of 5'-Deiodinase (5'-DI) in Liver

The activity of type 1 iodothyronine 5'-DI in the liver microsomal fraction was increased in both the E and PF groups compared to AC (Fig. 2). This enzyme activity in E was also higher than that in PF.

TRH Content in PVN, ME, and Septum

There were no significant changes in the TRH content of the ME and PVN in all groups (Table 3). In contrast, the septal TRH content was 51 and 83% higher in E and PF, respectively, than in AC.

TRH Secretion from PVN, ME, and Septum In Vitro

In the ME, there was no difference between groups in basal or KCl-stimulated TRH secretion (Fig. 3A). In the PVN, PF had increased basal, but not stimulated secretion compared to the other groups (Fig. 3B). There was a signifi-

Table 3
TRH Content in the Septum and in the Hypothalamic ME and PVN after 4-wk of Treatment[a]

	TRH content, pg		
	ME	PVN	Septum
AC	183.1 ± 28.8	146.5 ± 8.7	60.3 ± 5.0
E	209.2 ± 40.7	143.6 ± 9.5	90.9 ± 1.4[b]
PF	201.8 ± 13.8	137.6 ± 7.6	110.6 ± 12.2[b]

[a]Differences between E and PF were not significant.
[b]$p < 0.05$ compared to AC.

cant decrease in both basal and stimulated TRH secretion from the septum in PF (Fig. 3C). Ethanol treatment did not affect in vitro TRH secretion in any structure investigated.

Discussion

This study was concerned with changes in the rat HPT axis, septum TRH, body-wt changes induced by long-term alcohol treatment, and the role of food intake reduction *per se*. Drinking ethanol resulted in a decrease in solid food consumption. Total energy intake was, however, similar in the AC and E groups owing to the caloric contribution of ethanol. Nevertheless, alcohol administration was associated with a smaller weight gain in E than in AC. The energy intake represented by ethanol ingestion did not fully restore the growth retardation resulting from food deprivation. Although the energy intake of the E and AC groups was nearly identical, alcohol consumption was associated with a lower nutritional level. Nevertheless, the weight gain in E was significantly higher than in PF, so at least part of the ethanol energy content was effective. A possible ethanol effect on growth hormone secretion *(16)* or decreased water intake cannot be excluded.

Long-term food reduction reduced plasma T_3 and TSH concentration, in agreement with van Haasteren et al. *(17)* and Cohen et al. *(18)*. A decreased TSH level despite reduced T_3 concentration indicates that a central mechanism may be involved in the inhibition of thyroid function. However, we did not find any changes in TRH content or in vitro secretion in the ME. The PVN TRH content was not changed, whereas in vitro basal secretion was increased. Hypothalamic pro-TRH mRNA was found to be either decreased *(19)* or not affected in food-restricted rats *(17)*. A possible explanation for the plasma TSH decrease in our experiments is an elevated level of plasma corticosterone in PF rats (average 4.2, 4.2, and 12 mg/100 mL in AC, E, and PF, respectively) *(20)*. Van Haasteren et al. showed that the decrease in plasma TSH in starved rats could be partially prevented if plasma corticosterone is kept constant by adrenalectomy and corticosterone substitution *(21)*.

Long-term alcohol administration did not change T_3 or T_4 plasma levels, but plasma TSH was decreased. As in the PF rats, the TRH content in the PVN and ME was not altered in E. Chronic ethanol treatment was reported to produce an increase in TRH mRNA in the PVN, but the thyrotropin response to TRH was blunted *(1)*. The drop in plasma TSH

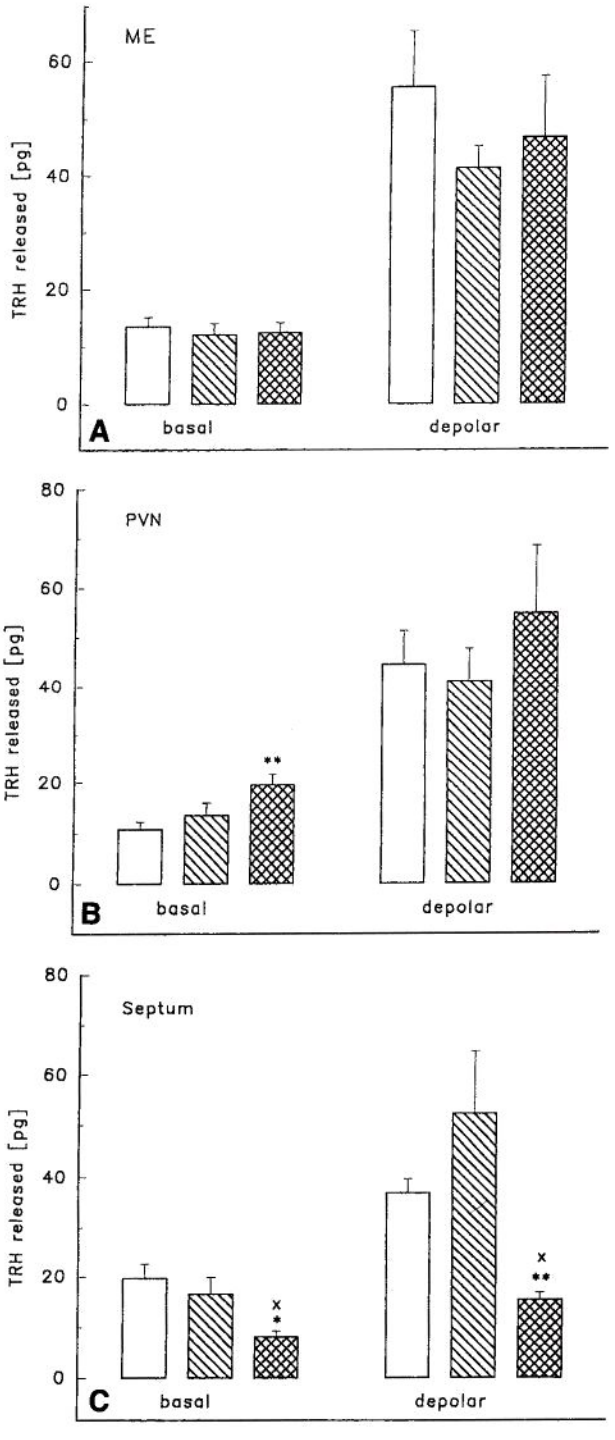

Fig. 3. TRH secretion in vitro from ME **(A)**, PVN **(B)**, and septum **(C)**. PVN and ME were incubated for 30-min successive periods alternately in basal or stimulating (depolarizing) medium. Secretion of TRH was stimulated with 56 m*M* KCl. The septum was incubated for 15-min successive periods using the same alternation schedule as with the PVN and ME. $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$ compared to AC, $^{x}p < 0.05$ compared to E. ▭, AC; ▨, E; ▦, PF.

in the E rats was not owing to increased corticosterone, because plasma corticosterone concentration is not changed by chronic drinking of 10% ethanol *(20)*. Recently, it was demonstrated that ethanol exerts thyrotropin-like activity in cultured porcine thyroid follicles *(22)*, which might explain the normal T_4 and T_3 level in E despite the TSH decrease. Ethanol affects thyroid hormone formation by stimulating the uptake and organification of iodide *(22)*.

The increased activity of type 1 iodothyronine 5'-deiodinase, which converts T_4 to T_3 in the liver, in both E and PF is of interest. Although the plasma level of T_4 was normal in both E and PF, T_4 concentration in tissues may be altered. The data of Kaptein at al. *(4)* indicate marked disturbances in T_4 transfer and distribution in patients with nonthyroidal illnesses, including chronic ethanol abuse. In the rat, chronic ethanol administration increased type 2 iodothyronine 5'-DI activity in brain frontal cortex *(10)*. In animals dependent on alcohol, after 3 mo of abstinence, the liver concentrations of T_3 and T_4 were significantly lower, whereas in the CNS, T_3 was normal *(9)*. The effects of food deprivation and alcohol consumption on 5'-DI in our experiments seem to be additive—the enzyme activity in E is increased even more than that in PF. This suggests that the effect of fasting differs from that of alcohol.

We reported earlier that acute ethanol in vitro increases basal and KCl-stimulated TRH secretion from PVN and ME in a concentration-dependent manner *(12)*. In the present study, we found that chronic administration of ethanol in vivo did not change TRH secretion in vitro from either the PVN or ME. TRH secretion may be affected differently by acute or chronic ethanol administration. Chronic alcohol administration produces tolerance to ethanol, which is reflected by a return toward a normal plasma corticosterone level after repeated administration of alcohol *(23,24)*.

The septum is a component of the limbic system that affects various motivational drives, including food and water intake *(25)*. Septal TRH increases after rats regain the righting reflex in following a sedating dose of alcohol *(26)*. Microinjection of TRH into the septum accelerates recovery from ethanol-induced hypothermia and narcosis *(27)*. Our present data indicate that chronic ethanol drinking causes a significant increase in TRH content in the septum. However, septal TRH in vitro secretion was not affected. Food deprivation resulted in a twofold increase in septal TRH content, and its in vitro secretion was distinctly lower under both basal and stimulated conditions. The increase in septum TRH content in PF therefore might be a consequence of its reduced release. The differences in the effect on in vitro secretion suggest that the mechanism of ethanol ingestion and food deprivation on septal TRH might be different. Although in PF animals a high TRH content is explainable by its low release, release in E was not lowered. An increase in TRH biosynthesis after ethanol treatment might be the eventual explanation. TRH in the septum is present in the perikarya as well as in neuron terminals *(28,29)*. Ethanol and food restriction could have affected different TRH pools in the septum, or TRH degradation might have been decreased inside the neurons of E rats. Chronic food deprivation, in contrast to chronic alcohol ingestion, is a stress stimulus associated with elevated plasma corticosterone in PF rats *(20)*. The septum has been identified as a primary target area for corticosterone *(30)*. Therefore, a direct corticosterone effect on septal TRH is also a possible reason for the difference between the PF and E groups.

In conclusion, chronic ethanol drinking was connected with reduced food intake and weight gain. Although drinking 10% ethanol compensated for the caloric deficit, it did not fully restore the weight gain. Both food restriction and alcohol consumption resulted in depressed plasma TSH, but plasma T_3 was affected only in food-restricted animals. The activity of type I iodothyronine 5'-deiodinase in liver was enhanced in both the E and PF groups. We demonstrated for the first time that chronic ethanol consumption and food deprivation significantly affect septal TRH. Most of the effects induced by ethanol were seen also in the pair-fed controls. Although the mechanism of induced changes might be different, the changed nutrition must be considered an important determinant of the ethanol effects.

Materials and Methods

Animals

Male Wistar rats initially weighing 200 g were individually housed in cages kept in a temperature-controlled room (20–24°C) and a 12-h light/12-h dark cycle. Ethanol (10% w/v) was administered to animals (E group) as the sole source of fluid for 4 wk with food *ad libitum*. There were two control groups—AC receiving tap water and food *ad libitum*, and PF, receiving tap water *ad libitum* and an amount of food corresponding to that consumed by E. Each group consisted of 20 animals.

The food and liquid intake was measured daily, and body weight was measured three times a week. After 4 wk of treatment, the animals were killed by decapitation. The brain was rapidly removed and the ME, PVN area *(31)*, and entire septum *(32)* for the in vitro experiments were dissected Aliquots of plasma and liver were frozen at –20 or –80°C, respectively, until assayed for hormones or measurement of type 1 iodothyronine 5'-DI.

Incubation of Brain Structures In Vitro

After a 30-min preincubation period, the ME (one ME/tube) and PVN (one PVN/tube) were incubated successively two times for 30-min periods in 150 µL medium alternately in basal or depolarizing medium at 37°C in 5% CO_2/95% O_2 atmosphere.

Septum (septum from 1 animal/tube) was preincubated for 60 min, and after that incubated for two successive 15-min periods in 150 µL medium in basal or depolarizing medium.

Solutions: basal medium—6 m*M* $NaHCO_3$, 130 m*M* NaCl, 5.6 m*M* KCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10 m*M* glucose, 1.5 m*M* ascorbic acid, 2 m*M* HEPES, pH = 7.4. To prevent TRH degradation, bacitracin (30 mg/100 mL) was added to the medium. Depolarizing medium—56 m*M* KCl, 79.6 m*M* NaCl (the content of NaCl was lowered to maintain physiological osmolarity), the rest was the same as in basal medium.

Measurement of Type I Iodothyronine 5'-DI

The activity of 5'-DI was measured in the liver microsomal fraction by determining the release of $^{125}I^-$ from ^{125}I-3,3',5'-triiodothyronine (rT_3) according to Leonard and Rosenberg *(33)*.

TRH Measurement

TRH released into the medium was determined by a specific radioimmunoassay *(31,34)*. The TRH antibody was prepared by immunization of rabbits with synthetic TRH coupled to bovine serum albumin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimid. Crossreactivity of the TRH antiserum with TRH degradation products or TRH precursor was <0.01%. TRH standards were prepared in each medium we utilized, and thus, correction for recovery was included.

TRH Extraction from Tissues

After incubation, the tissues were placed into ice-cold water and sonicated. Aliquots of the homogenates were used for protein determination. The same volume of 2 *M* acetic acid was added to the remaining homogenate, and the tubes were kept at –20°C overnight. The samples were then centrifuged at 7*g* for 10 min, and supernatant was stored. After addition of 0.5 mL 50% methanol to the sediment, the samples were recentrifuged under the same conditions. Both supernatants were pooled and lyophilized. The lyophilizates were kept at –20°C and reconstituted in assay buffer on the day of radioimmunoassay (RIA).

TSH, T_3, T_4 Assays

Thyroid hormones in nonextracted plasma were assayed with RIA T_3 and T_4 kits (Immunotech, Prague). Plasma TSH was assayed with reagents (rat TSH-RP-3, AFP-55 12B, rat TSR-I-9 AFP-11542B and anti-rat TSH-RIA-6, AFP-329661 Rb) obtained from the NIDDK through the National Hormone and Pituitary Program.

Statistical Analysis

The data are presented as mean ± SE. The experimental groups were compared using ANOVA followed by Newman-Keuls multiple comparisons for independent groups.

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References

1. Zoeller, R. T., Fletcher, D. L., Simonyl, A., and Rudeen, P. K. (1996). *Alcohol Clin. Exp. Res.* **20,** 954–960.
2. Baumgartner, A., Rommelspacher, H., Otto, M., Schmidt, L. G., Kurten, I., Graf, K. J., et al. (1994). *Alcohol Clin. Exp. Res.* **18,** 284–294.
3. Zoeller, R. T. and Rudeen, P. K. (1992). *Brain Res. Mol. Brain Res.* **13,** 321–330.
4. Kaptein, E. M., Kaptein, J. S., Chang, E. I., Egodage, P. M., Nicoloff, J. T., and Massry, S. G. (1987). *J. Clin. Endocrinol. Metab.* **65,** 606–616.
5. Ylikahri, R. H., Huttunen, M. O., Harkonen, M., Leino, T., Helenius, T., Liewendahl, K., et al. (1978). *J. Clin. Endocrinol. Metab.* **46,** 715–720.
6. Pamenter, R. W. and Boyden, T. W. (1985). *J. Lab. Clin. Med.* **106,** 141–146.
7. Holmberg, B. and Ekstrom, T. (1995). *Toxicology* **96,** 133–145.
8. Hegedus, L., Rasmussen, N., Ravn, V., Kastrup, J., Krogsgaard, K., and Aldershvile, J. (1988). *Metabolism* **37,** 229–233.
9. Baumgartner, A., Heyne, A., Campos-Barros, A., Kohlr, R., Muller, F., Meinhold, H., et al. (1994). *Alcohol Clin. Exp. Res.* **18,** 295–304.
10. Jimenez, J., Osuna, C., Rubio, A., and Guerrero, J. M. (1993). *Biosci. Rep.* **13,** 91–98.
11. Seeley, R. J., Sharon, L. M., and Woods, S. C. (1997). *Pharmacol. Biochem. Behav.* **56,** 379–382.
12. Nikodémová, M., Weismann, P., Filipčík, P., Mráz, P., Greer, M. A., and Štrbák, V. (1997). *Neuroscience* **80,** 1263–1269.
13. Lechan, R. M. (1993). *Thyroid Today* **16,** 1–11.
14. Breese, G. R., Frye, G. D, McCown, T. J., and Mueller, R. A. (1984). *Pharmacol. Biochem. Behav.* **21,** 145–149.
15. McCown, T. J., Moray, L. J., Kizer, J. S., and Breese, G. R. (1986). *Pharmacol. Biochem. Behav.* **24,** 1269–1274.
16. Sozynski, P. A. and Frohman, L. A. (1992). *Endocrinology* **131,** 2603–2608.
17. van Haasteren, G. A. C., Linkels, E., van Toor, H., Klootwijk, W., Kaptein, E., de Jong, F. H., et al. (1996). *J. Endocrinol.* **150,** 169–178.
18. Cohen, J. H., III, Alex, S., DeVito, W. J., Braverman, L. E., and Emerson, C. H. (1989). *Endocrinology* **124,** 3025–3029.
19. Legradi, G., Emerson, C. H., Ahima, R. S., Flier, J. S., and Lechan, R. M. (1997). *Endocrinology* **138,** 2569–2576.
20. Štrbák, V., Benický, J., Macho, L., Je_ová, D., and Nikodémová, M. (1998). Metabolism, in press.
21. van Haasteren, G. A. C., Linkels, E., Klootwijk, W., van Toor, H., Rondeel, J. M. M., Themmen, A. P. N., et al. (1995). *J. Endocrinol.* **145,** 143–153.
22. Nasu, M. and Sugawara, M. (1993). *Endocrinology* **132,** 155–160.
23. Spencer, R. L. and McEwen, B. S. (1997). *Neuroendocrinology* **65,** 353–359.
24. Seeley, R. J, Hawkins, M. H., Ramsay, D. S., Wilkinson, C. W., and Woods, S. C. (1996). *Pharmacol. Biochem. Behav.* **55,** 268–273.
25. Morgane, P. J. (1969). *Ann. NY Acad. Sci.* **157,** 806–848.
26. Morzorati, S. and Kubek, M. J. (1993). *Brain Res. Bull.* **31,** 301–304.
27. Widdowson, P. S. (1987). *Brain Res.* **27,** 281–289.

28. Ishikawa, K., Taniguchi, Y., Kurosumi, K., and Suzuki, M. (1986) *Neuroendocrinology* **44,** 54–58.
29. Merchenthaler, I., Csernus, V., Csontos, C., Petrusz, P., and Mess, B. (1988). *Am. J. Anatomy* **181,** 359–376
30. Knizley, H., Jr. (1972). *J. Neurochem.* **19,** 2737–2745.
31. Nikodémová, M. and Štrbák, V. (1995). *Life Sci.* **56,** 1511–1521.
32. Palkovits, M. and Brownstein, M. J. (1988). *Maps and Guide to Microdissection of the Rat Brain*. Elsevier: New York.
33. Leonard, J. L. and Rosenberg, I. N. (1980). *Endocrinology* **107,** 1376–1383.
34. Oliver, C., Eskay, R. L., Ben-Jonathan, N., and Porter, J. C. (1974). *Endocrinology* **96,** 540–546.