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Physiology

Role of neuromedin B in control of the release of thyrotropin in hypothyroid and hyperthyroid rats

(third ventricular injection/anterior pituitary incubation/thyroxine/triiodothyronine)

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ABSTRACT **Neuromedin B (NB) is a recently discovered neuropeptide related to bombesin. It is localized to thyrotropes and we have previously shown that it directly inhibits thyrotropin (TSH) release from the anterior pituitary gland of euthyroid rats. In the current studies, we further evaluated the action of NB and antiserum directed against it in euthyroid rats and compared the actions with those in hypo- and hyperthyroid rats. Rats were rendered hypothyroid by treatment with propylthiouracil and hyperthyroid by treatment with thyroxine. In euthyroid rats, NB suppressed TSH release from hemipituitaries *in vitro*. Incubation of these pituitaries with highly specific antiserum against NB produced a stimulation of TSH release, whereas normal rabbit serum had no effect on the output of TSH. Thus, in euthyroid animals NB is a physiologically significant inhibitor of TSH release from the pituitary. In hypothyroid as in euthyroid animals, NB inhibited TSH release when microinjected into the third ventricle (3V) in the same dose (0.5 μg; 0.44 nmol) as in euthyroid rats. TSH release from hemipituitaries of hypothyroid animals was also suppressed by NB as in euthyroid animals. In hypothyroid animals, anti-NB antiserum was ineffective both *in vivo* after its microinjection into the 3V and *in vitro* on hemipituitaries, which suggests that the peptide has little physiologic significance in this condition, presumably because of its reduced release from the thyrotropes associated with diminished NB content in the pituitary of the hypothyroid rat. Intraventricular injection of NB failed to lower plasma TSH in hyperthyroid rats, which suggests that the action of the peptide is already maximal in hyperthyroidism. When antiserum to NB was microinjected twice into the 3V, there was a delayed increase in plasma TSH manifest 24 hr after the initial injection. TSH release from pituitaries of these animals was markedly increased in the presence of NB antiserum. Thus, NB has a physiologically significant TSH release-inhibiting action at the pituitary in the hyperthyroid as well as in the euthyroid rat. We conclude that in the euthyroid animal NB acts in an autocrine fashion to suppress TSH release from the thyrotropes directly. In hypothyroidism, NB synthesis and presumably release from the pituitary is decreased, such that there is no physiologic significance to the residual NB release, although the responsiveness to the inhibitory action of the peptide is increased, possibly via upregulation of its postulated receptors on the thyrotrope. In hyperthyroidism, the concentration of NB in thyrotropes and presumably its release is increased so that it has a physiologically significant TSH release-inhibiting action.**

Neuromedin B (NB) is a neuropeptide that was first isolated from porcine spinal cord by Minamino *et al.* (1). Since it is related chemically to bombesin (B), another neuropeptide, it was given the name NB. Immunocytochemical studies in the rat have shown that NB is present in the hypothalamus and anterior pituitary gland (2). Steel *et al.* (2) have shown that NB is localized in the thyrotropes of the anterior pituitary gland and that its content paralleled that of thyrotropin (TSH) when the rats were made hypo- or hyperthyroid.

Therefore, our initial studies evaluated the role of NB in modulating TSH secretion in euthyroid rats. NB suppressed TSH release when injected intravenously. Since the dose required after intracerebral injection into the third ventricle (3V) was much less than the minimal effective intravenous dose, we concluded that it acted centrally. The peptide also suppressed TSH release from anterior pituitaries incubated *in vitro*, which indicated that it acted directly on the thyrotropes to inhibit TSH release (3).

In the present experiments, we determined the effect of altered thyroid hormone levels on the response to the peptide by examining the effects of NB and antiserum directed against it on TSH release both *in vivo* after its 3V injection in conscious, freely moving rats and *in vitro* after its incubation with anterior pituitaries of euthyroid, hypothyroid, and hyperthyroid rats. The results indicate that NB has powerful, physiologically significant direct actions on the release of TSH that vary depending on the thyroid status of the animal. On the basis of these results, we propose that NB be renamed thyromodulin.

MATERIALS AND METHODS

Male Sprague–Dawley rats ranging in weight from 220 to 250 g were purchased from Simonsen Laboratories (Gilroy, CA). The animals were housed in group cages in a room with controlled lighting (on from 0500 to 1900) and temperature (22°C–24°C). They had free access to rat chow and water.

Hyperthyroidism was induced by giving the animals subcutaneous injections of thyroxine (T_4) (10 μg per 100 g of body weight) daily for 5 days. The control animals were given daily injections of 0.9% NaCl (saline). Hypothyroidism was induced by giving the animals 0.05% propylthiouracil in the drinking water for 3 weeks. Control animals drank tap water only.

***In Vivo* Studies.** A stainless steel cannula (23 gauge) was implanted into the 3V as described (4) and the rats were housed in single cages thereafter. Five to 8 days after implantation of the cannula, a jugular catheter was implanted (5). The next morning, extension tubing was attached to the jugular catheter and the animals were left undisturbed for 1 hr, after which a basal heparinized blood sample was col-

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Abbreviations: NB, neuromedin B; TSH, thyrotropin; 3V, third ventricle; aNB, anti-NB antiserum; NRS, normal rabbit serum; T_4, thyroxine; T_3, triiodothyronine.

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Table 1. Plasma hormone concentrations in rats in different thyroid states

Rats	T_4, μg/dl	T_3, ng/dl	TSH, ng per ml
Euthyroid	3.13 ± 0.18	42.3 ± 4.33	222 ± 24
Hypothyroid	1.2 ± 0.13	6.0 ± 0.55	4427.5 ± 342
Hyperthyroid	>6.0 ± 0.01	73.0 ± 0.55	25.3 ± 3.85

lected, and a solution of 0.5 μg of NB in 2 μl of saline or an equal volume of saline in control animals was injected into the 3V. NB was purchased from Peninsula Laboratories. Blood samples were obtained 15, 30, 60, 120, and 180 min after the injection. After removal of each blood sample (0.5 ml), an equal volume of a 40% suspension of washed rat erythrocytes in saline was injected to maintain blood volume.

In another series of experiments, 2 μl of the highly specific anti-NB antiserum (aNB) used previously (3), diluted 1:10 with saline, was injected into the 3V of conscious rats. Since the antiserum was raised in rabbits, control animals were injected with 2 μl of normal rabbit serum (NRS) diluted 1:10 with saline. Blood samples (0.5 ml) were taken just before and at hourly intervals for 6 hr after intraventricular injections of antiserum or NRS. After the last blood sample was drawn (6 hr), the rats were again injected intraventricularly with the same dose of aNB or NRS and 18 hr later a final blood sample was obtained. All blood samples were centrifuged at low speed and plasma was stored frozen until hormone assays were performed.

***In Vitro* Studies.** For *in vitro* studies, the anterior pituitaries were cut longitudinally into halves and preincubated for 1 hr in Krebs–Ringer bicarbonate medium (pH 7.4) at 37°C in an atmosphere of 95% oxygen/5% CO_2 in a Dubnoff metabolic shaker. After removal of preincubation medium, the hemipituitaries were resuspended in 1 ml of medium alone or medium containing NB at final concentrations of 10^{-11} to 10^{-7} M.

Radioimmunoassay. TSH was determined with kits supplied by the National Institute of Diabetes, Digestive and Kidney Diseases and is expressed in terms of the reference preparation (RP2) provided. Triiodothyronine (T_3) and T_4 were measured by RIA as described by Taurog and coworkers (6).

Statistics. Analysis of variance with repeated measures followed by the Student–Newman–Keul multiple comparison test was used for assessment of significance. Student's *t* test was used for comparison of two means.

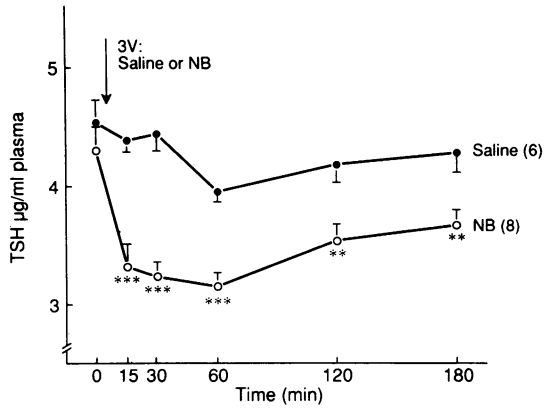

FIG. 1. Effect of 3V microinjection of NB on plasma TSH in hypothyroid rats. Values in this and subsequent figures are means ± SEM. Numbers in parentheses equal number of rats per group. **, $P < 0.01$; ***, $P < 0.001$ vs. control at this time point.

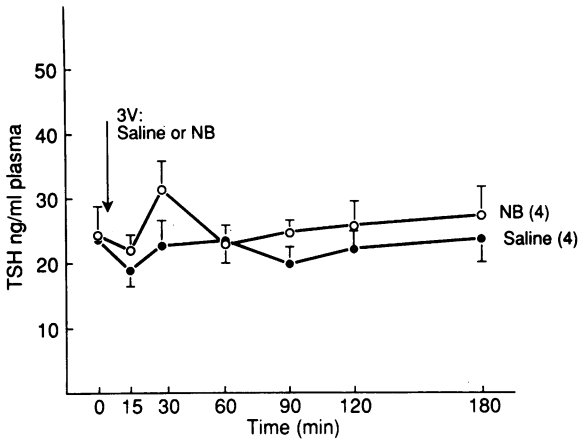

FIG. 2. Effect of 3V microinjection of NB on plasma TSH in hyperthyroid rats.

RESULTS

***In Vivo* Studies.** *Effect of intraventricular injection of NB on TSH release.* (*i*) Hypothyroid rats. As expected plasma TSH was almost 20-fold higher than in euthyroid rats (3) and levels of T_4 and T_3 were markedly lower (Table 1). Microinjection of the saline diluent into the 3V produced no significant changes in plasma TSH during the 3-hr duration of the experiments (Fig. 1). In contrast, 3V injection of 0.5 μg (0.44 nmol) of NB induced a large decline below initial values in levels of plasma TSH at 15, 30, and 60 min ($P < 0.001$). At 120 and 180 min, the levels of plasma TSH had increased significantly but were still significantly lowered ($P < 0.01$).

(*ii*) Hyperthyroid rats. Plasma TSH levels were greatly reduced below values of euthyroid rats, whereas T_4 and T_3 were elevated above values of euthyroid rats (Table 1). The injection of the saline diluent into the 3V produced no significant changes in plasma TSH during the 3-hr experiment (Fig. 2). In contrast to the inhibition of TSH secretion found in hypothyroid rats, the injection of 0.5 μg of NB produced no significant changes in plasma TSH at any time during the experiment in hyperthyroid rats.

Effect of 3V injection of aNB on plasma TSH levels. (*i*) Hypothyroid rats. In rats injected with NRS (2 μl; 1:10 dilution), levels of TSH did not change during the first 6 hr postinjection or 18 hr after the second injection of NRS given 6 hr after the first injection (Fig. 3). After 3V injection of aNB (2 μl; 1:10 dilution), there was a slight decline in the level of plasma TSH only at 5 and 6 hr. These values were statistically different ($P < 0.05$) from NRS-injected rats at these times but not from the initial values. Eighteen hours after the second

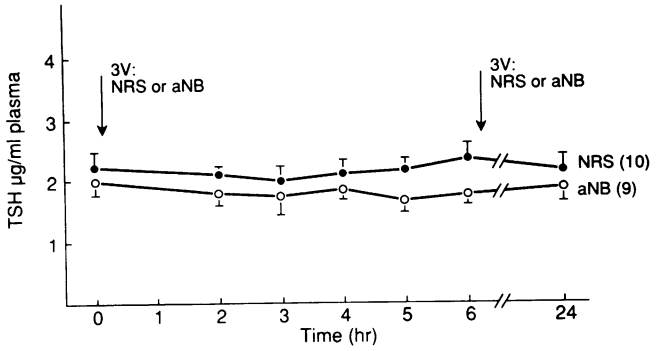

FIG. 3. Effect of 3V microinjection of aNB or NRS on plasma TSH in hypothyroid rats. Note that a second injection was given 6 hr after the first, and plasma levels of TSH were determined 0–6 hr and 24 hr after the first injection.

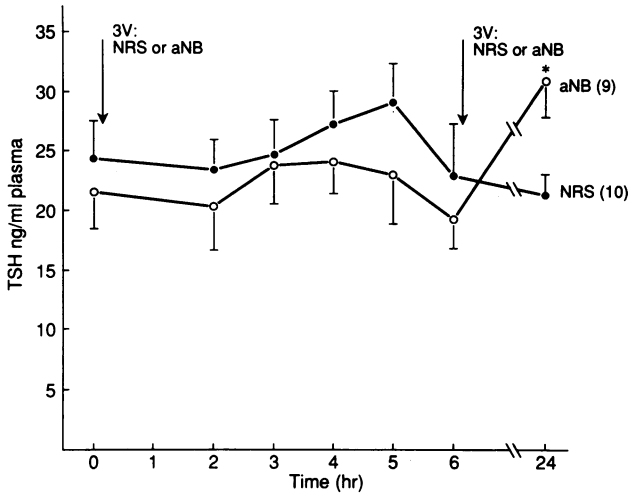

FIG. 4. Effect of 3V microinjection of NRS or aNB on plasma TSH in hyperthyroid rats. Protocol was the same as with hypothyroid rats in Fig. 3. *, $P < 0.05$ vs. control.

injection of aNB, plasma levels of TSH were not significantly different from those at time 0 or from those of NRS-injected rats.

(*ii*) Hyperthyroid rats. In rats injected into the 3V with NRS, the levels of TSH did not change significantly during the duration of the experiment (Fig. 4). After aNB injection into the 3V, the plasma levels of TSH did not change for the first 6 hr but, after the second injection of aNB, the levels of TSH were significantly higher ($P < 0.05$) than the initial values 24 hr after the first injection. They were also significantly increased above the values in NRS-injected animals at this time.

***In Vitro* Studies.** *Effect of NB on basal TSH release by hemipituitaries.* (*i*) Euthyroid status. Increasing concentrations of NB produced a decrease of basal TSH release that became significant at a concentration of 10^{-7} M (Fig. 5). This concentration of NB produced a 60% reduction in TSH release.

(*ii*) Hypothyroid status. Basal TSH release from pituitaries of hypothyroid rats was not significantly altered from that observed in euthyroid glands. NB produced an inhibition of basal TSH release from hypothyroid glands that became significant at NB concentrations of 10^{-9} and 10^{-7} M (Fig. 6). These two concentrations produced 38% and 54% reductions in TSH release, respectively.

(*iii*) Hyperthyroid status. Basal TSH release from glands of hyperthyroid rats was diminished below that of eu- or hypothyroid glands. In contrast to the previous results in eu- and hypothyroid rats, NB produced a significant increase of basal

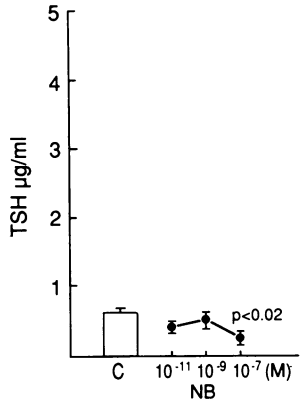

FIG. 5. Effect of NB on TSH release from pituitaries of euthyroid rats. There were seven pituitaries in each group. *P* value is vs. control release.

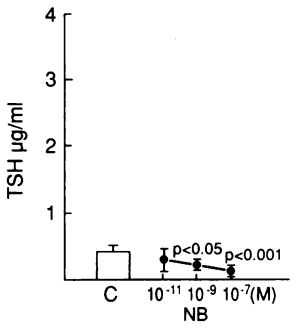

FIG. 6. Effect of NB on TSH release from hemipituitaries of hypothyroid rats. There were eight hemipituitaries per group; otherwise the format is the same as in Fig. 5.

TSH release at all three concentrations (10^{-11}–10^{-7} M) (Fig. 7). The slope of the response curve was significantly negative.

Effect of aNB on TSH release by hemipituitaries. (*i*) Euthyroid status. NRS incubated with control pituitaries at the same dilutions as aNB did not modify the basal release of TSH (data not shown). The addition of aNB at 1:500 and 1:2000 dilutions for 1 and 2 hr produced a highly significant increase in TSH release as compared to the release of the NRS-incubated group (Fig. 8). There was no significant difference between the response to the two dilutions of aNB. The basal TSH release was approximately doubled at 2 hr as compared to the 1-hr incubation but the increase of TSH release in the presence of aNB was proportionally similar after the first or second hour of incubation (Fig. 8). Therefore, in all subsequent experiments we used aNB and NRS at a 1:2000 dilution.

(*ii*) Hypothyroid status. In this thyroid state, in contrast to the euthyroid state (Fig. 9 *Left*), aNB did not modify the basal release of TSH as compared to that of the NRS-incubated control group (Fig. 9 *Center*).

(*iii*) Hyperthyroid status. In this thyroid state, aNB induced a dramatic increase in basal TSH secretion as compared to TSH release from pituitaries incubated with NRS (Fig. 9 *Right*).

DISCUSSION

For greater clarity, we shall discuss the results in euthyroid rats obtained in this and our previous paper (3) first and then compare and contrast the results in altered thyroid states with those in euthyroid animals. In previous work, we showed that 3V injection of NB lowered plasma TSH in conscious, euthyroid rats. The physiological significance of the peptide was indicated by the elevation in plasma TSH that followed intraventricular injection of highly specific antiserum directed against the peptide.

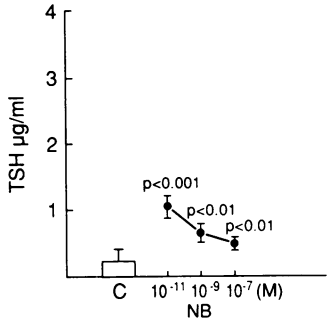

FIG. 7. Effect of NB on TSH release from hemipituitaries of hyperthyroid rats. There were eight glands per group.

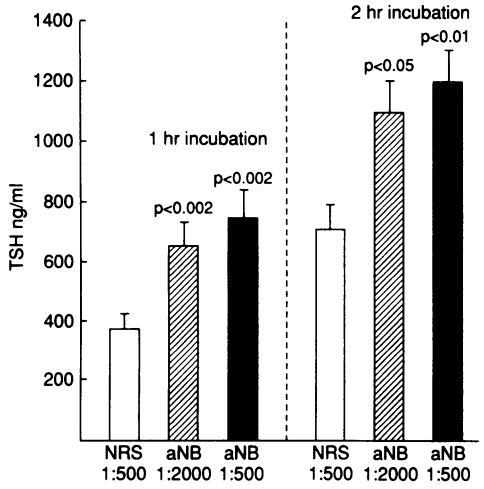

FIG. 8. Effect of incubation of pituitaries with diluted aNB or similarly diluted NRS on TSH release from hemipituitaries incubated for 1 hr (*Left*) or 2 hr (*Right*). There were eight hemipituitaries per group and *P* values are vs. the NRS-incubated control.

In the present work, we further evaluated the *in vitro* actions of the peptide in hemipituitaries from euthyroid rats incubated *in vitro* and found as before that the peptide also directly inhibited TSH release *in vitro*. NB exercises a physiologically significant suppressive effect on TSH release from the pituitaries of euthyroid animals *in vitro* since incubation with highly specific antiserum against NB produced a stimulation of TSH release that persisted for 2 hr. Thus, in the euthyroid animal NB has a physiologically significant inhibitory action on TSH release. Since the action is manifested directly on the anterior pituitary *in vitro*, we conclude that NB is a physiologically significant TSH release-inhibiting factor and we propose to rename it thyromedin (Fig. 10).

In hypothyroid as in euthyroid animals, NB had a pronounced inhibitory effect on the release of TSH when injected intracerebroventricularly in the same dose as in euthyroid rats. The percentage decrease in plasma TSH was lower in hypothyroid rats (25%) than was previously found in euthyroid rats (40%) (3); however, in view of the massive, 20-fold increase in plasma TSH concentrations, the result of decreased negative feedback from thyroid hormones (7), the decrease in the quantity of TSH released is estimated to be at least 15 times greater in hypothyroid than in euthyroid rats. The release of TSH was also suppressed more effectively by NB *in vitro* than from hemipituitaries of euthyroid animals. In hypothyroid rats, there is a reduced concentration of not only

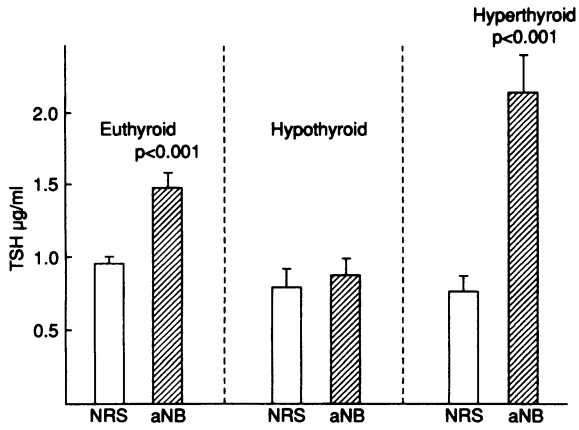

FIG. 9. Effect of aNB on TSH release from hemipituitaries *in vitro*. Glands were incubated for 1 hr in either NRS or aNB at 1:2000 dilution. There were seven or eight hemipituitaries per group.

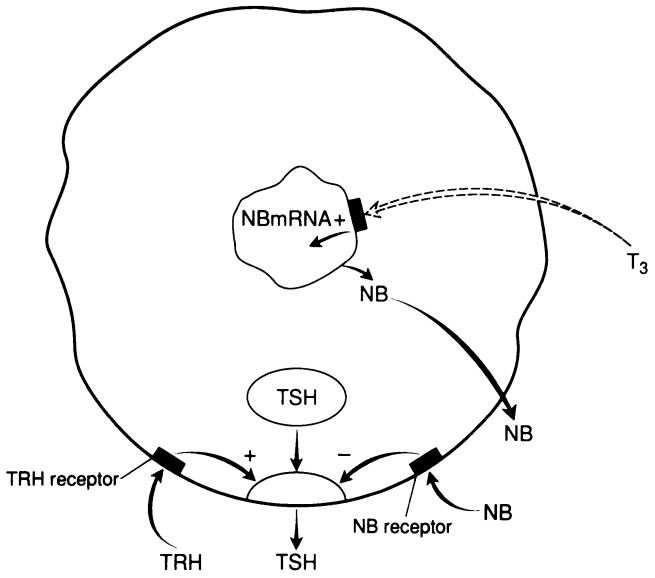

FIG. 10. Proposed action of NB on thyrotropes. For details, see text.

TSH but also NB in the pituitary gland (2). Therefore, we postulate that the pronounced inhibitory action of NB on TSH secretion *in vivo* and *in vitro* in the hypothyroid state is the result of increased responsiveness to NB. The decreased stored NB in the hypothyroid gland may lead to decreased release of endogenous NB resulting in upregulation of putative NB receptors on the surface of the thyrotropes. In contrast to the elevation of TSH release observed *in vivo* and *in vitro* after treatment with aNB in the euthyroid animal, there was virtually no effect of the antiserum either *in vivo* or *in vitro* in hypothyroid animals, suggesting that the peptide has little physiologic significance under this condition, presumably because of its reduced release as suggested above. The reduced quantity of NB released from the thyrotropes may be insufficient to inhibit TSH release *in vivo* or *in vitro*.

In the opposite state of thyroid function—namely, hyperthyroidism—the excess thyroid hormone feeds back at hypothalamic and pituitary levels to suppress the release of TSH (7). This is accompanied by an increase in NB concentration in the anterior pituitary (2). We postulate that the increased NB stored in the gland is also associated with increased release of the peptide, which then acts to suppress TSH release. We found no further lowering of TSH after intraventricular injection of NB, suggesting that the action of the peptide is already maximal under these conditions. Conversely, when the antiserum was injected into the 3V there was a delayed increase in plasma TSH manifest within 24 hr of the initial injection. This suggests that the TSH suppressive action of NB in the hyperthyroid animal is physiologically significant. The delay in the elevation of plasma TSH after injection of antiserum against the peptide may be related to the fact that the quantity of antiserum injected initially was not sufficient to immunoneutralize the increased levels of endogenous NB. After the second injection, these levels were significantly antagonized and, consequently, TSH levels increased. The physiological significance of NB in the hyperthyroid rat is further shown by the marked increase in TSH release in the presence of aNB from pituitaries of these animals. Thus, NB has a physiologically significant TSH release-inhibiting action at the pituitary level in the hyperthyroid as in the euthyroid rat.

The most puzzling result was the paradoxical dose-related elevation of TSH release from the anterior pituitaries of hyperthyroid rats *in vitro* induced by NB with a negative slope. We have no explanation for this except to draw a

parallel between this alteration in response to NB and the altered response of luteinizing hormone to various peptides when plasma gonadal steroid concentrations are altered. For example, neuropeptide Y, which inhibits luteinizing hormone release in the ovariectomized rat, stimulates it in the estrogen-primed animal (8). The altered responses to NB may be related to alterations in the receptors for NB induced by altered thyroid states.

In conclusion, in the euthyroid animal NB acts in an autocrine fashion to suppress TSH release from the thyrotropes directly (Fig. 10). In hypothyroidism, NB synthesis and presumably release from the pituitary is dramatically decreased, as evidenced by dramatic decreases in neuromedin mRNA (P. M. Jones, personal communication) and pituitary NB concentrations (2). The responsiveness to the inhibitory action of the peptide is increased, probably via upregulation of its postulated receptors on the thyrotrope induced by decreased release of the peptide. In the absence of appreciable release of the peptide, it has no significant inhibitory action on TSH release in this thyroid state. In hyperthyroidism, the concentration of NB in the thyrotropes, and presumably its release, is increased, so that it has a physiologically significant TSH release-inhibiting action.

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1. Minamino, N., Kangawa, K. & Matsuo, H. (1983) *Biochem. Biophys. Res. Commun.* **114,** 541–548.
2. Steel, J. H., Van Noorden, S., Ballesta, J., Gibson, S. J., Ghatei, M. A., Burrin, J., Leonhardt, U., Domin, J., Bloom, S. R. & Polak, J. M. (1988) *Endocrinology* **122,** 270–282.
3. Rettori, V., Milenkovic, L., Fahim, A. M., Polak, J., Bloom, S. R. & McCann, S. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4789–4792.
4. Antunes-Rodrigues, J. & McCann, S. M. (1970) *Proc. Soc. Exp. Biol. Med.* **133,** 1464–1470.
5. Harms, P. & Ojeda, S. R. (1974) *J. Appl. Physiol.* **36,** 391–392.
6. Regard, E., Taurog, A. & Nakashima, T. (1978) *Endocrinology* **102,** 674–684.
7. Reichlin, S. (1985) in *Williams Textbook of Endocrinology*, eds. Wilson, J. & Foster, D. W. (Saunders, Philadelphia), pp. 492–567.
8. McCann, S. M., Rettori, V., Milenkovic, L., Riedel, M., Aguila, M. C. & McDonald, J. K. (1989) in *Nobel Conference on NPY*, eds. Mutt, V., Hokfelt, T. & Fuxe, K. (Raven, New York), pp. 215–227.