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Impaired serum thyrotropin response to hypothyroidism in mice with disruption of neuromedin B receptor

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Abstract

Neuromedin B (NB), a neuropeptide highly concentrated in pituitary, has been proposed to be an inhibitor of thyrotropin (TSH) secretion. Previous study showed that mice with disruption of neuromedin B receptor (NBR-KO) have higher TSH release in response to thyrotropin-releasing hormone (TRH), although TSH seems to have decreased bioactivity. Here we examined in NBR-KO mice the response of TSH to thyroid hormone (TH) deprivation, obtained by methimazole treatment, or excess, obtained by acute and chronic TH administration. In response to hypothyroidism NBR-KO mice exhibited a lower magnitude increase in serum TSH compared to wild-type (WT) mice (1.7 *vs.* 3.3-times increase compared to euthyroid values, respectively, $P<0.001$). One hour after a single T4 injection (0.4µg/100 g BW), WT and NBR-KO hypothyroid mice presented similar degree of serum TSH reduction (54%, $P<0.05$). However, 3 h after T4 administration, WT mice presented serum TSH similar to hypothyroid baseline, while NBR-KO mice still had decreased serum TSH (30% reduced in comparison to hypothyroid baseline $P<0.05$). T3 treatment of euthyroid mice for 21 days, with progressively increasing doses, significantly reduced serum TSH similarly in WT and NBR-KO mice. Also, serum T4 exhibited the same degree of suppression in WT and NBR-KO. In conclusion, disruption of neuromedin B receptor did not interfere with the sensitivity of thyroid hormone-mediated suppression of TSH release, but impaired the ability of thyrotroph to increase serum TSH in hypothyroidism, which highlights the importance of NB in modulating the set point of the hypothalamus–pituitary–thyroid axis at hypothyroidism.
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1. Introduction

The set point of thyrotropin (TSH) secretion is determined mainly by the balance of the stimulatory effect of hypothalamic thyrotropin-releasing hormone (TRH) and the potent inhibition exerted by thyroid hormones. However, other modulators of TSH secretion have been described, including neuromedin B (NB). NB is a bombesin-like peptide [1] that has several biological actions [2]. NB and its receptor (NBR) have a wide tissue distribution, with predominant expression at the central nervous system [3] and gastrointestinal cells [4]. NB is also highly expressed in humans, rat and mouse pituitary [5–8] and in rats, the pituitary is the tissue with the highest NB content [5], as a result of local synthesis [6].

NB has an inhibitory action on TSH secretion, demonstrated both in pituitary explants and after a single administration into normal and hypothyroid rats [9–11]. Endogenously produced-NB exerts a tonic inhibitory effect on TSH secretion, as demonstrated by experiments employing a specific antiserum, which induced a rise in TSH secretion, not only in animal experiments but also when incubated with isolated pituitaries

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[11,12]. In addition, pituitary NB expression is up-regulated by thyroid hormones and down-regulated by TRH [6,13,14]. Taken together, the data led us to propose that NB is a constitutive inhibitor of TSH release, acting mainly as autocrine/paracrine factor, and that the control of its pituitary expression by TSH secretagogues or TSH release inhibitors may serve to modulate the final action of these hormones [15].

Recently we studied the thyrotroph axis of mice that has a disruption of neuromedin B receptor (NBR-KO) and we were able to demonstrate that TSH inhibitory action of NB is mediated by this receptor. NBR-KO mice presented a facilitated TSH release, at basal state, with serum TSH slightly elevated, and more importantly, after TRH, which is consistent with the proposed role for NB on TSH release. However, NBR-KO mice revealed that neuromedin B may be involved in other aspects of thyrotroph axis function, since they showed reduced serum T3 and evidence of a less-bioactive TSH [8]. In the present study, we further investigated the thyrotroph axis of NBR-KO mice focusing in the regulation of TSH by thyroid hormones deficiency or excess.

2. Materials and methods

2.1. Experimental animals

All experiments were conducted in adult male mice, at 3–4 months old, homozygous for the deletion in NB-R (NBR-KO) and wild-type littermates. Heterozygous NB-R+/− mice generated as described previously [16], were interbred to generate litters containing homozygous NB-R−/− and NB-R+/+ progeny. To confirm the genotype of the mice, genomic DNA was obtained from tail samples and analyzed by polymerase chain reaction (PCR) using specific primers as described previously [16].

Animals were maintained under controlled temperature (22 °C ± 1 °C) and 12 h alternating darkness and artificial light cycles (light on at 7 am) and fed laboratory chow and water *ad libitum*. All animals were sacrificed in the morning between 9:30 and 11:30 am. In all experiments body weight of NBR-KO mice was similar to wild-type (25–28 g).

All procedures were performed in accordance with the Fund for the Replacement of Animals in Medical Experiments Guide for the care and use of laboratory animals and protocols were approved by the Institutional Committee on Animal Care and Use.

2.2. Experimental hypothyroidism

Hypothyroidism was induced by treatment with 0.1% methimazole (2-mercapto-1-methylimidazole, Sigma) in the drinking water for 28 days. Twenty-four hours before starting the treatment, blood from the tail vein was obtained for measuring baseline euthyroid serum TSH. Mice were sacrificed by CO_2 inhalation, followed by decapitation and trunk blood was collected for TSH evaluation.

2.3. Evaluation of acute TSH response to T4

In order to evaluate the acute effect of thyroid hormones on TSH secretion, we used T4 since this hormone has a better physiological correlation with serum TSH than T3 [17]. Hypothyroid mice produced as described before, were divided in two groups that received a single subcutaneous injection of T4 (thyroxine, Sigma) 0.4 µg/100 g BW, 1 or 3 h before sacrifice. We employed a low T4 dose in order to be able to better discriminate the T4 suppressive effect on serum TSH in the two groups of animals. Twenty-four hours before T4 injection, a sample of blood from the tail vein was obtained for TSH baseline hypothyroid measurements. After sacrifice as described before, trunk blood was collected for TSH measurement.

2.4. Evaluation of TSH suppression by T3 chronic treatment

The ability of thyrotroph to respond to T3 (triiodothyronine) reducing TSH secretion was tested by a protocol of progressive increasing doses of T3, previously described [18]. By using T3 treatment we were able to measure TSH suppression and the consequent T4 reduction. Euthyroid mice received daily intraperitoneal injections of T3 (Sigma) for 3 weeks at doses of 0.2 µg/100 g BW during the first week, 0.5 µg/100 g BW during the second week, and 1.0 µg/100 g BW during the third week. Twenty-four hours before starting T3 administration, a blood sample from the tail vein was obtained for total T4 baseline measurements. During T3 treatment, tail vein blood samples were obtained at weekly intervals for T4 measurements. Experiments were terminated 24 h after the last T3 injection. Mice were sacrificed by CO_2 inhalation, followed by decapitation and trunk blood was collected for T4 and TSH determinations and their pituitaries were taken and frozen until processed to measure TSH content.

2.5. Hormone measurements

In the experiments described previously, tail and trunk blood samples were centrifuged, and serum was stored at −20 °C for T4 and TSH determinations by specific radioimmunoassays. Serum TSH and pituitary TSH content were determined by specific mouse radioimmunoassay using a mouse TSH/LH reference preparation (AFP51718MP), a mouse TSH antiserum (AFP98991), and rat TSH antigen for radioiodination (NIDDK-rTSH-I-9, AFP-11542B), as previously described [8]. All reagents were obtained from Dr. A.F. Parlow at the National Hormone and Peptide Program (Harbor University of California at Los Angeles Medical Center, Torrance, CA). Minimum assay detection was 30 ng/mL. The intra-assay variations were less than 7%. Serum total T4 concentration was measured by coated tubes radioimmunoassay from ICN Pharmaceuticals (Costa Mesa, CA) using 25 µl of serum. The sensitivity of assay is 0.76 µg/dl. The intra-assay variation was 5.1%.

2.6. Statistical analysis

Data are expressed as mean ± SEM. Data differences were assessed by two-way ANOVA followed by post-hoc tests to evaluate difference between groups employing software GraphPad Prism and Graph Pad Calculator (GraphPad Software, Inc., San Diego, CA). TSH intrapituitary content was analyzed by a two-tailed unpaired *t*-test for assessment of significance. Serum

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