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Analysis of the responsible region of TRH in the hypothalamus-pituitary-thyroid axis using the Paraventricular nucleus-specific TRH deficient mice.

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Objectives: Thyrotropin-releasing hormone (TRH) was the first hypothalamic hormone isolated that stimulates pituitary thyroid-stimulating hormone (TSH) secretion. TRH was later found to be distributed throughout the brain, gastrointestinal tract and pancreaticβ-cells. We previously reported the TRH null mice (conventional TRHKO) which exhibit characteristic tertiary hypothyroidism, however the responsible regionfor the hypothalamic-pituitary-thyroid (HPT) axis remained obscure because of the broad expression of TRH in hypothalamus. Previous studies suggested that TRH in the hypothalamic paraventricular nucleus (PVN) was secreted through the median eminence (ME), however it was not directly demonstrated . To determine the region functionally responsible for the HPT axis, we established and analyzed PVN-specific TRH knock-out (PVN-TRHKO) mice. **Methods:** For the targeting vector, a Neo-loxP cassette was inserted into the 3′-UTR of the Trh gene in ES cells. Another loxP site was placed at intron 1. After we obtained the Trh +/lox [neo+] founder mice, we crossed them with the CAG-FLPe transgenic mice to remove the Neo cassette. The offspring were backcrossed to wild-type mice, producing conditional knock-out mice without the Neo cassette, which were labeled as Trh +/lox . We crossed Trh +/lox with transgenic mice expressing Single-minded homolog (Sim)1-Cre recombinase and created PVN-TRHKO mice (Sim1-Cre; Trh lox/lox mice). We confirmed that most Sim1 was expressed in the PVN using Sim1-Cre/tdTomato mice. We performed the following experiments using PVN-TRHKO andTrh lox/lox mice as control (Ct). 1) immunostaining of TRH using antibody forproTRH in cerebrum, 2)qPCR analysis detecting the expression of preproTrh mRNA in hypothalamic PVN, 3) measurement of the level of serum free-T4 and TSH, 4) qPCR analysis detecting theexpression levels of Tshβ, Prl, Cga, Gh, Pomc and Lhβ gene mRNAin the anterior pituitary and 5)immunohistochemical analysis with antibodies for TSHβ and Prolactin in anterior pituitary. **Results:** In the PVN-TRHKO mice, 1) ProTRH-immunopositive cells were lost in the area of PVN and ME, whereas proTRH detected in the medial preoptic area(MPA) and medial preoptic nucleus (MPO) in the hypothalamus.2)The expression level of proTRHmRNA was decreased to7.7% compared with Ct.3) Free-T4 levels decreased to 50% and the TSH levels increased to 170% of those in Ct. 4) TshβmRNA levels were decreased by 65% and thePrlmRNA levels were reduced to 55% of those in Ct. 5) The number of TSH-positive andProlactin-positive cells in the pituitary significantly decreased to 50% and 60% of those in Ct. **Conclusions:** PVN-TRHKO mice exhibited tertiary hypothyroidism similar to conventional TRHKO. The pathway of TRH neuron from PVN to ME was lost in the PVN-TRHKO mice. These findings are conclusive evidence that the TRH neuron in the PVN is the center of the HPT axis.

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