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TRH Receptor Type 2 Deficient Mice are Euthyroid and Exhibit Increased Depression and Reduced Anxiety Phenotypes

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Abstract

Thyrotropin-releasing hormone (TRH) is a neuropeptide that initiates its effects in mice by interacting with two G protein-coupled receptors, TRH receptor type 1 (TRH-R1) and TRH receptor type 2 (TRH-R2). Two previous reports described the effects of deleting TRH-R1 in mice. TRH-R1 knockout mice exhibit hypothyroidism, hyperglycemia, and increased depression and anxiety-like behavior. Here we report the generation of TRH-R2 knockout mice. The phenotype of these mice was characterized using gross and histological analyses along with blood hematological assays and chemistries. Standard metabolic tests to assess glucose and insulin tolerance were performed. Behavioral testing included elevated plus maze, open field, tail suspension, forced swim and novelty-induced hypophagia tests. TRH-R2 knockout mice are euthyroid with normal basal and TRH-stimulated serum levels of thyroid-stimulating hormone (TSH, thyrotropin), are normoglycemic and exhibit normal development and growth. Female, but not male, TRH-R2 knockout mice exhibit moderately increased depression-like and reduced anxiety-like phenotypes. Because the behavioral changes in TRH-R1 knockout mice may have been caused secondarily by their hypothyroidism whereas TRH-R2 knockout mice are euthyroid, these data provide the first evidence for the involvement of the TRH/TRH-R system, specifically extrahypothalamic TRH/TRH-R2, in regulating mood and affect.

Keywords

Thyrotropin-releasing hormone; Thyrotropin-releasing hormone receptors; Depression; Anxiety; Extrahypothalamic TRH; Behavioral changes; Neurotransmitter; Neuromodulator; TRH-R2-deficient mouse

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INTRODUCTION

Thyrotropin-releasing hormone (TRH) is a tripeptide agonist that initiates its effects by activating two G protein-coupled receptors, TRH receptor type 1 (TRH-R1) and TRH receptor type 2 (TRH-R2) (see (Sun *et al*, 2003) for review). Activation of these receptors appears to initiate a number of effects within the central nervous system. TRH receptors are expressed in the anterior pituitary and respond to hypothalamic TRH by activating the hypothalamic-pituitary-thyroid (HPT) axis and consequently regulating metabolism. TRH and its corresponding receptors are also expressed extrahypothalamically in various brain regions including the limbic system and cortex. Because of its presence outside of the HPT axis, TRH is also considered a neurotransmitter or neuromodulator (Gary *et al*, 2003). The function of extrahypothalamic TRH has been inferred from pharmacological studies using TRH and its analogues showing a wide array of actions including arousal, increased locomotor activity, antidepressant and anxiolytic effects (Pekary *et al*, 2005; Sattin, 1999; Gutierrez-Mariscal *et al*, 2008).

Studies have linked TRH and its receptors to psychiatric disorders. Hypothyroidism causes depression, loss of energy and weight gain and within the HPT axis, a number of subtle abnormalities have been found in individuals with major depression including blunted TSH response to TRH and absence of nocturnal TSH surge (Bartalena *et al*, 1990). Also, decreased *TRH* gene expression was observed in the hypothalamus of depressed patients (Alkemade *et al*, 2003). TRH and its receptors in the limbic system and cortex are believed to mediate the endogenous and exogenous effect TRH on affect, mood and arousal (Gary *et al*, 2003; Yarbrough *et al*, 2007) but it has been notoriously difficult to separate the hypothalamic and extrahypothalamic functions of TRH and to assign specific functions and behaviors to extrahypothalamic TRH.

TRH-R1 was initially cloned from a mouse pituitary tumor cDNA library (Straub *et al*, 1990) and then orthologous receptors were cloned from a number of different species, including rat (de la Pena *et al*, 1992; Zhao *et al*, 1992; Sellar *et al*, 1993), chicken (Sun *et al*, 1998), cow (Takata *et al*, 1998), *Catostomus commersoni* (Harder *et al*, 2001) and humans (Duthie *et al*, 1993; Matre *et al*, 1993). The human TRH-R1 is 90% and 89% homologous to the mouse and rat receptors at the DNA level, respectively; the three receptors exhibit approximately 95% homology at the amino acid level. TRH-R2 was identified in rat (Cao *et al*, 1998; Itadani *et al*, 1998; O'Dowd *et al*, 2000), mouse (Harder *et al*, 2001) and *Catostomus commersoni* (Harder *et al*, 2001); TRH-R2 has not been described in humans. Amino acid sequence alignments of the two types of TRH receptors from the same species reveal a 50% overall identity. TRH-R1 and TRH-R2 bind TRH with equal affinities but exhibit a different distribution of expression in rats. In particular, TRH-R1 is expressed at high levels whereas TRH-R2 is not expressed or expressed at very low levels within the anterior pituitary gland, and the distribution of their expression within the rat brain is mostly non-overlapping. Of note, TRH-R2 is expressed within the limbic system (O'Dowd *et al*, 2000).

Two previous reports described the generation and characterization of TRH-R1-deficient mice (Rabeler *et al*, 2004; Zeng *et al*, 2007). TRH-R1-deficient mice exhibit "tertiary

hypothyroidism" with low levels of thyroid hormones, and mild hyperglycemia. Zeng *et al.* found that the TRH-R1-deficient mice they generated, in contrast to those generated by Rabeler *et al.*, did not exhibit developmental abnormalities but displayed increased depression-like and increased anxiety-like behaviors. Because the mice studied by Zeng *et al.* were hypothyroid, it is not possible to determine whether the depression- and anxiety-like signs were caused by TRH-R1 deficiency outside of the HPT axis or the changes associated with tertiary hypothyroidism. TRH-deficient mice have been generated and characterized also (Yamada *et al*, 1997). Similar to TRH-R1-deficient mice, TRH-deficient mice are hypothyroid and hyperglycemic. To our knowledge, no behavioral studies of TRH-deficient mice have been reported.

Herein we report the generation and characterization of TRH-R2 knockout mice. We show that TRH-R2-deficient mice are euthyroid with normal thyroxine and normal basal and TRH-stimulated serum levels of thyroid-stimulating hormone (TSH, thyrotropin), are normoglycemic and exhibit normal development and growth. Interestingly, female, but not male, TRH-R2 knockout mice exhibit moderately increased depression-like and reduced anxiety-like phenotypes.

MATERIALS AND METHODS

Construction of targeting vector and generation of TRH-R2 knock-out mice

Genomic clones spanning the entire mouse TRH-R2 gene were isolated by screening a BAC mouse genomic library (129/SvJ, Bac Mouse I PCR library screening service, Genome Systems Inc., St. Louis, Missouri) with primers specific for the third exon (sense primer: 5'-TTTCCTGGACCCCTGGGGTCCTGCTG-3'; anti-sense primer: 5'-GGGAAGCAGCTGCTGCTGGACCTGAGAC-3'). The clones were characterized in detail by standard molecular biology techniques. A 13 kb *Bgl II-Bgl II* DNA fragment including the TRH-R2 gene missing the first exon and the first 130 bp of the first intron were subcloned into the *Bam*H I site of the pBluescript SK II (-) vector (Stratagene). A 1243 bp DNA fragment including the second exon and the second intron was subcloned into the *Eco*R I-*Bam*H I sites of the pLoxpneo vector (Yang *et al*, 1998). The resulting construct was cleaved with *Hpa* I and *Not* I followed by insertion of a 9 kb DNA fragment which is 3' to the third exon of the TRH-R2 gene. The replacement vector finally contained 10.2 kilobases of homologous genomic DNA in which the third exon of the TRH-R2 gene was removed and replaced by a loxp-Neo-loxp cassette. The finished construct, *p*TRH-R2*neo*, is shown in Fig. 1.

TC1 ES cells (Deng *et al*, 1996) were transfected with *Not* I-digested *pTRH-R2neo* and selected with G418 and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl-5-iodouracil (FIAU). ES cell colonies that were resistant to both G418 and FIAU were analyzed by Southern blotting for homologous recombination events within the TRH-R2 locus. Genomic DNAs from these clones and the parental TC1 cell line were digested with *Eco*R I and *Eco*R V and then probed with a 5'-flanking probe specific to the TRH-R2 gene. The 5' probe is a 416 bp *Sma* I-*Sma* I fragment. Homologous recombinant cell clones were identified by Southern blot analysis as described below. These ES cells were injected into 3.5 day old 129/Svev blastocysts that were subsequently implanted into pseudopregnant recipients. The resulting

chimeras were mated with 129/Svev females and germline transmission of the mutant allele was identified by Southern blot analysis. The mutant mice were backcrossed 4 times to the 129 background.

Genotype determination

For DNA preparation, ES cells or mouse tails were incubated overnight at 55°C in extraction buffer (100 mM Tris·HCl pH 8.5, 200 mM NaCl, 5 mM EDTA, 0.2% SDS, 200 μg/ml Proteinase K) followed by 15 min at 80°C.

Southern blot

The DIG High Primer DNA Labeling and Detection Starter Kit II (Roche Molecular Biochemicals, Mannheim, Germany) was used for Southern blot assay. Briefly, DNA (10 μg) was digested with *Eco*RI and *Eco*RV, size separated on a 0.8% agarose gel, and then capillary-transferred to a nylon membrane (Hybond N, Amersham Bioscience, Braunschweig, Germany). The membranes were hybridized using a probe which was a digoxigenin-labeled 416 bp *Sma* I-*Sma* I fragment specific for a region upstream of the third exon of the TRH-R2 gene that was not included in the targeting vector. Signals were analyzed by exposing to X-ray film (Eastman Kodak Company, Rochester, NY).

PCR analysis

PCR was carried out by using the Expand Long Template PCR System (Roche Molecular Biochemicals). The primer pair generating an 800 bp product specific for the wild-type gene was: sense: 5'-GGGCAGCCTTCCTGAAACTGTGCTGGTGCCGGGCAG -3'; anti-sense: 5'-CTTGCACATTCGGGGTCAAGTGGCTTCTTGATGGAG -3'. The primer pair generating a 1200 bp product specific for the neo gene was: sense: 5'-GACGTGACAAATGGAAAGTAGCACGTCTCACTAGTC-3'; anti-sense: 5'-CAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATG-3'.

Quantitative RT-PCR

Total RNA was purified, reverse-transcribed and transcript levels of selected genes were measured by qRT-PCR. In brief, total brain RNA was purified using an RNeasy Mini Kit (Qiagen) and first strand cDNA was prepared using a High Capacity cDNA Archive Kit (Applied Biosystems). PCR was performed in 25 μl reactions in 96-well plates using cDNA prepared from 100 ng of total RNA and Universal PCR Master Mix (Applied Biosystems). Primers and probes were Assay-on-Demand (Applied Biosystems). The Ct values for TRH-R mRNAs were normalized to GAPDH and the levels measured in wild type mice (Ct = 25 for TRH-R1 and TRH-R2) were set at 100%. The levels of TRH-R2 mRNA in TRH-R2-/- mice were undetectable (Ct > 37).

Animal care

All animal protocols used in these studies were approved by the National Institute of Diabetes and Digestive and Kidney Diseases and the Weill Cornell Medical College Animal Care and Use Committees. We housed mice in microisolator cages (Labproducts) in a specific pathogen-free barrier facility with an air shower entrance.

Pathology analysis and blood chemistries

Gross and histological analyses, and blood hematological assays and chemistries were performed by the Office of Research Services/Division of Veterinary Resources, National Institutes of Health.

Metabolic testing

Analysis of growth and glucose/insulin homeostasis including glucose and insulin tolerance tests on normal and high fat diets were performed by the Mouse Metabolism Laboratory of the National Institute of Diabetes and Digestive and Kidney Diseases Intramural Research Program under the direction of Dr. Oksana Gavrilova. Glucose tolerance test: Mice were fasted for 14-16 hours. Sterile glucose (2 g/kg; dissolved in phosphate buffered saline; volume: 0.1-0.2 ml/mouse) was injected at time zero (i.p. glucose tolerance test). Blood samples (20-30 μl) were taken from the tail vein just prior to the injection (0 min), 15, 30, 60 and 120 min after the glucose injection. Blood glucose and insulin levels were determined. Insulin tolerance test: Freely fed mice were injected i.p. with human insulin (0.75 U/kg). Blood samples (20-30 μl) were taken from the tail vein just prior to the injection (0 min) and 15, 30, 60 and 120 min after the insulin injection. Blood glucose levels were measured with Glucometer Elite (Bayer Healthcare).

Thyroxine, TSH and Prolactin

Serum thyroxine was assayed by Gammacoat Total T4 Radioimmunoassay Kit (DiaSorin Inc., Stillwater, MN). Serum TSH levels were measured in blood obtained by retro-orbital sampling before (basal) and 30 min after intraperitoneal injection of TRH (5 μg/kg). TSH was measured by radioimmunoassay using highly purified rat TSH (AFP11542B) for radioiodination, guinea pig anti-TSH antibody (AFP98991) and mouse TSH reference preparation (AFP51718MP). Serum prolactin was assayed using mouse prolactin (AFP10777D) for radioiodination, rabbit anti-mouse prolactin antibody (AFP131078) and mouse prolactin reference standard (AFP6476C). TSH and prolactin measurements were performed by Dr. A.F. Parlow of the NIDDK National Hormone and Pituitary Program.

Behavioral testing

Eight to twelve weeks old TRH-R2+/+ and -/- male and female littermates were tested in elevated plus maze (EPM), open field (OF), tail suspension test (TST), forced swim test (FST) and in the novelty-induced hypophagia test (NIHT). Mice were also tested for locomotor activity (LA) and prepulse inhibition (PPI). Three cohorts were tested in the following order: Cohort 1, (i) EPM, (ii) OF, (iii) LA, (iv) PPI, (v) FST; Cohorts 2 and 3, (i) FST, (ii) TST, (iii) NIHT. EPM, OF, LA and PPI data are not reported because no differences between the groups were found. Only one procedure was conducted per day.

TST

Mice were individually suspended by the tail using adhesive tape for 6 min. The session was videotaped and immobility was scored between 2 and 6 min by a trained observer who was blind to the genotype and male and female data were analyzed separately by *t*-test.

FST

Mice were placed in a clear, water-filled cylinder (diameter, 8 inch; depth, 5 inch) for 6 min (Porsolt *et al.*, 1978) and immobility time of the mice was measured between 2 and 6 min from a recorded videotape. Water temperature was equilibrated to room temperature of 21°C. Mice were tested by an observer who was blind to the genotype of the mice. The male and female data were analyzed separately by *t*-test.

NIHT

The test consists of 3 days of training in the home cage followed by testing in the home cage and then in a novel cage environment. Littermates were single-housed for 3 days before training began. Then, for 3 consecutive days mice were presented with sweetened condensed milk (Carnation) diluted 1 to 3 with water for 30 min each day in LM Animal Farms Quick Quench Universal Water Bottle (5 oz, Petco) essentially as described by Dulawa et al. (Dulawa *et al*, 2004). Home cage testing occurred on day 4 by presenting the milk for 10 min in the dark and the latency to drink and the duration of drinking were recorded (dark or home cage latency and duration). Novel cage testing was on day 5 under bright lighting conditions and by placing the mice into new clean cages of the same dimensions as the home cage but without bedding. Latency to drink and duration of drinking were again recorded (light or novel cage latency and duration). All sessions were videotaped and scored by a trained observer who was blind to the genotype of the mice. Mice were tested only once. Statistical significance in the measures of latency and duration of drinking between TRH-R2+/+ and -/- littermates was established by *t*-test. Males and females were analyzed separately.

RESULTS

Generation of TRH-R2-deficient mice

As depicted in Figure 1a, the targeting vector was designed to delete exon 3 of the TRH-R2 coding region. Exon 3 was replaced by the neomycin-resistance gene. Homologous recombinants of the stably transfected embryonic stem (ES) clones obtained after antibiotic selection were identified by Southern analysis. Male chimeras generated by blastula injection were bred with 129Svev females with successful germ line transmission. The heterozygous mice (TRH-R2+/-) were apparently normal and subsequently gave rise to mice homozygous (TRH-R2-/-) for the mutant TRH-R2 locus with the expected frequency, indicating that the deletion of the TRH-R2 gene did not result in embryonic lethality. The animals were genotyped by Southern blot (Fig. 1b) and by PCR (Fig. 1c) using the probe and primers described in *Experimental Procedures*, respectively. Quantitative RT-PCR of brain RNA showed that TRH-R1 mRNA levels were the same in wild type, heterozygous and homozygous mutants whereas heterozygous mice exhibited 55% of the level of TRH-R2 mRNA found in wild type mice and the knockout mice did not express any TRH-R2 mRNA (Fig. 1d). It is noteworthy that TRH-R2-/- mice exhibited the same level of TRH-R1 as wild type mice even though they did not express TRH-R2.

General characterization of TRH-R2-/- mice

Male and female fertility in the TRH-R2-/- mice appeared normal; litter size was not affected. There was no difference in body weight at birth irrespective of the genotype of the mothers. Wild type and TRH-R2-/-littermate mice of both genders exhibited similar growth rates on normal and high fat diets. Adult TRH-R2-/- mice exhibited no pathology different from wild type littermates. There were no differences in body weights and organ weights of adult wild type and TRH-R2-/- mice. Routine blood chemistries were within normal limits and there were no differences between wild type and TRH-R2-/- mice.

Metabolic testing

On both a normal and high fat diet, both male and female TRH-R2-/- mice exhibited normal glucose and insulin levels (Fig. 2). TRH-R2-/- mice exhibited normal glucose and insulin tolerance tests also (data not shown).

Thyroxine, TSH and Prolactin

Serum thyroxine levels in male and female TRH-R2-/-mice were not different from controls: male wild type, 4.7±0.21; male ko, 4.0±0.20; female wild type, 2.4±0.10; and female ko, 2.60±0.33 μg/dl. Serum TSH levels were not different basally and responded to TRH with a similar increase in TSH in wild type and TRH-R2-/- mice of both genders (Fig. 3). Serum prolactin levels were not different from controls: male wild type, 8.5±2.3; male ko, 5.5±0.71; female wild type, 49±29; and female ko, 62±19 ng/ml.

Behavioral testing

Since TRH has been linked to mood and anxiety disorders (Gary *et al*, 2003; Sattin, 1999; Gutierrez-Mariscal *et al*, 2008), TRH-R2-/- mice were tested in depression and anxiety-related behavioral paradigms. The tail suspension test (TST) and forced swim test (FST) are validated behavioral assays for assessing antidepressant activity. In these tests, animals are subjected to short term inescapable stress that normally elicits a rapid adaptation to immobile posture sometimes called “behavioral despair”. Immobility time is reduced by the acute administration of antidepressants and, as an extension of this finding, increased immobility time has been interpreted as depression-like behavior. Interestingly, female but not male TRH-R2-/-mice exhibited a significant increase in immobility time in TST (t-test; female: t=2.42, p=0.012, N=11 +/+ and 14 -/-; male: t=1.24, p=0.114, N=11 +/+ and 8 -/-) (Fig. 4). In FST, no significant difference was found but both male and female TRH-R2-/- mice showed a trend for increased immobility (t-test; female: t=1.62, p=0.057; male: t=1.47, p=0.076) (Fig. 4). These data suggest that female TRH-R2-/- mice have a mild depression-like behavior.

A number of anxiety-related tests are available but only a few of them are validated by various classes of clinically relevant anxiolytics (including benzodiazepines and serotonin reuptake inhibitors). One of these assays is the novelty-induced hypophagia test (NIHT) (Dulawa *et al*, 2004). In this test, animals are provided with a familiar and highly palatable food (sweetened milk in our experiments) in a novel and brightly lit environment. In these conditions, animals are conflicted on whether to avoid or approach the food. Heightened fear

and anxiety are reflected by an increase in the latency and a decrease in the duration of feeding and clinically used anxiolytics elicit opposite responses (Merali *et al*, 2003; Dulawa and Hen, 2005). Duration of feeding was significantly increased in female but not in male TRH-R2-/- mice (t-test; female: t=2.60, p=0.008, N=11 +/+ and 14 -/-) (Fig. 5). Consistent with a reduced anxiety level in females, the latency to eat was also reduced in female but not in male TRH-R2-/-mice but this effect was only a trend (t-test; female: t=1.47, p=0.077) (Fig. 5). In the familiar home cage environment in the dark, no difference in latency and duration was measured between TRH-R2+/+ and TRH-R2-/- littermates in either gender (data not shown) indicating that the observed novel cage effect in females was not due to a difference in motivation to feed.

DISCUSSION

The role of the TRH/TRH-R system in regulation the HPT axis is well known and the results of the previous studies in TRH-deficient (Yamada *et al*, 1997) and TRH-R1-deficient mice (Rabeler *et al*, 2004; Zeng *et al*, 2007) and the current studies in TRH-R2-deficient mice show that TRH-R1 is the physiological target of TRH in the TSH-producing cells of the anterior pituitary gland. The role of this system in regulation of glucose homeostasis is less well understood but both TRH-deficient and TRH-R1-deficient mice exhibit hyperglycemia. In contrast, TRH-R2-knockout mice exhibit normal glucose homeostasis even when given a high fat diet. Therefore, TRH regulation of glucose metabolism in rodents (Kulkarni *et al*, 1995; Yamada *et al*, 2000; Luo and Yano, 2004) is mediated by TRH-R1. Furthermore, it was suggested that the effect of TRH on glucose homeostasis is mediated, at least in part, by TRH-R1 expressed on insulin-secreting cells. It is noteworthy, therefore, that although TRH-R1 has been reported to be expressed in adult rodent islets at the mRNA and protein levels (Kulkarni *et al*, 1995; Luo and Yano, 2004), we have found no TRH-R mRNA in islets of Langerhans from adult humans (Mulla *et al*, 2008).

Besides its established neuroendocrine role in controlling TSH release, the TRH/TRH-R system has been proposed as a major regulatory system within the central nervous system including a role in the modulation of mood, arousal and circadian rhythm; functions thought to be impaired or altered in depression and anxiety (Gary *et al*, 2003; Yarbrough *et al*, 2007; Sattin, 1999; Pekary *et al*, 2006; Gutierrez-Mariscal *et al*, 2008). Here we tested if lack of TRH-R2 results in changes in depression- and anxiety-related behaviors. Both FST and TST indicated a moderate depression-like behavior in female but not male TRH-R2-/- mice. Although no animal model can reproduce the complex symptomatology of depression, it is believed that some aspects or endophenotypes of the disease can be reproduced in animals. In particular, disturbances in the monoamine system have long been implicated in depression and it is thought that FST and TST are assays to detect changes in monoaminergic activity. Indeed, depletion of serotonin increases while antidepressant-induced activation of serotonergic and/or noradrenergic neurotransmission reduces immobility time in FST and TST (Porsolt *et al*, 1978; Page *et al*, 1999; Cryan *et al*, 2002). We speculate that the increased immobility of TRH-R2-/- mice in the FST and TST indicates reduced serotonergic and/or adrenergic neurotransmission in these mice (Sattin *et al*, 2008). The depression-like behavior of TRH-R2-/- mice is also consistent with the reported antidepressant effect of TRH (Sattin, 1999) although not all studies support this

effect (Gary *et al*, 2003). Another interesting aspect of the depression-like behavior of the TRH-R2 mice is the gender specificity of the phenotype. Not only is depression more prevalent in females, symptoms of major depression show gender differences (Khan *et al*, 2002).

A large body of human imaging studies and animal experiments suggest that depression and depression-like behavior are associated with a neuronal circuit composed of the anterior cingulate cortex and its connections with the limbic system that includes the amygdala and the hippocampus among other brain regions. TRH-R2 is expressed in these brain areas and it is particularly interesting that this receptor is the sole TRH-R in the cingulate cortex in the rat (O'Dowd *et al*, 2000). Since lesion of the anterior cingulate increases immobility in FST (Bissiere *et al*, 2008) and because FST activates the immediate early gene c-fos in this area (Duncan *et al*, 1996), we speculate that the absence of TRH-R2 in this region is especially relevant in the depression-like behavior of TRH-R2-/- mice. However, TRH-R2, together with TRH-R1, is also expressed in the amygdala and in the bed nucleus of the stria terminalis, other important regions in the regulation of mood and the role of this receptor pool may also play a role in the emergence of the phenotype in TRH-R2-/- mice. Indeed, the moderate phenotype we observed in TRH-R2-/- mice may be because TRH-R1, which signals via the same pathways as TRH-R2, is present in these regions.

The level of anxiety of TRH-R2 mice was tested in NIHT. Again, only female TRH-R2-/- mice showed abnormalities, specifically increased duration and reduced latency of feeding that are consistent with reduced anxiety. Although the depression-like and reduced anxiety-like behaviors of TRH-R2-/-mice seem contradictory because of the comorbidity of mood and anxiety disorders, these phenotypes represent only specific dimensions of these disorders and are uncoupled in some animal models. Indeed, 5-HT1A receptor and CREB deficient mice exhibit antidepressant-like and increased anxiety-like phenotypes (Parks *et al*, 1998; Sibille *et al*, 2000; Gur *et al*, 2007).

It is noteworthy that there are prominent similarities between control of central nervous system function by TRH-R1 and TRH-R2 and the better studied two receptor system for corticotropin–releasing hormone (CRH) (see (Steckler and Holsboer, 1999), for review). Like TRH-R1/TRH-R2, CRH receptor 1 (CRH1) and CRH receptor 2 (CRH2) exhibit different patterns of expression and activate multiple signaling pathways, and have been shown to mediate overlapping but different behaviors including control of mood.

In conclusion, female TRH-R2 knockout mice exhibit increased depression-like and reduced anxiety-like phenotypes. Although TRH-R1 knockout mice were reported to exhibit increased depression-like and increased anxiety-like behaviors they were hypothyroid and their behavioral changes may have been caused indirectly by hypothyroidism. TRH-R2 is not expressed in the anterior pituitary and TRH-R2-/- mice are euthyroid; thus, the data reported herein provide the first evidence of the involvement of extrahypothalamic TRH in regulating depression/anxiety-like behavior in mice and show that TRH-R2 mediates these behaviors.

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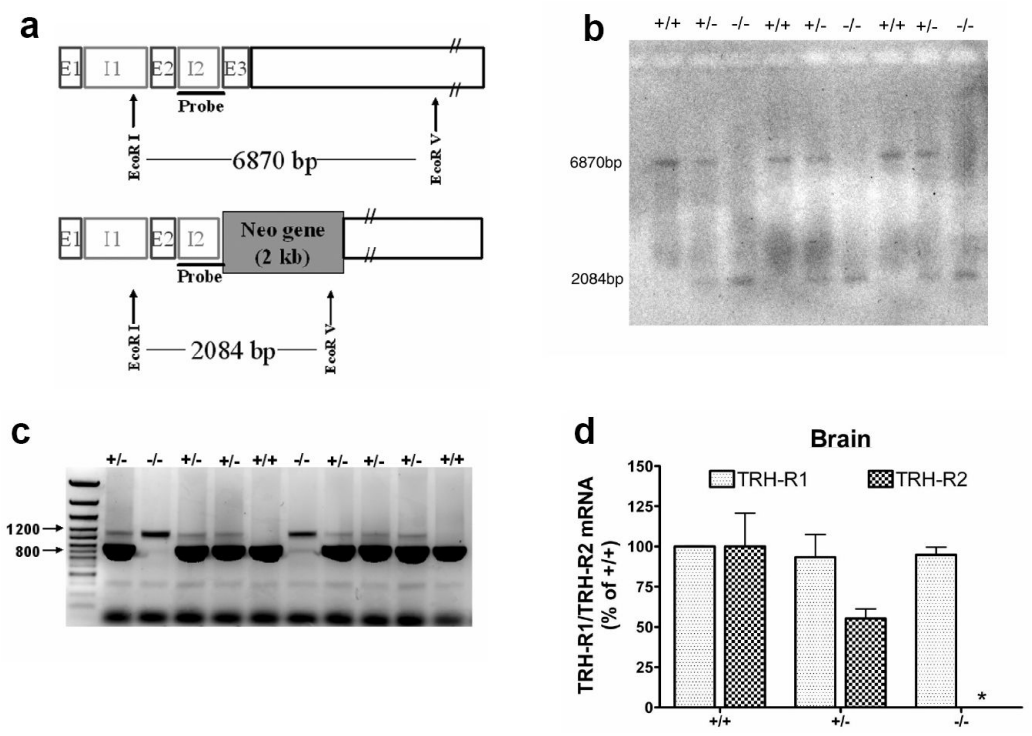

Figure 1.

Targeting vector for TRH-R2 knockout and genotyping. Schematic representation of the targeting vector (**a**). E1, 2, 3 – Exon 1, 2, 3; I1, 2 – Intron 1, 2. Representative Southern blot (**b**) and PCR (**c**) analysis of wild type (+/+), heterozygous (+/-) and homozygous mice (-/-). qRT-PCR analysis of whole brain mRNA (**d**). The asterisk indicates that no TRH-R2 mRNA was detected in homozygous mice (-/-).

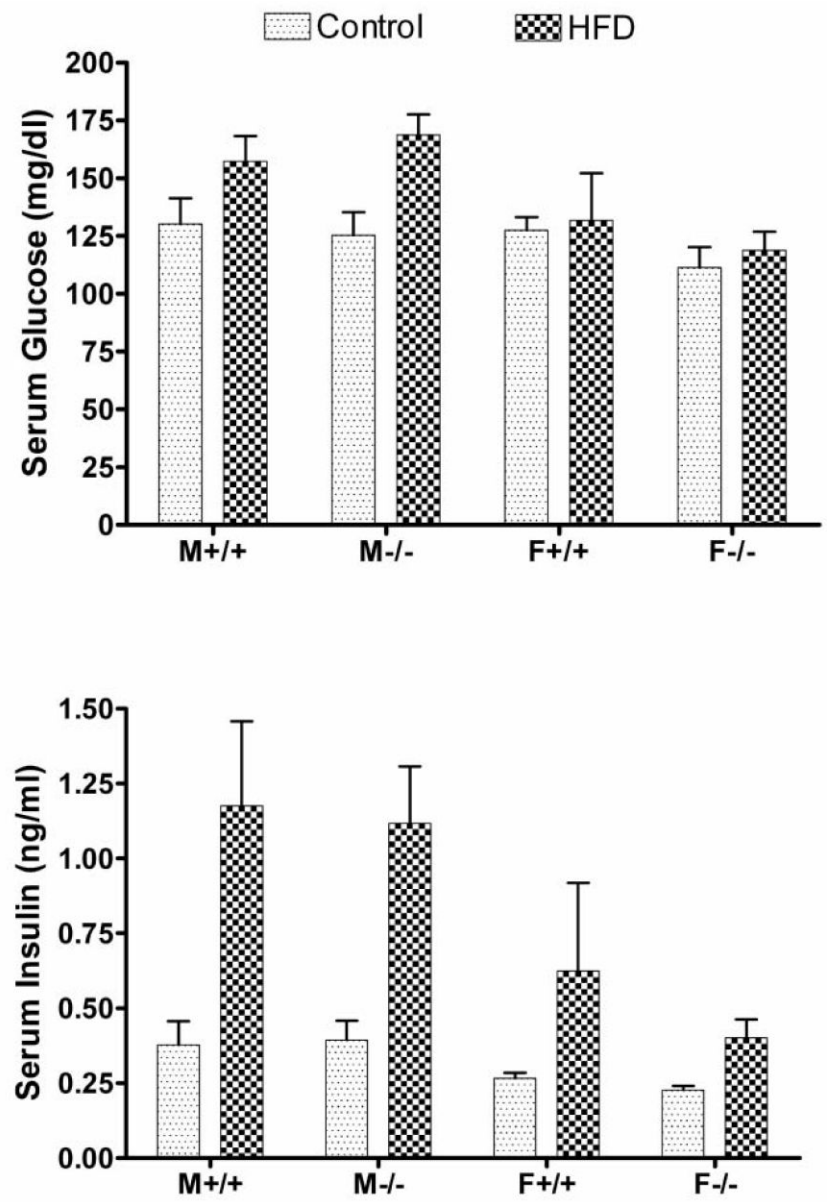

Figure 2.

Serum glucose and insulin levels in wild type and TRH-R2-deficient mice on normal (Control) and high fat diets (HFD). TRH-R2 knockout mice exhibit normal glucose (upper panel) and insulin levels (lower panel) on Control and HFD. There was no difference in any of the values in wild type compared to TRH-R2-deficient mice.

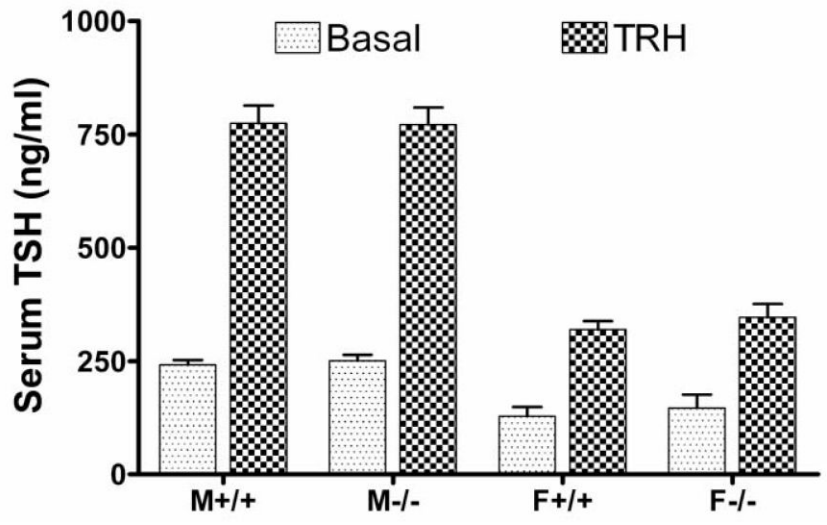

Figure 3.

Basal and TRH-stimulated serum TSH levels in wild type and TRH-R2-deficient mice. Serum for TSH measurements were obtained just before TRH (5 μg/kg) administration i.p. (Basal) and 30 min after (TRH). There was no difference in any of the values in wild type compared to TRH-R2-deficient mice.

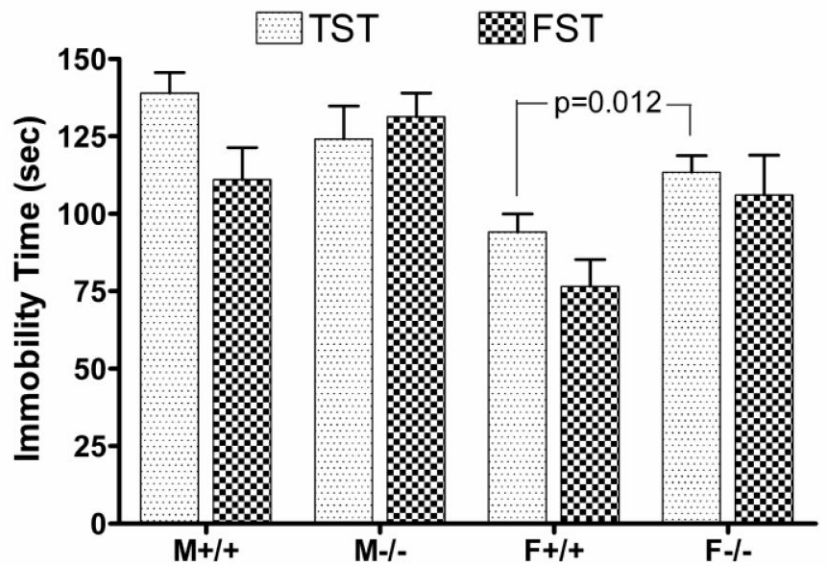

Figure 4.

Results of tail suspension and forced swim tests in wild type and TRH-R2-deficient mice. Forced swim (FST) and tail suspension tests (TST) were performed as described in Experimental Procedures. Male and female data were analyzed separately by *t*-test.

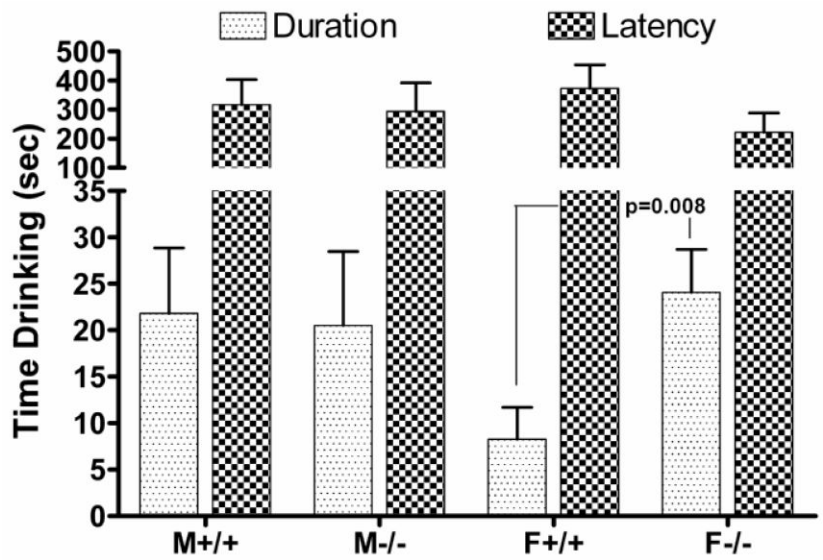

Figure 5.

Results of the novelty-induced hypophagia test in wild type and TRH-R2-deficient mice. The novelty-induced hypophagia test (NIHT) was performed as described in Experimental Procedures; durations and latencies of drinking in novel cages were measured. Male and female data were analyzed separately by *t*-test.