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Increased TRH and TRH-like peptide release in rat brain and peripheral tissues during proestrus/estrus

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ABSTRACT

Women are at greater risk for major depression, PTSD, and other anxiety disorders. ERβ-selective agonists for the treatment of these disorders are the focus of pharmacologic development and clinical testing. Estradiol and its metabolites contribute to the neuroprotective effects of this steroid class, particularly in men, due to local conversion of testosterone to estiradiol in key brain regions which are predisposed to neurodegenerative diseases. We have used young adult female Sprague–Dawley rats to assess the role of TRH and TRH-like peptides, with the general structure pGlu-X-Pro-NH2 where "X" can be any amino acid residue, as mediators of the neurobiochemical effects of estradiol. The neuroprotective TRH and TRH-like peptides are coreleased with excitotoxic glutamate by glutamatergic neurons which contribute importantly to the regulation of the estrus cycle. The levels of TRH and TRH-like peptides during proestrus and/or estrus in the 12 brain regions analyzed were significantly decreased (due to accelerated release) 106 times but increased only 25 times when compared to the corresponding levels during diestrus days 1 and 2. These changes, listed by brain region in the order of decreasing number of significant decreases (↓) and/or increases (↑), were: striatum (20↓,1↑), medulla oblongata (16↓,2↑), amygdala (14↓,1↑), cerebellum (13↓,1↑), hypothalamus (12↓,1↑), entorhinal cortex (6↓,6↑), posterior cingulate (10↓,1↑), frontal cortex (3↓,5↑), nucleus accumbens (5↓,3↑), hippocampus (5↓,2↑), anterior cingulate (2↓,1↑), and piriform cortex (1↑). In peripheral tissues the corresponding changes were: ovaries (23↓), uterus (16↓,1↑), adrenals (11↓,3↑), and pancreas (1↓,6↑). We conclude that these peptides may be downstream mediators of some of the therapeutic effects of estrogen.

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1. Introduction

Women are at greater risk for major depression, PTSD and anxiety disorders than men [27]. Estradiol and its metabolites contribute more neuroprotection in men, due to local conversion of testosterone to high levels of estradiol in key brain regions which are predisposed to the neurodegenerative effects of stress, trauma, ischemia, hypoglycemia, and aging [2,3,8,9,11,16,18,19,25,41–43,45,48,72,75–77,80].

Normal females differ from males not only in the composition but also the temporal pattern of change in their hormonal milieu. For this reason the female rat estrus cycle represents a convenient model for assessing the role of estradiol in the regulation of neurochemical processes involved in the regulation of mood and anxiety without the need for any surgical or pharmacologic interventions. We have been engaged in studies of the role of TRH (pGlu-His-Pro-NH_2) and TRH-like peptides (X-TRH) with the general structure pGlu-X-Pro-NH_2 where "X" can be any amino acid residue, in male rats. We, and others, have found that these peptides are neuroprotective, antidepressant, anti-epileptic, anxiolytic, analeptic and anorexogenic [46,53–55,57–60,64]. They are colocalized and coreleased with glutamate in glutamatergic neurons and modulate its neurotoxicity [46,53–55,57–60,64]. The rapid fluctuations of estradiol, along with progesterone, during the estrus cycle are the result of major changes in glutamatergic neuronal activity in the olfactory bulb, nucleus of the solitary tract, and preoptic nucleus of the hypothalamus which stimulates the LH surge during the afternoon of proestrus [14,21,22,65].

The two major estrogen receptors, ERα and ERβ, mediate reproductive, metabolic, behavioral, neuroprotective, cognitive,

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neuroendocrine, immunomodulatory, and neurodevelopmental functions of estrogenic steroids [78]. ERβ mediates more of the cognitive and neuroprotective effects of estrogens and less of the familiar reproductive, metabolic and secondary sexual responses mediated by ERα [3,5,9,11,13,16,23,36,38,43,78,80]. ERβ-selective agonists for treatment of major depression, PTSD, and other anxiety disorders are the focus of pharmacologic development and clinical testing [29,74–77].

The present aim is to determine whether the co-release of TRH and TRH-like peptides by glutamatergic neurons before, during and after the LH surge on the afternoon of proestrus can be a used to further understand the mood and behavior changes experienced by normal and depressed females. We hypothesize that fluctuations in estrogenic hormone levels have a major impact on the supra-hypothalamic brain resulting in highly significant changes in the biosynthesis and release of TRH and TRH-like peptides throughout the reproductive cycle. The present studies represent a necessary first step toward understanding the role of TRH and TRH-like peptides in neuropsychiatric illnesses of females and suggest therapeutic potential for this new class of neuropeptides.

2. Materials and methods

2.1. Animals

Thirty-four young adult female Sprague–Dawley rats (Harlan, Indianapolis, IN) were used for the present experiment. These animals were group housed (3–4 animals per cage), maintained with standard Purina rodent chow #5001 and water ad libitum during a standard one week initial quarantine in a controlled temperature and humidity environment; lights on: 6 am–6 pm. All animals were weighed on the day of receipt and on the morning of each experiment. Initial and final body weights did not differ between experimental groups. Research was approved by the VA Greater Los Angeles Healthcare System Animal Care and Use Committee and conducted in compliance with the Animal Welfare Act and the federal statutes and regulations related to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and use of Laboratory Animals, Eighth Edition, NRC Publication, 2011. All efforts have been made to minimize the number of animals used and their suffering. Daily vaginal smears were obtained for 2.5 weeks in the Veterinary Medical Unit (VMU) following the end of the quarantine period and analyzed microscopically [28]. Only animals with 2 consecutive, normal, 4-day estrus cycles were used. All animals were decapitated between 9 and 11 am [diestrus day 1 (D1), diestrus day 2 (D2), proestrus AM, and estrus], or between 4 and 6 pm (proestrus PM). All animals were transferred from the VMU to the laboratory 12 h before decapitation to minimize the stress of a novel environment.

2.2. Dissection of rat brain, pancreas and reproductive organs

The 14 week-old female rats weighed 223 ± 10 g on the day of decapitation. Nucleus accumbens (NA), amygdala (AY), frontal cortex (FCX), cerebellum (CBL), medulla oblongata (MED), anterior cingulate (ACNG), posterior cingulate (PCNG), striatum (STR), piriform cortex (PIR), hippocampus (HC), hypothalamus (HY), entorhinal cortex (ENT), pancreas, adrenals, uterus, and ovaries were hand dissected, weighed rapidly, and then peptides were extracted as previously described [46,53–55,57–60,64]. The dissection procedure has previously been described in detail [57].

2.3. Serum hormone assays

Serum rat LH, leptin, insulin, active ghrelin, total ghrelin, oxytocin, estradiol, testosterone, progesterone, corticosterone, insulin-like growth factor-1 (IGF-1), total T_3, and free T_4, were measured with the following RIAs: rat LH (National Pituitary Agency, Torrance, CA), leptin, insulin, active and total ghrelin (Linco Research, Inc., St. Charles, MO), estradiol, progesterone, testosterone, free T_4, and total T_3, (DPC Coat-A-Count, Los Angeles, CA), corticosterone (MP Biomedical, Irvine, CA), and IGF-1 (ALPCO, Salem, NH). Serum glucose was measured with the OneTouch Ultra Meter (LifeScan, Milpitas, CA).

2.4. HPLC and RIA procedures, HPLC peak identification and quantitation of TRH and TRH-like peptides

HPLC and RIA procedures, peak identification, and quantitation by co-chromatography with synthetic TRH and TRH-like peptides, relative potency analysis of multiple antibodies to TRH and TRH-like peptides, mass spectrometry and statistical methods for comparing HPLC peak areas have been previously reported in detail [46,53–55,57–60,64].

Briefly, after boiling, tissues were dried, re-extracted with methanol, dried and defatted by water-ethyl ether partitioning. Dried samples were dissolved in 0.1% trifluroacetic acid (TFA), and loaded onto reverse phase C_{18} Sep-Pak cartridges (Water, Milford, MA). TRH and TRH-like peptides were eluted with 50% methanol. Dried peptides were again dissolved in TFA, filtered and then fractionated by HPLC using a 4.6 mm × 150 mm Econosphere, 3 μm C_{18} reverse phase column (Alltech Associates, Deerfield, IL) and a linear 0.2%/min gradient of acetonitrile in TFA starting at 0 M acetonitrile. The 0.5 ml fractions collected were dried completely and reconstituted with 0.10 ml of 0.02% NaN_3 just before RIA.

The antiserum used (8B9) cross-reacts with TRH and eight TRH-like peptides with the following relative potency of displacements relative to Tyr-TRH: 2.31 (Lys-TRH); 1.14 (TRH); 1.02 (Phe-TRH); 0.990 (Glu-TRH); 0.620 (Leu-TRH); 0.590 (Val-TRH); 0.288 (Ser-TRH); 0.247 (Gln-TRH) [55]. One of the regularly observed peaks (peak 2) consists of a mixture of unidentified TRH-like peptides. Of the seven observed peptides three have so far been confirmed by mass spectrometry: TRH, Glu-TRH and Tyr-TRH [46].

The mean recovery of TRH and TRH-like peptide immunoreactivity from all tissues studied was 84 ±15% (mean ± SD). The within-assay and between-assay coefficient of variation for measuring 333 pg/ml TRH was 4.8% and 16.9%, respectively. All HPLC fractions obtained from a given brain region or peripheral tissue was analyzed in the same RIA. The minimum detectable dose for TRH was 5 pg/ml. The specific binding of [^{125}I]TRH (Bo/T) was 25%.

Table 1
Reproductive hormone levels versus phases of the rat estrus cycle.

Phase	LH (ng/ml)	Estradiol (ng/ml)	Progesterone (ng/ml)	(*n*)
D1	4.1 ± 0.9	20 ± 2	17 ± 6	5
D2	5.6 ± 1.1	18 ± 1	13 ± 8	5
PRO (AM)	10.0 ± 0.8	35 ± 5*	10 ± 3	3
PRO (PM)	1500 ± 700*	39 ± 19	54 ± 36	3
EST	9 ± 2	19 ± 0.2	8 ± 5	5

* $p < 0.05$ by one way ANOVA.