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Rapid modulation of TRH and TRH-like peptide release in rat brain, pancreas, and testis by a GSK-3β inhibitor

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ABSTRACT

Antidepressants have been shown to be neuroprotective and able to reverse damage to glia and neurons. Thyrotropin-releasing hormone (TRH) is an endogenous antidepressant-like neuropeptide that reduces the expression of glycogen synthase kinase-3β (GSK-3β), an enzyme that hyperphosphorylates tau and is implicated in bipolar disorder, diabetes and Alzheimer's disease. In order to understand the potential role of GSK-3β in the modulation of depression by TRH and TRH-like peptides and the therapeutic potential of GSK-3β inhibitors for neuropsychiatric and metabolic diseases, young adult male Sprague–Dawley (SD) rats were (a) injected ip with 1.8 mg/kg of GSK-3β inhibitor VIII (GSKI) and sacrificed 0, 2, 4, 6, and 8 h later or (b) injected with 0, 0.018, 0.18 or 1.8 mg/kg GSKI and bled 4 h later. Levels of TRH and TRH-like peptides were measured in various brain regions involved in mood regulation, pancreas and reproductive tissues. Large, 3–15-fold, increases of TRH and TRH-like peptide levels in cerebellum, for example, as well as other brain regions were noted at 2 and 4 h. In contrast, a nearly complete loss of TRH and TRH-like peptides from testis within 2 h and pancreas by 4 h following GSKI injection was observed. We have previously reported similar acute effects of corticosterone in brain and peripheral tissues. Incubation of a decapsulated rat testis with either GSKI or corticosterone accelerated release of TRH, and TRH-like peptides. Glucocorticoids, via inhibition of GSK3-β activity, may thus be involved in the inhibition of TRH and TRH-like peptide release in brain, thereby contributing to the depressogenic effect of this class of steroids. Corticosterone-induced acceleration of release of these peptides from testis may contribute to the decline in reproductive function and redirection of energy needed during life-threatening emergencies. These contrasting effects of glucocorticoid on peptide release appear to be mediated by GSK-3β.

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1. Introduction

Glycogen synthase kinase-3β (GSK-3β) phosphorylates a number of key regulatory proteins that have been implicated in diabetes, stroke, Alzheimer's disease, tumor progression, major depression, bipolar disorder, and microtubule assembly in neurons [3–5,12,14,21,26,29,35,48,58,59,62,63]. We and others have previously reported that TRH (pGlu-His-Pro-NH_2) and TRH-like peptides (pGlu-X-Pro-NH_2 or X-TRH, where "X" can be any amino acid residue), have neuroprotective, antidepressant, analeptic, and anti-amnesic properties [16,23,24,29,37,42,44–46,61]. In the case of TRH, this is due, at least in part, to potent inhibition of GSK-3β gene expression [29]. TRH and TRH-like peptide levels in brain and peripheral tissues can be profoundly modulated by antidepressants, thyroid and steroid hormones, proinflammatory cytokines, neuropharmacologic agents, psychostimulants, electroconvulsive shock, and the photoperiod [16,18,37,40,42,44–47].

As part of a systematic exploration of the involvement of this family of endogenous neuropeptides in both the pathogenesis and treatment of psychiatric diseases, we hypothesized that (a) many of the extrapituitary effects of TRH and TRH-like peptides involve the modulation of GSK-3β activity, and (b) the well-established decline in levels of TRH and responsiveness to TRH in brain and peripheral tissues with aging [1,17,36] contributes to age-related GSK-3β

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hyperactivity and consequent CNS and metabolic pathology and reproductive decline [54,63].

For these reasons we have used a specific GSK-3β inhibitor (GSKI) to study both the in vivo and in vitro effects of acute GSK-3β inhibition on TRH and TRH-like peptide levels in, and release from, rat brain and peripheral tissues.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats (Harlan, Indianapolis, IN) were used for all experiments. These animals were group housed (4 animals per cage), maintained with standard Purina rodent chow #5001 and water ad libitum during a standard 1 week initial quarantine in a controlled temperature and humidity environment; lights on: 6 am to 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, NRC Publication, 1996 edition. All efforts have been made to minimize the number of animals used and their suffering. All animals were transferred from the Veterinary Medical Unit to the laboratory 12 h before the start of experiments to minimize the stress of a novel environment. Decapitation occurred between 9 am and 11 am to minimize the diurnal variation in TRH and TRH-like peptide levels [45].

2.2. Time-dependent in vivo effects of GSKI

Ten rats received a single 0.5 ml ip injection of 1.0 mg/ml DMSO of the cell permeable and antidepressant-like [15] GSK-3 inhibitor VIII (GSKI, IC50 = 104 ± 27 nM, Calbiochem, San Diego, CA), which acts in an ATP-competitive manner (K_i = 38 nM, [6]), at 0, 2, 4, 6, and 8 h (n = 2 for each time point) before sacrifice. This does not significantly inhibit cdk2 or cdk5 (IC50 > 100 μM) or 26 other kinases demonstrating high specificity for GSK3. The mean body weights were 272 ± 12 g (mean ± SD). Thus 1.8 mg GSKI/kg body weight was administered. Uptake values for [^{11}C]GSKI into rat brain, corrected for radioactivity in the vascular compartment, was 0.06% at 30 min [57]. Even though this uptake is very poor, the GSKI concentration in brain at this time would be 1.0 μM, 10 times the IC50 for GSK-3.

2.3. Concentration-dependent in vivo effects of GSKI

Sixteen rats were ip injected with 0.5 ml of 0, 0.01, 0.1 or 1.0 mg GSKI/ml DMSO (n = 4 for each concentration). The mean body weight was 281 ± 14 g (mean ± SD). All rats were decapitated 4 h after injection.

2.4. Dissection of rat brain, pancreas and reproductive organs

After decapitation, Nucleus accumbens (NA), amygdala (AY), frontal cortex (FCX), cerebellum (CBL), medulla oblongata (MED), anterior cingulate (ACNG), posterior cingulate (PCNG), striatum (STR), pyriform cortex (PYR), hippocampus (HC), entorhinal cortex (ENT), pancreas (PAN), prostate, epididymis, and testes (T) were hand dissected, weighed rapidly, and then extracted as previously described [37,42,44–47].

2.5. In vitro effects of GSKI and corticosterone

GSKI is extremely photosensitive requiring that all possible measures be used to protect it from light exposure during the in vitro experiments described below. Lights were turned out and window shades were closed. Bacitracin from Bacillus licheniformis (3.0 mg, Sigma) and 1.0 mg of a potent TRH-degrading ectoenzyme inhibitor (pGlu-Asn-Pro-d-Tyr-d-Trp-NH_2, Phoenix Pharmaceuticals, Belmont, CA [52]) were dissolved in 40 ml of Ham's F-10 medium with HEPES buffer (MP Biomedicals, Irvine, CA) and equilibrated with 95% O_2–5% CO_2 for 15 min at room temperature. Maximum dissolved O_2 of 31.2 mg/L was reached at 10 min as measured with a Fisher Brand Portable Dissolved Oxygen Meter (Fisher Scientific, Pittsburg, PA). Three 20 ml glass scintillation vials were labeled as A, B, and C. DMSO (150 μl) was added to vial A. Corticosterone hemisuccinate (150 μl of 10 mg/ml DMSO) was added to vial B (224 μM corticosterone final concentration) and 150 μl of 1.0 mg GSKI/ml DMSO was added to vial C wrapped in aluminum foil (37 μM GSKI final concentration). Thirteen ml of the Ham's F-10 mixture were then added to each vial. Contents of vials A was distributed equally between four 13 mm × 100 mm glass test tubes, 3.0 ml/vial; likewise for vials B and C. The "C" test tubes were wrapped in black paper. 95% O_2–5% CO_2 was bubbled into each vial continuously to maintain the oxygenation and pH balance of the incubation mixture using a Thermolyne Dri-Bath evaporator (Barnstead, Dubuque, IA) set at 35 °C. One 350 g male SD rat was then decapitated. Each decapsulated testis was divided into 6 equal pieces. One testis fragment was transferred into each of the 12 glass test tubes. A 100 μl aliquot from each of the four replicate glass tubes containing medium from Vial A were pooled together; similarly for tubes containing medium from Vial B and C. These pooled aliquots was centrifuged at 100 × g for 5 min at 4 °C to remove Leydig cells dislodged from tubules by the continuous oxygenation at 0.5, 1.0, 1.5, 2.0, and 2.5 h after the addition of the testes. The supernatant was decanted into a 12 mm × 75 mm glass test tube containing 2.0 ml of methanol. All tubes were then centrifuged at 1000 × g, the supernatant decanted and dried on a heater block with a fan blowing air across the top of the tubes to accelerate drying. The dried residues were reconstituted with 0.5 ml of 0.02% NaN_3 prior to TRH RIA in duplicate.

2.6. HPLC and RIA procedures, HPLC peak identification and quantitation

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 resolution of overlapping peaks by least squares fitting of a 2-Gaussian statistical model have been previously reported in detail [39,43].

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 30% 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 0.33% min^{-1} gradient of acetonitrile. The 0.5 ml fractions collected were dried completely and reconstituted with 0.15 ml of 0.02% NaN_3 just before RIA.

The antiserum used (8B9) cross-reacts with TRH and eight TRH-like peptides with a relative potency of displacement ranging from 2.31 (Lys-TRH) to 0.288 (Ser-TRH) relative to Tyr-TRH (Table 2, [42]). Two of the regularly observed peaks (2a and 2b) consist of a mixture of unidentified TRH-like peptides. Of the seven observed