Research report

Electroconvulsive seizures modulate levels of thyrotropin releasing hormone and related peptides in rat hypothalamus, cingulate and lateral cerebellum

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Abstract

We have studied the neuroanatomic extent of electroconvulsive (ECS)-responsive prepro-TRH and TRH-related gene expression and its possible interaction with forced swimming. Young adult male Wistar rats were treated in a 2 × 2 Latin square protocol of swimming, no swimming, three daily ECS or sham ECS. Sixteen different brain regions were dissected and immunoreactivity measured for TRH (pGlu–His–Pro–NH₂), TRH–Gly, a TRH precursor; Ps4, a prepro-TRH-derived TRH-enhancing decapeptide, and EEP (pGlu–Glu–Pro–NH₂). ECS, in addition to elevating TRH–immunoreactivity (TRH–IR), TRH–Gly–IR, Ps4–IR and EEP–IR levels in the limbic regions, as we have previously reported, also significantly increased Ps4–IR levels in hypothalamus, posterior cingulate and lateral cerebellum, and increased TRH–Gly–IR levels in hypothalamus. Interestingly, the combination of ECS and swimming significantly reduced the levels of TRH–Gly–IR in the anterior cingulate compared to the sham ECS-no swim group. The combined use of high-pressure liquid chromatography and the EEP radioimmunoassay (RIA) revealed that pGlu–Tyr–Pro–NH₂ and/or pGlu–Phe–Pro–NH₂ occur in amygdala, anterior cingulate, frontal cortex, entorhinal cortex, lateral cerebellum and striatum and make a substantial contribution to the EEP–IR and TRH–IR. We conclude that ECS can alter the expression and secretion of TRH-related peptides in the hypothalamus, cingulate and lateral cerebellum. Such effects have not previously been reported in these limbic and extra-limbic regions which are increasingly implicated in the autonomic, behavioral and volitional changes which accompany severe depression and its treatment.

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1. Introduction

Electroconvulsive therapy (ECT) is an effective method for the treatment of severe unipolar and bipolar depression as well as schizophrenia and Parkinson’s disease [42, 47]. Understanding the mechanism by which ECT ameliorates these serious mental disorders should lead to safer and more cost-effective treatments. Evidence has accumulated that electroconvulsive seizures (ECS) modify the expression of several genes in various regions of the brain associated with regulation of mood, including the amygdala, hippocampus, entorhinal cortex, and pyriform
cortex, important components of the limbic system [3,14,17,34,43,44]. Genes which undergo a sustained increase in expression include those for thyrotropin releasing hormone (TRH, pGlu–His–Pro–NH₂) and a closely related peptide, pGlu–Glu–Pro–NH₂ (EEP) [31].

Increased transcription, translation and post-translational processing of the prepro-TRH gene leads to the production of not only more TRH, but also other prepro-TRH-derived peptides which may have a role in alleviating depressive symptoms. These include Ps₄, a TRH-enhancing decapeptide and Ps₅, corticotropin releasing hormone (CRH) inhibiting factor (CRIF, a 22 amino acid residue peptide) [32,37–39]. Ps₄ greatly enhances the pituitary TSH [5] and gastric acid releasing activity [48] of TRH but lacks any TRH-like activity itself. It is likely that Ps₄ also enhances the CNS effects of TRH since it is a necessary byproduct of TRH biosynthesis and would be cosecreted with TRH by TRH-containing synaptic vesicles. Ps₅ inhibition of release of CRH, should also contribute to the antidepressant effect of ECT, because excessive CRH may contribute to anxiety components of depressive syndromes [26].

TRH has been reported to alleviate depressive symptoms [20], but the necessity of intrathecal administration to minimize rapid inactivation by serum enzymes, is a serious limitation for clinical use of this peptide. Numerous analogs of TRH with greatly increased in vivo stability and enhanced CNS versus TSH-stimulating effect exist [11]. Interestingly, EEP, an endogenous TRH-like peptide, is not metabolized by thyroliberinase, a blood enzyme which specifically inactivates TRH [25]. EEP does not bind to TRH receptors isolated from the pituitary of mammals, as evidenced by the inability of high dose EEP to release T₃ from the thyroid gland [19]. Nevertheless, EEP does have antidepressant activity similar to that for TRH in the Porsolt Forced Swim Test [19]. Interaction of EEP with an EEP-specific receptor is a possible mechanism of action.

The symptomatology which is included in depressive syndromes might involve pathophysiologic changes in brain areas outside the classical limbic structures. For example, insomnia, anorexia, and alterations in normal diurnal rhythms alter neuroendocrine functions associated with the hypothalamus, midbrain and other autonomic control centers [36,40]. Structural and functional imaging studies have consistently shown a deficit in the volume and function of the anterior cingulate in unipolar depression [24,41]. The cerebellum, in addition to the prefrontal cortex and anterior and posterior cingulate, are involved in volition, which is impaired in depressive disease [7,13].

For the above reasons we have extended the neuroanatomical scope of our studies. We have also increased the number of relevant assays to include those for TRH, TRH–Gly (pGlu–His–Pro–Gly, a TRH precursor peptide), Ps₄, and EEP. We find that ECS has a profound effect on the expression of TRH-related peptides in numerous regions of the brain, which may help to explain the efficacy of ECT for a variety of mental disorders including depression.

2. Materials and methods

2.1. Peptides

Ps₄ (Ser–Phe–Pro–Trp–Met–Glu–Ser–Asp–Val–Thr) was obtained from Bachem California (Torrance, CA, USA), [Phe²]–TRH and Tyr₆–Ps₄ were prepared by Peninsula Laboratories (Belmont, CA, USA). EEP (pGlu–Glu–Pro–NH₂) and TRH (pGlu–His–Pro–NH₂) were purchased from Sigma Chemical Co. (St. Louis, MO). [Tyr²]–TRH was custom synthesized by the UCLA Peptide Synthesis Laboratory.

2.2. Immunization procedure

Ps₄ and EEP were conjugated to keyhole limpet hemocyanin (KLH, Sigma Chemical Co., St. Louis, MO, USA) by the carbodiimide method [1]. TRH was conjugated to KLH by the dinitrodi fluorobenzene method [48]. New Zealand white rabbits (Universal Animal Care, Bloomington, CA) were immunized subcutaneously with a stable emulsion prepared from equal volumes of conjugate (1.5 mg/ml saline) and Freund’s complete adjuvant. After shaving a 10×10-cm area of the back or flank, rabbits received 10–12 subcutaneous injections, equalling 1.0 ml of emulsion prepared with the Ps₄– and EEP–KLH conjugates, as previously described [31,32]. Three weeks later, all rabbits received injections of immunogen identical to that received initially. The rabbits were bled from an ear vein prior to the second and subsequent immunizations.

2.3. Experimental design

Male Wistar rats weighing 160–180 g on arrival (Simonsen Laboratories, Gilroy, CA, USA), maintained in a temperature and light-controlled environment (lights on: 6 am to 6 pm) were provided water and standard rodent lab chow (Purina #5001 and water ad libitum. After a 1-week quarantine period, three corneal ECS or sham ECS treatments were administered between 8:00 and 10:00 h on consecutive days, one ECS given on each day. Stimuli were given using the Ugo Basile model 7801 unipolar square-wave ECS pulse generator (Stoelting Co., Wood Dale, IL) via hand-held brass electrodes that were applied to the corneas after instillation of artificial tears. Polishing the smooth, rounded electrode tips between applications prevented behavioral distress. The currents on each of the three successive days were 9.43, 23.5, and 33.0 mA [44]. Stimulus strength was elevated by increasing the pulse rate from 85 to 142 to 200 pulses/s, across the three stimulus days. The graded increase in current-dose mimics the clinical paradigm of ECT although the rat and human
paradigms are not directly comparable [44]. Their effect on swim immobility time in the Porsolt forced swim test was measured 24 h after the last ECS.

2.4. Forced swim test

The Porsolt forced swim test involves two phases. In the first phase (pre-swim), the rats are individually placed in a tank of water for 15 min, from which they cannot escape. During the second phase (swim test), the animals are returned to the tank for 5 min, and the duration of immobility is recorded. Antidepressant treatments (ECS) are administered between the first (pre-swim) and second (swim test) phases of the Porsolt procedure. The antidepressant treatments typically increase the swimming activity (decreased immobility). Decreases in mean immobility time (during swim test), relative to sham-treated animals, are predictive of antidepressant effects of drugs and ECS [6,31,32,35,44]. Twenty-four hours before the first ECS, all rats were placed in a cylindrical polycarbonate tank of water (25±0.5°C) for 15 min (pre-swim). The tank was 22 cm in diameter and filled to a depth of 30 cm. At this depth the rat is not able to contact the bottom of the cylinder while floating. One day after the third (final) ECS, all rats were placed into the tank for 300 s (swim test). All conditions were the same. The cumulative duration of immobility (absence of swimming movements) was recorded [31,32,44].

2.5. Dissection and extraction

Olfactory bulbs, vermis, lateral cerebellum, medulla, pons, interpeduncular nucleus, frontal cortex, anterior and posterior cingulate, septum, ventral striatum (nucleus accumbens and olfactory tubercle), striatum, pyriform cortex, hypothalamus, hippocampus, entorhinal cortex, amygdala, thalamus, rest of cortex and midbrain were hand dissected as previously described [21,22], weighed rapidly, and then boiled in 1.0 M acetic acid at 95°C for 15 min in glass test tubes [31,32,44]. After Polytron (Brinkman Instruments, Westbury, NY, USA) homogenization, the samples were lyophilized to a damp pellet, redispersed in 2.0 ml methanol, centrifuged, and the supernatant transferred to glass test tubes and dried completely. Dried samples from a given experiment were stored at −20°C and reconstituted with 1.0 ml 0.02% NaNO₃ prior to Ps4, EEP, TRH and TRH–Gly radioimmunoassay (RIA) and high-pressure liquid chromatography (HPLC).

2.6. HPLC

The corresponding tissue extracts remaining after RIA measurements (20% of each initial extract preparation) were pooled within each treatment group, defatted by water–ethyl ether partitioning, centrifuged, and the aqueous phase was lyophilized. After dissolving the residue in 0.1% trifluoroacetic acid (TFA), each pooled extract was loaded onto a C18 Sep-Pak cartridge (Waters, Milford, MA) which had been previously activated by washing with methanol and water. The cartridges were eluted with 3 ml of methanol and the combined sample pass-through and methanol eluates were dried completely on a heater block and redissolved in 0.1% TFA. Particulates were removed with a HPLC-certified Acro LC3A filter ( Pall Gelman Laboratories, Ann Arbor, MI). These pooled extracts were then injected into a programmable HPLC system (Shimadzu Corp, Kyoto) equipped with a 4.6×150 mm Econosphere, 3 μm C18 reverse phase column (Alltech Associates, Deerfield, IL) previously equilibrated with 0.1% TFA. At the time of injection, a 0.33%/min gradient of acetonitrile at a flow-rate of 1 ml/min was started. At 30 min the gradient was increased to 1%/min and at 70 min the gradient was further increased to 5%/min. The 1 ml fractions collected were dried completely and reconstituted with 1 ml of 0.02% NaNO₃ just before EEP RIA. The optical density profile for a mixture of EEP, TRH, pGlu–Tyr–Pro–NH₃, and pGlu–Phe–Pro–NH₃ (25 μg/ml each, 0.5 ml injected volume, 1.0 absorbance units full scale) was used to determine the HPLC retention times for these four peptides (results not shown).

2.7. RIA procedure

TRH, Tyr-o–Ps4 and TRH–Gly were labeled with 125I using the chloramine-T method [30–32]. Using a small column of 2% BSA-treated Sephadex G10 packed in a 5-ml pipet, the tracer was separated from unreacted 125I by eluting with phosphate-buffered saline (PBS), pH 7.5. It was then diluted in 0.325% normal rabbit serum, 0.025 M EDTA, and 0.02% sodium azide (NaNO₃) to 15 000 cpm/0.2 ml. Ps4, EEP, TRH or TRH–Gly standards and samples were diluted in 0.02% NaNO₃. Standards and tissue extracts or HPLC fractions, in duplicate 100-μl aliquots, tracer (200 μl), Ps4 antiserum 1128B7 diluted 1:2000, EEP antiserum 580B4 diluted 1:100, TRH antiserum 465B12 diluted 1:5000 or TRH–Gly antiserum 898B6 diluted 1:200 in 0.02% NaNO₃ in PBS to give 20% specifically bound counts/total counts (Bₛ/T), and goat antirabbit IgG in 0.02% NaNO₃ in PBS (100 μl) were added together, vortex mixed, and incubated at 4°C for 24 h. Tubes were centrifuged at 1000 g for 30 min, aspirated, and counted for 2 min. The minimum detectable dose was 20 pg/ml. Each of these RIAs has previously been described in detail [30–33,44]. All samples from an experiment were run in the same assay. pGlu–Tyr–Pro–NH₃ (Tyr²–TRH) and pGlu–Phe–Pro–NH₃ (Phe²–TRH) are at least 100% cross-reactive in the EEP and TRH RIAs. Since EEP cross-reacts less than 1% in the TRH RIA [30], the EEP levels are calculated as the difference between the EEP RIA and TRH RIA results [31].
2.8. Statistical analysis

Statistical comparisons were made with the aid of Statview (Abacus Concepts, Inc., Berkeley, CA), a statistical software package for the Macintosh computer. All multigroup comparisons were carried out by one way analysis of variance using post-hoc Scheffe contrasts with the control group.

3. Results

The combined use of HPLC and EEP RIA (see Fig. 1 for representative EEP–IR profiles following HPLC of pooled tissue extracts) demonstrated that EEP, TRH, Tyr2–TRH, and Phe2–TRH all occur in rat brain but their relative concentrations vary markedly between specific brain regions. A minor EEP–IR peak with a retention time of about 15 min has not yet been identified. It is apparent that in lateral cerebellum and striatum (Fig. 1) and in amygdala, anterior cingulate, entorhinal cortex, and frontal cortex (results not shown), the EEP–IR (and TRH–IR) consists of a mixture of two or more different TRH-like peptides. Following ECS the peak corresponding to Phe2–TRH in lateral cerebellum increased six-fold (results not shown). We have not been successful in our attempts to produce more specific antibodies to EEP, Tyr2–TRH and Phe2–TRH using currently available methodologies [28]. This is the first report of Tyr2–TRH and Phe2–TRH occurring in mammalian brain.

Three ECS administered on consecutive days with progressively increasing current, with or without forced swimming, did not affect total body weights or tissue weights in the brain regions that were dissected (results not shown). Post-ECS hindlimb paralysis reduced the number of ECS animals. This was most likely due to the use of 65-day-old, 320 g body weight, instead of 55-day-old, 250 g animals. The three rats that received both ECS and a swim score were not sufficient for meaningful correlation of swimming behavior with levels of TRH and TRH-related peptides in various brain regions.

Nevertheless, highly significant increases in the levels of Ps4–IR, TRH–IR, and TRH–Gly–IR were observed in a variety of brain regions, not only in the limbic system as we have previously reported [31,32,44], but also in a number of non-limbic areas as summarized in Table 1. Consistent with our previous reports [31,32,44], ECS increased Ps4–IR, TRH–IR and TRH–Gly–IR levels in pyriform cortex, hippocampus, entorhinal cortex, and amygdala. TRH–Gly–IR levels in the amygdala were at least 10-fold higher than the corresponding levels in any other brain region studied. In addition, we found that ECS increased Ps4–IR levels in hypothalamus, posterior cingu late and lateral cerebellum and raised the TRH–Gly–IR concentration in the hypothalamus.

The combined effect of ECS and swimming was to decrease the level of TRH–Gly–IR in the anterior cingulate (Fig. 2), an observation that has recently been confirmed in a similar protocol using vagal nerve stimulation instead of ECS (S. Krahl, A.E. Pekary, A. Sattin, unpublished results). This result is consistent with increased conversion of TRH–Gly–IR (for anterior cingulate, a mixture of EEP–Gly and Tyr–TRH–Gly) to EEP and Tyr–TRH, respectively, the major EEP–IR peaks found after HPLC and EEP RIA of this tissue (result not shown), and release of these peptides [4,30]. This combined suppressive effect of ECS and forced swimming on TRH–Gly–IR concentration in the anterior cingulate is in
Table 1
Effect of electroconvulsive seizure (ECS; three swim plus three non-swim) versus sham ECS (control; six swim plus six non-swim) treatment on levels of Ps4 immunoreactivity (Ps4–IR); TRH–IR, and TRH–Gly–IR, (ng/g wet weight), levels in various brain regions of male Wistar rats, 300 to 350 g body weight.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Pyriform cortex</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Entorhinal cortex</th>
<th>Amygdala</th>
<th>Posterior cingulate</th>
<th>Lateral cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH–IR</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>ECS (6)</td>
<td>0.9 ± 0.3</td>
<td>27.9 ± 4.3</td>
<td>2.3 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>3.6 ± 0.7</td>
<td>0.42 ± 0.14</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>Control (12)</td>
<td>0.4 ± 0.2</td>
<td>25.6 ± 4.9</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>2.6 ± 0.4</td>
<td>0.45 ± 0.15</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>t</td>
<td>4.97</td>
<td>0.96</td>
<td>8.87</td>
<td>2.4</td>
<td>3.46</td>
<td>0.48</td>
<td>1.08</td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;N.S.</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>N.S.</td>
<td>N.S.</td>
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<td>TRH–Gly–IR</td>
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<tr>
<td>ECS (6)</td>
<td>32.4 ± 10.9</td>
<td>19.0 ± 9.9</td>
<td>18.0 ± 6.5</td>
<td>28.6 ± 15.1</td>
<td>322 ± 172</td>
<td>32 ± 16</td>
<td>10.3 ± 4.9</td>
</tr>
<tr>
<td>Control (12)</td>
<td>19.7 ± 11.0</td>
<td>10.0 ± 6.1</td>
<td>7.2 ± 3.0</td>
<td>15.4 ± 7.2</td>
<td>133 ± 82</td>
<td>30 ± 13</td>
<td>8.1 ± 4.1</td>
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<tr>
<td>t</td>
<td>2.30</td>
<td>2.40</td>
<td>4.92</td>
<td>2.55</td>
<td>3.12</td>
<td>0.27</td>
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<tr>
<td>P</td>
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<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>N.S.</td>
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<tr>
<td>Ps4–IR</td>
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<tr>
<td>ECS (6)</td>
<td>7.2 ± 3.7</td>
<td>33.6 ± 16.5</td>
<td>4.8 ± 2.5</td>
<td>5.8 ± 2.7</td>
<td>10.6 ± 3.4</td>
<td>5.4 ± 2.9</td>
<td>2.5 ± 0.7</td>
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<tr>
<td>Control (12)</td>
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<td>16.3 ± 8.2</td>
<td>1.3 ± 0.8</td>
<td>2.7 ± 1.4</td>
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<tr>
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<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>

*p-values are for the two-tailed Student’s t-test.

marked contrast with the observed increase in TRH–Gly–IR, TRH–IR and Ps4–IR levels in other brain regions, summarized in Figs. 3–5.

TRH, TRH–Gly and Ps4 are all prepro-TRH-derived peptides. The TRH-like peptides, EEP, Tyr–TRH, and Phe–TRH are the products of other, as yet uncharacterized, precursor proteins [31]. Nevertheless, expression of these peptides appears to vary in a similar manner to the prepro-TRH-derived peptides as evidenced by the significant correlation of EEP–IR and Ps4–IR levels in a variety of brain regions as displayed in Figs. 6 and 7.

4. Discussion

Previous studies clearly indicate that ECS increases the expression, in rat limbic system, not only of the prepro-TRH gene [17] but also the enzymes [3] responsible for the processing of the corresponding protein to a variety of prepro-TRH-derived peptides including TRH–Gly, TRH, Ps4, and Ps5 [31,32,44]. In this and our previous studies, ECS consistently increases the levels of TRH–Gly–IR, TRH–IR and Ps4–IR in rat pyriform cortex, hippocampus, entorhinal cortex, and amygdala [31,32,44]. We report for the first time that ECS also increases Ps4–IR levels in the lateral cerebellum, posterior cingulate and hypothalamus. TRH–Gly–IR levels are also increased in the hypothalamus and Phe–TRH concentration increased in lateral cerebellum.

The cerebellum receives its inputs from the sensory, motor and parietal cortex by way of the relay nuclei in the pons. From the cerebellar hemispheres (lateral cerebellum) the output is projected to the cerebellar deep nuclei then to the ventrolateral nucleus of the thalamus, and then feeds...
Fig. 3. Effect of electroconvulsive seizures (ECS), sham ECS, forced swimming (swim) and no swim on TRH–Gly levels in hypothalamus and three limbic regions of male Wistar rats.

back to the motor cortex. This circuit plays an important role in planning and initiating movement [8]. Localization of the ECS-responsive prepro-TRH and TRH-like precursor expression to this most recently evolved region of the cerebellum might have therapeutic implications for electroconvulsive treatment (ECT) because impaired volition is one of the hallmarks of severe depression [13].

The striking difference in the effect of ECS and forced swimming on TRH–Gly–IR levels in the anterior cingulate compared to the 15 other brain regions studied may relate to the central role played by this brain region in the regulation of mood [7,13,24,41,43]. Recent neuroanatomic studies have found a significant post-mortem depletion of glial cells in the anterior cingulate of patients with a familial form of major depressive disorder or bipolar disorder [24]. Post-mortem depletion of glia and neuronal abnormalities have also been found in lateral regions of the prefrontal cortex of depressives [36]. The acute administration of ECS and forced swimming is not likely to reduce the number of glia in the anterior cortical regions of rats. However, the critical function of these cells in maintaining potassium homeostasis in the extracellular fluid, for coupling of neuronal activity and energy metabolism and for the uptake and recycling of neuronally released glutamate may have been altered sufficiently to change the biosynthesis and release of prepro-TRH-derived peptides as
Fig. 4. Effect of electroconvulsive seizures (ECS), sham ECS, forced swimming (swim) and no swim on TRH levels in three limbic regions of male Wistar rats.

Fig. 5. Effect of electroconvulsive seizures (ECS), sham ECS, forced swimming (swim) and no swim on Ps4 levels in hypothalamus and five limbic regions of male Wistar rats.
well as mood-regulating biogenic amines and other neuropeptides or the responsiveness of the cognate receptor and second messenger systems [6,16,24].

Interneuronal signalling by biogenic amines, amino acids, neuropeptides and protein hormones is mediated primarily by G-protein-coupled receptors [10]. The great diversity and specificity of signalling between cells is due to the variety of somatic and dendritic couplings between neurons [8], the large number of receptor subtypes with varying G-protein coupling preferences [2,9,10,12,15,18] and the heterogeneous distribution of G-proteins within different brain regions, cell types and subcellular domains [2,23]. ECS, as discussed above, alters prepro-TRH gene transcription, translation and post-translational processing. Levels of the TRH receptor [17] and G-proteins [2,14] within a variety of limbic and extra-limbic regions of the mammalian brain are also modified by ECS. Given this pleotropic action of ECS, it should not be surprising that in the anterior cingulate, at least, an atypical reduction in TRH–Gly–IR level was observed following the combined treatment with ECS and the depressogenic forced swim test.

Inability to detect an increase in ECS-induced TRH levels in hypothalamus is most likely due to the rapid rate and high efficiency of prepro-TRH processing to form tripeptide TRH in the TSH-regulating periventricular neurosecretory neurons projecting to the hypothalamic–pituitary portal circulation [45]. The occurrence of less abundant TRH-containing neurons within the hypothalamus that are less efficient in converting TRH–Gly to form TRH but are susceptible to upregulation of prepro-TRH expression by ECS, is evidenced by the ECS-induced increase in TRH–Gly–IR, and Ps4–IR. These cells do not, however, contribute enough TRH to alter the high level of this tripeptide produced for neurosecretion into the portal system, which is primarily under the control of thyroid hormone negative feedback [4].

TRH injected intrathecally almost immediately reduces depressive symptoms in 50% of severely depressed patients [20]. The therapeutic potential of this tripeptide, however, is limited by the necessity of intrathecal injection to minimize rapid degradation by TRH-degrading enzymes in the circulation and to maximize restricted transport across the blood–brain barrier by a non-saturable, enzyme degradation-coupled mechanism [25,50]. These limitations would not apply, of course, to ECT-induced intracerebral synthesis of TRH and other prepro-TRH-derived peptides that might augment the antidepressant action of TRH. For example, Ps4 is a TRH-enhancing peptide that lacks any inherent TRH-like effects but greatly increases the activity of TRH in pituitary, brain and extra-CNS systems [5,27,49].

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**Fig. 6.** Correlation of EEP levels and Ps4 concentration in three posterior brain regions and ventral striatum of male Wistar rats. All correlations are $P<0.01$. EEP–IR calculated as the difference between EEP RIA results, which gives sum of EEP and TRH levels [31], and the specific TRH RIA.
Fig. 7. Correlation of EEP levels and Ps4 concentration in four limbic brain regions, of male Wistar rats. All correlations are $P<0.02$. EEP–IR calculated as the difference between EEP RIA results, which gives sum of EEP and TRH levels [31], and the specific TRH RIA.

In summary, corneal ECS increases the expression of prepro-TRH-derived and other TRH-like peptides in limbic, including hypothalamic and cingulate, regions and lateral cerebellum of the rat brain in addition to its previously demonstrated effects in multiple limbic regions. The levels of prepro-TRH-derived and related peptides vary proportionately in all brain regions studied, whether or not their expression is inducible by ECS. The levels of TRH–Gly–IR in the amygdala are at least 10-fold higher than in the other brain regions studied. We conclude that ECS induction of TRH and TRH homolog expression in rat limbic and extra-limbic regions contributes to the accumulating evidence for TRH and TRH-like peptides as mediators of the antidepressant effects of ECT [43].

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References


