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TRH in Therapeutic vs. Nontherapeutic Seizures: Affective and Motor Functions

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SATTIN, A., A. E. PEKARY AND R. L. LLOYD. *TRH in therapeutic vs. nontherapeutic seizures: Affective and motor functions.* PHARMACOL BIOCHEM BEHAV **62**(3) 575–583, 1999.—We have modeled some aspects of electroconvulsive therapy (ECT) in rats. In addition to sham-treated controls, one group received two electroconvulsive (ECS) current–doses at grand mal seizure threshold. Two more groups received three additional ECSs at two higher current–doses. Only the two suprathreshold groups showed significant antidepressant (AD) effects in the forced-swim test, but all three seizure groups showed significant increases in TRH and related peptides in anterior cortex (AC), pyriform cortex (PYR), amygdala/entorhinal cortex (AY), and hippocampus (HC). In motor cortex (MC), TRH appeared to be increased only in the lower dose suprathreshold ECS condition. No condition increased TRH in striatum (STR). These results fell short of directly implicating limbic TRH in AD effects, but in HC, MC, and STR, correlations of peptide levels with individual swim scores raise the possibility that this peptidergic system might be involved in motor as well as affective functions. Other peptides related to TRH might also be implicated in affective regulation and antidepressant effects. © 1999 Elsevier Science Inc.

Thyrotrophin-releasing hormone TRH Affect Emotion Antidepressant Electroconvulsive Seizures Parkinsonism Forced swim Limbic THR-enhancing peptide Motor

THE antidepressant (AD) efficacy of electroconvulsive therapy (ECT) is dependent on the degree to which the electrical stimulation exceeds the threshold needed to initiate seizures within a particular patient. Further increase in current–dose might increase memory side effects, while lower currents too close to the threshold result in failure to achieve the therapeutic effects (65–67). In an attempt to examine the mechanisms underlying the relationship between seizure threshold and AD efficacy of clinical ECT (64), we have studied the change in immobility time during the Porsolt forced-swim test of young adult male Wistar rats in response to threshold electroconvulsive shock (ECS) or higher current suprathreshold ECS. Suprathreshold ECS has previously been shown to elevate TRH and its immediate precursors in limbic and forebrain regions of rat brain (73).

The seizure-induced increased synthesis and action of TRH in multiple limbic regions has been correlated (72,73) with increased forced swimming (7,57), an established neuropharmacological predictor of AD effects. Direct IP administration of TRH to rats produces a dose-related AD effect in the forced-swim test (46). In humans, direct assessment of TRH content or synthesis in CNS in vivo has not yet been achieved, but a role for TRH in AD treatment is supported by observations of rapid remission of major depression, within hours, following intrathecal injection of 500 μg of TRH (10, 41). These new clinical results have continued to sustain the possibility that TRH generated endogenously by seizures might contribute to the AD effects of ECT. If so, this might be a unique mechanism, because several reports have indicated that acute and chronic treatment with antidepressant drugs fail to alter TRH levels in the limbic forebrain (34,48,69). TRH is also known to be antiepileptic and antiepileptogenic [reviewed in (29)]. Its endogenous synthesis and action might, therefore, also contribute to the clinical coincidence of recovery from depression, together with the shorter lasting increase in seizure threshold following a course of ECT [reviewed in (67)].

TRH is biosynthesized from prepro-TRH. In rats, this is a protein with five repetitive sequences of -Lys-Arg-Gln-His-

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Pro-Gly-(Lys/Arg)-Arg- (32). Following endopeptidase cleavage at dibasic residues by endopeptidases, the C-terminal basic amino acid residues are removed by zinc-dependent carboxipeptidase H (56), the N-terminal Gln is cyclized to pyroglutamate (9), and the remaining C-terminal Gly is cleaved by the copper, ascorbate, and oxygen-requiring peptidylglycine α-amidating mono-oxygenase (PAM) enzyme, which carries out the final conversion of TRH-Gly to TRH (16). This processing may begin at the trans-Golgi network and continue in the secretory vesicle during transport to the site of neurosecretion (45,46). Because our previous results with ECS given to rats demonstrated rate limitation at the final (PAM enzyme) step in TRH synthesis in the limbic forebrain, we have continued to utilize our RIA for TRH-Gly and its N-terminal extensions in the present study (50,73). These data were previously presented in abstract (72).

METHOD

Male Wistar rats, 160–180 g on arrival (Simonsen Labs, Gilroy, CA), were divided into four groups of 12–13, housed in pairs on baked wood-chip bedding. After 1 week of handling and quarantine (normal 12-h/12-h light/dark cycle), all were individually immersed for 15 min in a 30-cm depth of water, 25°C, for the "preswim" portion of the Porsolt forced-swim test (7,57) in polycarbonate cylinders (22 cm i.d., 45 cm high). Each cylinder was rinsed and refilled with fresh water prior to each swim. Each rat was hand dried with towels and returned to its home cage.

The following day, excluding one sham-treatment group, seizure threshold was determined on each rat in 1-millicoulomb (mC) increments from 1 mC, every 10 min, ending in a grand mal seizure, which usually occurred at 5 mC (80% of rats. The rest were 5 ± 1 mC). Using these 1-mC increments in current–dose, when a seizure occurred, there was never any ambiguity about its behavioral type (kindling type vs. generalized grand mal type).

All ECS employed the Ugo Basile Model 7801 Unipolar square-wave ECS pulse generator, Stoelting Co., Wood Dale, IL.

On each of the next 3 days sham ECS, Flat ECS, or Ramp ECS was administered to each of the four groups (Table 1). The electrodes were polished brass rods in a wooden holder. They were held onto the corneas after application of artificial tears while gently restraining the rats with gloved hands. [Of the two available noninvasive routes of seizure induction in rats, the corneal route simulates bifrontotemporal ECT more closely than the pinnate route. Corneally induced increases in TRH in anterior limbic regions correlated better with swim behavior than pinnate-induced changes (69)]. Flat ECS consisted of 9.43 mC (0.9 ms pulse width) of current applied corneally. Ramp ECS employed 9.43, 23.5, and 33 mc on the 3 successive days. The threshold tests were repeated a day after the last ECS (Table 1).

An attempt to mimic the clinical practice of ECT provided the rationale for the choice of these currents. It has been established by clinical research that grand mal seizures induced at just-threshold levels are not reliably therapeutic. It has also been established that, with suprathreshold stimulation, current–dose is directly related to the magnitude of the therapeutic effect. The Ramp ECS condition provided a condition of maximal current–dose, as well as mimicking typical clinical application of current–dose increments across the sequence of treatments. All of these clinical considerations have been recently reviewed (64).

The day after the last ECS all rats were forced-swim tested in 16-cm water depth for 5 min while visually scored by two simultaneous observers for immobility time (seconds). The "AD effect" by this test is decreased immobility, i.e., the rats are more active in the water (less immobile) after effective treatment. This is quantitated by the cumulated duration of immobility during the 5 min immersion (73). The grouped mean results of this test are highly predictive of the AD effects of drugs and ECS (7,57). The occurrence of false positives for stimulant drugs can be screened out by adding tests of the locomotor function (7). However, ECS does not increase locomotor function in rats (68).

Although rats are natural swimmers, the swim experience does constitute a mild stress. Previous data comparing TRH and TRH-Gly levels in limbic and cortical regions in swim vs. no-swim conditions failed to reveal any generalizable effect of swim. A no-swim control group was, therefore, not included (73).

The day after the forced-swim test all rats were sacrificed. Six limbic and forebrain regions were freehand dissected on an ice-chilled plate (2). Motor cortex (MC) was defined as the

TABLE 1

FLOW SHEET FOR THE FOUR EXPERIMENTAL PROTOCOLS

	S+S Shm ECS/Shm T-hld	S+T Shm ECS/T-hld	F+T ECS/T-hld (Flat)	R+T Ramp ECS/T-hld
Day 1	Preswim, 15 min	→	→	→
Day 2	Sham threshold	Determine threshold (∼5 mc)	→	→
Day 3	Sham ECS	→	ECS-9.43 mc	ECS-9.43 mc
Day 4	Sham ECS	→	ECS-9.43 mc	ECS-23.5 mc
Day 5	Sham ECS	→	ECS-9.43 mc	ECS-33.0 mc
Day 6	Sham threshold	Determine threshold (∼5 mc)	→	→
Day 7	Forced-swim test, 5 min	→	→	→
Day 8	Sacrifice	→	→	→

S+S: Sham ECS and sham threshold determination. S+T: Sham ECS and threshold determination. F+T: Three suprathreshold ECS at fixed (flat) current-dose and threshold determination. R+T: Three suprathreshold ECS given at increasing (ramped) current–dose and threshold determination. See text for method of threshold determination.

region, excluding pyriform, between the anterior and posterior margins of the underlying striatum (coronal cuts). In addition, two medulla regions, ventrolateral quadrant, and dorsomedial quadrant containing the locus coeruleus were taken. All regions were bilaterally pooled before weighing. Variation in mean weights of the freehand-dissected regions between the four protocol groups was 10% (NS). Tissues were boiled in 1-M acetic acid at 95°C then homogenized with a Polytron™ probe. The samples were lyophilized to a damp residue, extracted with 2 ml of methanol, centrifuged, and the supernatant dried on a heater block with a fan blowing air across the tubes. Dried extracts were stored at −20°C until reconstitution with 1.0 ml of 0.2% NaN_3 just prior to RIA.

Radioimmunoassay (RIA) Procedures

TRH and TRH-Gly RIAs were performed on tissue extracts as previously described (49,55). Extraction efficiencies are 94 and 71% for TRH (53) and TRH-Gly (52), respectively. All samples were measured in duplicate. The TRH RIA is highly specific for pGlu-His-Pro-NH_2 (TRH; crossreactivity with TRH-Gly <0.01%). The TRH-Gly RIA reacts with pGlu-His-Pro-Gly (TRH-Gly) and its N-terminally extended precursor peptides with the carboxy (COOH) terminal sequence -His-Pro-Gly. Therefore, RIA results reported as "TRH-Gly" refer to TRH-Gly itself plus all of its N-terminally extended peptides, although the TRH-Gly tetrapeptide predominates in brain tissue after ECS (50). However, the longer N-terminal extensions of TRH-Gly are also precursors of TRH. Peptide results are given as ng/g wet weight of tissue (identical to pg/mg wet weight of tissue) (49).

Data Presentation and Statistical Analysis

All data are presented as mean ± SEM unless otherwise indicated. Correlational analyses were carried out with the aid of Statview 512+ (BrainPower, Inc., Calabasas, CA). The Sheffe test was used for group comparisons of mean levels of TRH and TRH-Gly. Group differences of $p < 0.05$ were considered significant.

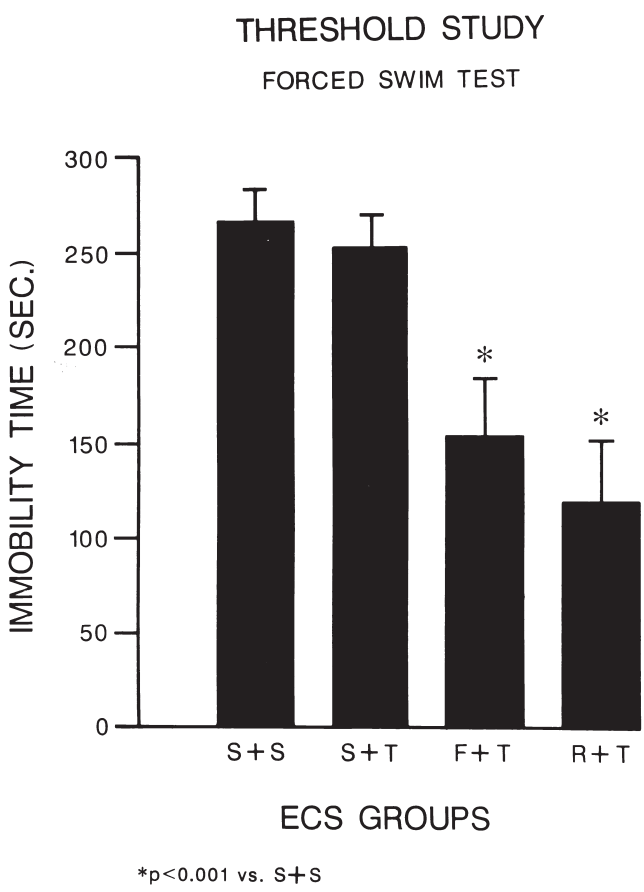

FIG. 1. Immobility time (s) in the Porsolt forced-swim test vs. treatment protocol (mean ± SEM). Treatment protocols: sham ECS—sham threshold ECS (S+S); sham ECS—threshold ECS (S+T); flat ECS—threshold ECS (F+T); ramp ECS—threshold ECS (R+T). See the Method section for details of treatment protocols.

RESULTS

Seizure Threshold

This did not change (not shown) following any of the series of ECSs in any of the ECS protocols. At currents approaching the threshold for grand mal seizures, the transcorneal stimuli resulted in seizures of the type seen during kindling, showing one or more features of the typical Racine (60) progression in facial, forelimb, and hindlimb responses.

ECS: Porsolt Forced-Swim Test

In the forced-swim test, the rats experiencing only the two threshold grand mal seizures (S+T treatment protocol) showed no significant reduction of immobility (Fig. 1). Both suprathreshold ECS schedules significantly reduced the immobility score (i.e., swim activity was increased), which is the indicator of the AD effect. The highest suprathreshold current dose (R+T) group showed the greatest reduction in immobility, but this was not statistically significantly lower than the reduction in the F+T group (Fig. 1).

ECS: Radioimmunoassay

As previously noted (73), basal levels of TRH and its peptide precursors vary greatly as a function of region. Also, as noted previously (73) levels of the precursors of TRH (TRH-Gly) are an order of magnitude higher (Fig. 2). This is explained by the fact that the enzyme peptidyl glycine-amidating mono-oxygenase (PAM) is rate limiting of the conversion of TRH-Gly to TRH in the extrahypothalamic brain and other tissues (55,73,75,76). Also, TRH might be differentially metabolized in the different brain regions, which might explain some of the apparent differences in its ratio to TRH-Gly in those regions. In general, experimental effects on TRH were paralleled by the effects on TRH-Gly (Fig. 2).

All seizure protocols produced statistically significant increases in the TRH-Gly (Fig. 3, upper panel) and TRH (Fig. 3, lower panel) concentrations for the anterior cortex (AC), pyriform cortex (PYR), amygdala/entorhinal cortex (AY), and hippocampus (HC) compared with the sham ECS-sham threshold (S+S) treatment protocol ($p < 0.05$). In AC, PYR and AY the TRH values in the F+T protocol were significantly higher than in either S+T or R+T. In HC, the TRH value in the F+T protocol was higher than in S+T. In PYR, the TRH-Gly values in the F+T protocol were significantly higher than in either S+T or R+T. In HC, the TRH-Gly value in the F+T protocol was significantly higher than in R+T.

The mean basal (S+S) levels of TRH in forebrain were, in descending order of TRH content: AY = 1.123, PYR = 0.636,

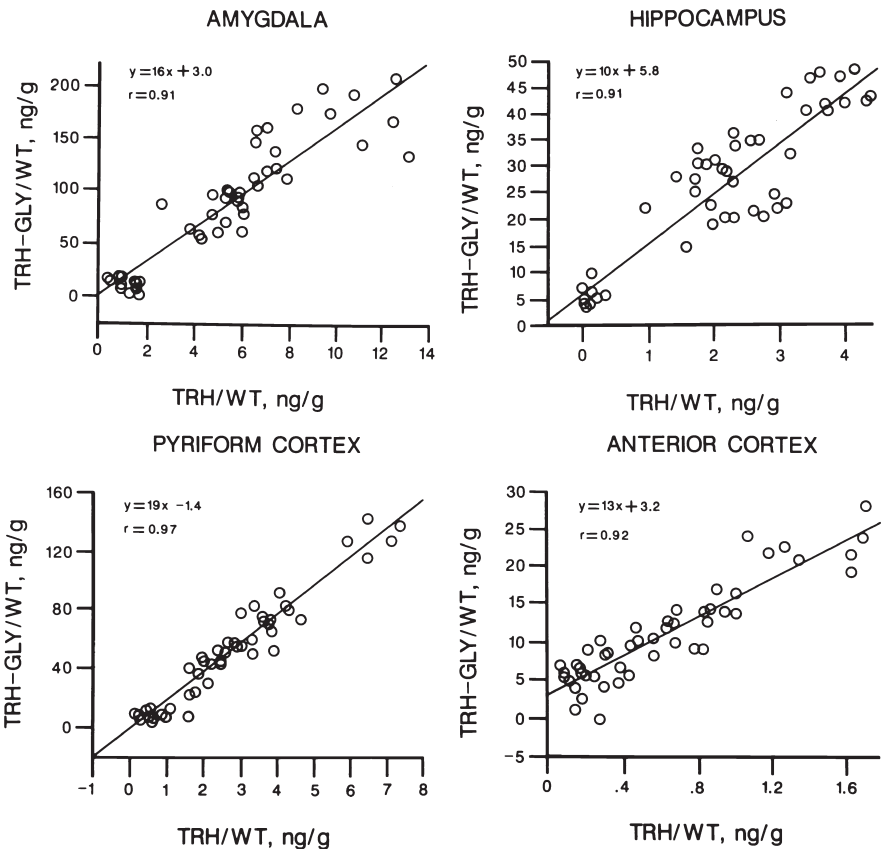

FIG. 2. Correlation of TRH-Gly and TRH results for the amygdala (AY), hippocampus (HC), pyriform cortex (PYR), and anterior cortex (AC) from all of the treatment groups. All correlations were significant at $p < 0.001$.

AC = 0.137, HC = 0.117, and MC = 0.032 ng/g tissue. The greatest regional ratio of TRH level, AY/MC was 35. The largest increases in TRH, relative to controls, following seizure induction were in HC (27.4-fold and 22.9-fold, for F+T and R+T, respectively). The next largest increase, relative to controls, was an 11.1-fold increase in MC for F+T, but at increased current (R+T) it was only 4.1-fold increased (NS, Fig. 4).

TRH content of MC increased with the number of grand mal seizures, except in the highest current group, R+T. In the S+T protocol, TRH appeared to increase in MC, but not significantly. Significantly increased TRH occurred only in the F+T protocol, i.e., three additional suprathreshold seizures were required (Fig. 4). Among the regions chosen for analysis, only in MC did the increase in TRH appear to be proportional to the number of grand mal seizures (S+T vs. F+T). However, other regional cortical localizations were not sampled, so similar changes might have occurred elsewhere.

Typical of all of our previous results, there were no seizure-induced changes in the measured TRH peptides in striatum (Fig. 3). No seizure-related peptide changes were seen in the regions encompassing locus coeruleus or ventrolateral medulla (not shown).

ECS: Correlation of Forced-Swim Scores and Regional Levels of TRH and TRH-Gly

In this analysis, data from the forced-swim test is utilized in a way that differs from the original Porsolt comparison of group means. Swim immobility times were negatively correlated with TRH levels in the HC and with TRH-Gly levels in STR and MC, using all data from the four treatment groups (Fig. 5). Stated differently, those animals (the individual points of Fig. 5) that were most active in the water had the highest amounts of the TRH peptides in those regions. These findings confirm and extend our previous observations of negative TRH-Gly correlations with swim immobility in AC, PYR, HC, and AY (73). Only in HC (Fig. 5) did this correlation hold for TRH itself, replicating our previous observation (73). Although the TRH-Gly/swim correlation reached statistical significance in STR, the functional significance for that region may be questioned, as mean peptide levels were unaffected by any of the seizure conditions, as has always been observed in this region. In all other regions correlations of immobility with TRH or with TRH-Gly did not reach the $p < 0.05$ level of significance.

DISCUSSION

The peptide data of this study represent single time point levels measured 1 day after the forced-swim tests (and 2 days after the final ECS). Nevertheless, it is valid to relate the swim results with the peptide levels taken 24 h later at sacrifice, because our previous work has shown that the seizure-induced changes in TRH last for many days after a series of three ECS, with substantial increases persisting for over 1 week

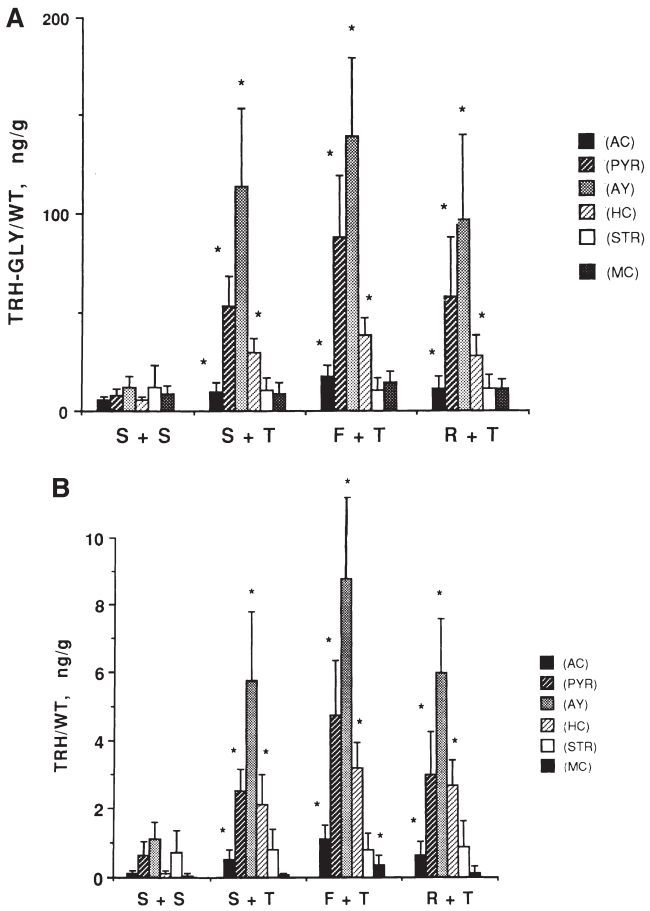

FIG. 3. Comparison of TRH-Gly/wet weight (upper panel) and TRH/wet weight (lower panel) ratios for various brain regions among the four treatment protocols. Brain regions examined: anterior cortex (AC); pyriform (olfactory) cortex (PYR), amygdala/entorhinal cortex (AY); hippocampus (HC); striatum (STR); motor cortex (MC) (*$p < 0.05$ by Sheffe *F*-test, compared to S+S). There were no significant differences between S+T and R+T for either peptide in any of the regions. See text for other comparisons.

(69,70). The physiological significance of these seizure-induced increases in TRH and its precursors are not fully understood, but seizures are known to induce the synthesis of the prepro-TRH protein (28), and there is evidence for TRH release (26) and TRH receptor downregulation following seizures (33,74) suggesting activation of TRH receptors.

As expected, the mean immobility of group S+T was not reduced. These rats experienced only two threshold-level grand mal seizures. Also, as expected, the mean immobilities of the two suprathreshold groups, F+T and R+T were significantly reduced (Fig. 1).

In most of the limbic forebrain regions (AY, HC, PYR, and AC), both TRH and its peptide precursors were increased in parallel following all three seizure conditions, threshold, and suprathreshold. In fact, while some regions showed significantly higher values in the F+T condition than either the S+T or R+T condition, none of the S+T (threshold-only) means differed from those of the highest current group, R+T. The evidence from the tissue levels of TRH in these important limbic regions does not support a contributory role of TRH in the antidepressant effect, because significant elevations of this peptidergic regulator occurred in the absence of any antiimmobility effect. Although this data is not supportive, it also cannot, by itself, rule out such a role for TRH in these regions. In the behaviorally positive groups it is possible that the TRH in these regions was turning over more rapidly than in the S+T group, or that TRH receptor activation was augmented. It is unclear whether those questions can be experimentally approached at this time. One of many technical problems is the lack of any TRH receptor antagonists.

The correlation of TRH levels in HC with immobility (Fig. 5) replicates a result from our previously reported study using suprathreshold seizures (73). Conditions were similar to the present, except that 1) the corneal ECS was entirely given according to a similar "ramp" schedule of increasing currents, and 2) no additional threshold stimuli were given. MC was not dissected, but a significant correlation of TRH-Gly with immobility was seen in AY. All correlations were in the same direction as those seen in this study (Fig. 5). Thus, we have been able to replicate this chemical/behavioral effect. The meaning of this effect remains unclear. If an antidepressant mechanism of TRH could be shown by other mechanisms, this would be supportive. However, it is possible that the behaviorally related changes in TRH are correlated with an unknown substance whose antidepressant effects might be shown to correlate more accurately with reduction of immobility. We have obtained the initial evidence for another candidate peptide that might fulfill this role (35,51), and correlative studies are planned.

The only brain region where the ECS-induced increase in TRH correlated with the reduced immobility on the forced-swim test was MC, and only in the F+T, but not the R+T condition (Fig. 4). As an isolated finding, this result might be spurious, but if replicated, it might indicate that MC is a region where PAM enzyme induction by ECS is required to overcome the rate limitation by that enzyme of the synthesis of TRH from its immediate precursor, TRH-Gly (50,73). This interpretation would be compatible with the failure of any of the ECS conditions to alter TRH-Gly levels in that region (Fig. 3, upper panel). Although it is conceivable that additional enzyme induction, that of a TRH-degrading enzyme, might explain the lack of effect of condition T+R on TRH level in MC, only additional studies correlating enzyme levels with the changes in the peptide levels would be capable of resolving this. Despite the lack of effect of ECS on TRH-Gly, MC displayed the highest and most significant negative correlation of TRH-Gly level with immobility (Fig. 5). Thus, if confirmed, the seizure-induced increase in TRH in this region might be more directly related to the change in immobility than the TRH changes in the limbic regions.

The lowest brain level measured in this study, the control level of TRH in MC, was only 23% of the TRH content of the rostrally adjacent AC. On the other hand, the control levels of TRH-Gly were the same in these two cortical regions. This phenomenon is also potentially explainable with reference to the PAM enzyme, but in this instance, to a regional difference in enzyme activity, which could be experimentally investigated. If low TRH level in MC implies decreased TRH activity there, seizure susceptibility may be enhanced (29,58). Diminished activity of TRH in MC might contribute to the clinical finding that ECT given at threshold levels of current often yields a seizure that is restricted to the motor system, i.e., convulsive motor movements may be seen in the absence of seizure generalization by EEG (65–67). Such seizures lack therapeutic efficacy probably because they are localized to

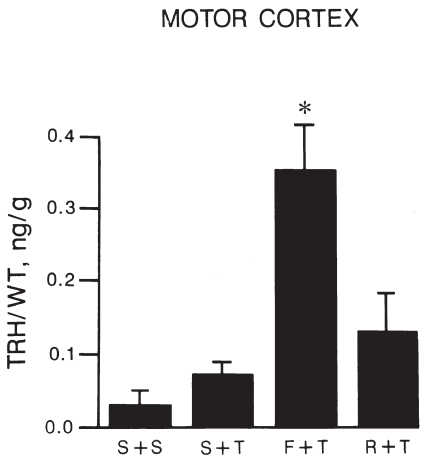

FIG. 4. Comparison of TRH/wet weight in motor cortex among the four treatment protocols (enlarged from Fig. 3, lower panel; $*p < 0.05$ by Sheffe *F*-test, compared to the three other protocols).

motor cortex and do not involve multiple limbic and forebrain regions. Thus, the low seizure threshold characteristic of MC might be consequent, in part, to the low basal level of TRH found there. TRH levels have been reported in multiple forebrain regions of Vervet monkeys, which were similar to rat (24), but there are no established data for the human brain.

The mean levels of TRH in STR were unchanged following all seizure conditions, as has been repeatedly observed in previous studies (30,31,70,73). Despite this, when the values from all protocols were plotted, TRH-Gly in STR correlated significantly with immobility (Fig. 5). It is possible that the large glutamatergic neurons in MC (12) that project to STR contain TRH as a cotransmitter, as has been seen in histochemical observations of limbic cortical regions (29,37,38). The large postseizure increases in TRH in MC might have contributed, via this pathway, to the result seen in STR (Fig. 5). There is precedent for anterograde transport of regulatory peptides from cortex to striatum in the case of BDNF (1). With respect to TRH itself, it was previously shown that TRH immunohistochemically localized to the fornix projection from the medial septum may account for part of the TRH content of ventral HC (37,38). This type of interregional transport remains to be investigated in the corticostriatal pathway.

There was no increase in seizure threshold across the progression of suprathreshold seizures in this study, although this is known to occur in rats, and it is usually seen in patients receiving ECT (58,65–67). However, a clinical series of ECTs is longer than the one used here, usually requiring 6–12 treatments, given over 2–4 weeks.

The first report of seizure-induced increases of forebrain TRH (27) led to the suggestion that TRH might function as an endogenous AD (30,31), because TRH given IV (500 μg) induced remissions in 1–3 h, lasting 1–2 days in some patients in early studies (22,59). Recently, 500 μg TRH injected intrathecally in Major Depressed humans induced similarly fast remissions lasting 2–3 days vs. no effect of vehicle placebo, and the effects were more reliable than in the early studies (10,41). TRH, given IP to rats, induced a dose-related AD effect by the forced-swim test (46). Thus although the TRH–behavior correlations in the present study fail to support a causal link (with the possible exception of MC) the new clinical findings are consistent with a contributory role for TRH in therapeutic

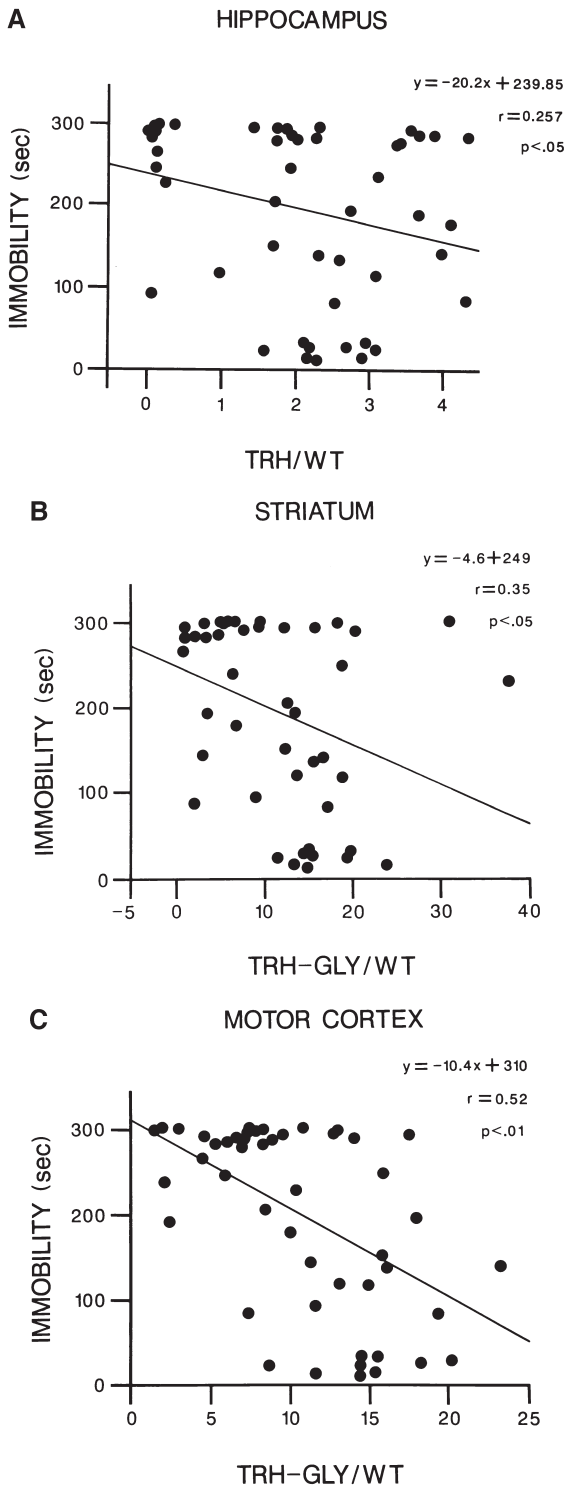

FIG. 5. Correlation of swim immobility time in the Porsolt forced-swim test with TRH/wet weight (hippocampus) and with TRH-Gly/wet weight (striatum and motor cortex). Units for all peptide/wet weight values are ng/g.

seizures. The correlated increases in TRH-Gly are also consistent with this idea, because TRH-Gly is the most immediate precursor of TRH. PS-4 (8), a coprocessed decapeptide product of prepro-TRH, also correlated with swim behavior as did TRH-Gly (54). PS-4 potentiates the endocrine effects of TRH on the release of TSH and acid secretion but it has no intrinsic activity on TSH release or acid secretion (8,78). It is not yet known whether PS-4 participates in the behavioral functions of forebrain TRH.

Another peptide coprocessed from the TRH precursor, prepro-TRH-(178–199) has been called corticotropin-release inhibitory factor (CRIF). The secretion of ACTH was inhibited at a site that differed from the receptor for corticotropin-releasing factor (CRF) (62,63). This might provide a basis for functional interaction between TRH and CRF, two peptidergic systems that are involved in affective disorders. A reported failure to confirm this (44) was based upon a commercial source of CRIF that had oxidized on the shelf of the supplier (E. Redei, personal communication). Using a rat model of stress designed to activate the release and action of CRF, ICV infusion of this 22-amino acid peptide reversed stress-induced behaviors (42). Therefore, like PS4, CRF may also have a physiological role that complements the putative AD role of TRH or a related peptide. CRF is a widely distributed endogenous neuropeptide in animals and humans that promotes or produces depressive behaviors. Excessive CNS activity of CRF is believed to underlie the failure to suppress plasma cortisol following infusions of dexamethasone in two-thirds of Major Depressed patients (3,11,18,19,25,47).

TRH has been shown to be released from hippocampal slices superfused with depolarizing concentrations of potassium, and significantly more TRH was released using tissues taken from rats previously receiving generalized seizures, which increased tissue levels of TRH (26). It would be of interest to know whether the peptides coprocessed with TRH are also coreleased with TRH.

The limbic/cortical TRH systems described here appear to be regulated differently from the hypothalamic TRH system, which functions in hypothalamic–pituitary–thyroid regulation. Peripheral thyroid hormones exhibit feedback regulation of TRH synthesis in the hypothalamus, but such regulation did not occur in limbic regions where kindled seizures induced the synthesis of mRNA for prepro-TRH (23). This suggests that peripheral thyroid hormones may not play a role in initiating synthesis of TRH or in processing the peptide precursors of TRH in limbic regions, but we cannot rule it out.

Triiodothyronine (T3) has been clinically demonstrated to potentiate or shorten the latency to clinical response of various AD drugs [see review, (21)]. The same clinical effects were also reported when 50 μg T3 was orally administered daily, in conjunction with ECT (77). New evidence suggests a novel mechanism through which thyroid hormone might affect behavior: uptake into nerve endings where it might function as a cotransmitter (40).

PET findings in human depression deviate from normal in the caudate as well as the limbic cortical and subcortical regions (13,15,17,43), but the motor neocortex has not yet been implicated. The present study suggests possible involvement of the primary motor cortex in the motor abnormalities associated with affective disorders. TRH is known to be present in all of these regions in Vervet monkeys (24) as well as rats. Other CNS regions and subregions which have not yet been systematically studied might also display seizure-induced changes in TRH and related peptides. Enlargement of this anatomical dimension may further enhance our understanding of affective and motor functions.

Seizure-induced increases in TRH in MC (Fig. 4), and the behavioral correlations with TRH levels in MC and striatum (Fig. 5) may also be relevant to the increasing utilization of ECT for improvement of motor function in end-stage Parkinsonian patients who are no longer responsive to levodopa/carbidopa [reviewed in (61)]. It has long been known that TRH can activate behavior through aminergic (including dopaminergic) mechanisms (4,5). Do the known projections from the motor cortex to the striatum include TRH-containing fibers that might augment DA release there? The means of TRH-Gly in MC and STR were similar (Fig. 3, upper panel). Although the STR group means did not differ, in the combined data of Fig. 5, the faster swimming rats might have augmented their striatal TRH-Gly via a possible corticostriatal TRHergic pathway, which merits investigation by histochemical and lesion studies.

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