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TRH Protection Against Memory Retrieval Deficits Is Independent of Endocrine Effects

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STWERTKA, S. A., G. P. VINCENT, E. R. GAMZU, D. A. MACNEIL AND A. G. VERDERESE. *TRH protection against memory retrieval deficits is independent of endocrine effects.* PHARMACOL BIOCHEM BEHAV **41**(1) 145–152, 1992.—An electrobrainshock (EBS)-induced memory retrieval deficit was produced in normal and hypophysectomized mice. In normal mice, thyrotropin-releasing hormone (TRH) (0.1 to 30 mg/kg) protected against this EBS disruption of memory after intraperitoneal but not oral (1.0 to 100 mg/kg) administration. In hypophysectomized mice, TRH (0.3 and 3.0 mg/kg) also protected against the retrieval deficit induced by EBS. The memory protection afforded by TRH was unrelated to its ability to elevate plasma levels of triiodothyronine (T_3) and thyroxine (T_4), nor was TRH's memory protection mediated through an anticonvulsive mechanism. These results support the notion that TRH may play an important role in memory modulation and may have therapeutic value in certain disease states in humans.

TRH Memory retrieval Triiodothyronine Thyroxine Avoidance behavior

PATHOLOGICAL and experimentally induced dysfunctions in the processes of memory storage and retrieval have been associated with the changes in the activity of a number of different neurotransmitters. For example, there is extensive literature implicating cholinergic systems in learning and memory in general, and particularly in the cognitive impairments of Alzheimer's disease, where there are decreased levels of cholinergic markers (3,4). Furthermore, impaired dopaminergic function has been correlated with losses in primate learning ability (16,35), whereas impaired adrenergic function has been shown to disrupt learning in rodents (48). Significant reductions in the levels of these two neurotransmitters have also been reported in Alzheimer's disease (1, 2, 9, 47). Additionally, there is decreased serotonergic functioning in the brains of Alzheimer's patients as measured by [^{3}H] ketanserin binding (34). In view of the involvement of multiple transmitter pathways in memory processes, a pharmacological agent that could modulate activity of a number of these relevant CNS neurotransmitters would be of considerable interest as a potential therapeutic entity for pathologies affecting learning and memory.

There are substantial biochemical and neurophysiological data to support the idea that thyrotropin releasing hormone (TRH) and its degradation-resistant analogs might be considered for this purpose. Potentiating effects of TRH and its analogs have been demonstrated for cholinergic, noradrenergic, serotonergic and dopaminergic systems (15, 16, 23, 31). TRH has been shown to facilitate cholinergic neurotransmission (44–46). Iontophoretic application of TRH onto populations of cortical neurons has been reported to potentiate excitation produced by acetylcholine (5, 37, 44). In striatal slices, TRH increases the uptake and conversion of choline into acetylcholine (28). TRH can also shorten barbiturate-induced sleep, an effect that is antagonized by atropine (25). TRH and its analogs also potentiate monoaminergic neurotransmission by increasing the turnover and release of dopamine (26,42) norepinephrine (19,21) and serotonin (33). The potentiating activity of TRH in many of the neurotransmitter systems implicated in memory storage and retrieval suggests that it might be effective in ameliorating some forms of memory disruption. TRH and its analogs have been reported to reverse behavioral impairments induced by: septohippocampal lesions in rats (17); cycloheximide, CO_2, or basal forebrain lesions in mice (27); and behavioral deficits in senescence-accelerated mice (43).

To assess the possible therapeutic potential of TRH-like peptides in cognitive disorders, we chose to evaluate the effects of TRH in an animal test of disrupted memory retrieval. The test employed the delivery of a brief subconvulsive electrobrainshock (EBS) to mice in order to disrupt retrieval of an avoidance response. This test has been shown to be sensitive to the memory-protective effects of a number of nootropic compounds including piracetam and aniracetam (11). In order to determine whether any protective effects of TRH in this procedure were mediated directly through the nervous system or indirectly through the pituitary/thyroid axis, experiments were performed in both normal and hypophysectomized mice. Plasma levels of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) were measured in all the experimental groups in order to evaluate any relation-

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ship between levels of these hormones and the observed behavioral effects.

EXPERIMENT 1

The first study evaluated the memory-protective effects of TRH in mice subjected to a disruptive electrobrainshock (EBS) administered just prior to retrieval testing for an avoidance response.

METHOD

Subjects

Male albino mice, CF1 strain, weighing 19 to 25 grams were housed in groups of 10 for one to two weeks prior to the start of testing.

Apparatus and Procedure

The testing apparatus consisted of a standard Coulbourn mouse chamber complete with a stainless steel grid floor. The procedure required separate training and testing days. Each animal was placed onto an elevated platform supported by a retractable lever mounted 7.5 cm above the grid floor, and immediately a house light and a masking noise were presented. After one minute, the platform was released by the retraction of a solenoid, the mouse was dropped onto the grid floor, and the platform was returned to an upright position. A loud tone and clicking noise were also presented at this time and served as part of the conditioned stimulus (CS). During the next 15 seconds, the animal could avoid a scheduled footshock by returning to the platform before the onset of shock (i.e., avoidance response). Following the CS period, shock (1.3 mA) was administered for 30 seconds (unconditioned stimulus—US), along with the tone and the clicking noise. During this period, the mouse could escape from the shock by jumping onto the platform. In order for an avoidance or an escape response to be scored as such, the animal had to remain on the platform for four seconds. The tone and clicking noise were turned off for four seconds as soon as the mouse jumped onto the platform, but came back on if the mouse failed to stay on the platform for the full four seconds. If the animal did not avoid or escape shock, an escape failure was recorded. Following shock, there was a 10-second safety period during which the tone and the clicking noise were inoperative; this was followed by the initiation of the next trial. The operation and recording of all jumps onto the platform (i.e., closures of the platform microswitch) were fully automated through programming of a BRS Interact and Data General computer system.

Brain shock was delivered transcorneally via stainless steel electrodes covered with saline-soaked cotton pads. A Grass S88 stimulator and a constant current unit delivered a 200-ms train of square-wave unipolar DC pulses (10 ms of 10 mA constant current at 60 Hz). Control animals receiving a sham shock were treated identically, but the power to the stimulator was disconnected.

Training

All mice were given two five-trial training sessions (five trials given between 9:00 a.m. and noon; five trials given between 1:00 p.m. and 4:00 p.m.) on the first day. For each animal, the morning and afternoon training sessions were separated by approximately a four-hour period.

Testing

On Day 2, approximately 22 hours after the last training session, the mice were randomly assigned to one of the treatment groups. Two vehicle control groups (n = 15 and 13) received an injection of physiological saline 30 minutes prior to testing. The first control group of mice received a sham shock (SHAM), whereas the second control group of mice received EBS transcorneally 5 minutes before testing (EBS). The remaining 8 groups (n = 5) of mice were given EBS plus one of eight different doses of TRH (0.03, 0.1, 0.3, 1.0, 3.0, 10, 30 or 100 mg/kg) 30 minutes before testing (25 minutes before EBS). The drug was dissolved in physiological saline and administered intraperitoneally. All mice were tested for 10 trials using the same procedure described for training. An identical experiment was run using doses of 1, 10 and 100 mg/kg TRH administered by gavage 60 minutes before testing, with five mice in each group.

Statistical Analysis

Statistical significance of the results was evaluated on the mean number of avoidance responses for all the groups that were exposed to EBS (sham control animals were not included), using a one-way analysis of variance (41) for overall evaluation of the drug effect. If the results of this analysis were significant at the $p<0.05$ level, an additional analysis of each of the individual dose groups versus the vehicle treated EBS group was conducted using a Dunnett t-test ($p<0.05$) (41).

In addition, to facilitate cross drug and cross experiment comparisons, we also used the mean number of avoidance responses in each dose group to compute the percent recovery of function (%RF) according to the formula:

$$\%RF = \frac{\text{Drug} - \text{EBS}}{\text{Sham} - \text{EBS}} \times 100$$

RESULTS

All mice receiving EBS prior to testing exhibited a Straub tail and were quiescent for up to 45 seconds following the administration of the EBS. In this state, the animals would move if touched and the Straub tail would be lost. After these initial effects of EBS had disappeared, the animals appeared normal for the remaining period before being introduced to the testing chambers. During testing, all mice were able to avoid or escape the footshock once it was initiated, even though in some treatment conditions, the number of avoidance responses was low (i.e., EBS group and for inactive doses of TRH). The ability of mice to make an avoidance response suggests that their motor performance was not impaired by the EBS. Figure 1 illustrates the mean number of avoidances and the %RF for the sham EBS group, EBS group, and each of the TRH treatment conditions. Intraperitoneal administration of TRH prior to EBS resulted in a significant increase in avoidance responding, $F(8,44) = 3.31$, $p<0.01$, during testing. The post hoc Dunnett t-test analysis indicated that all doses of TRH between 0.1 and 30 mg/kg were significantly different from the vehicle-treated EBS group ($p<0.05$ for the dose of 3 mg/kg; $p<0.01$ for the doses of 0.1, 0.3, 1.0, 10, and 30 mg/kg). Although TRH did not completely restore the level of avoidance behavior of EBS-exposed mice to that of sham EBS mice, there was a considerable recovery of function which peaked at 64 %RF at the dose of 0.1 mg/kg. The overall inverted U-shaped dose-response profile of TRH (see Fig. 1) is

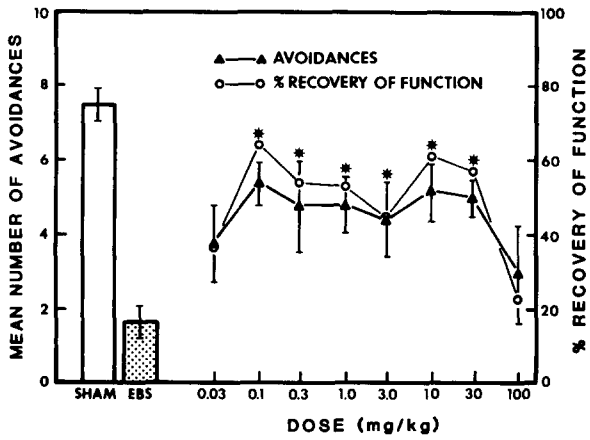

FIG. 1. Mean (±SEM) number of avoidances for mice in the sham EBS, vehicle plus EBS, and each of the TRH plus EBS treatment conditions. The percent recovery of function is shown for each of the TRH plus EBS-treated groups. Vehicle or TRH was administered IP 30 minutes prior to the 10-trial testing sessions. EBS occurred 5 minutes before testing.

characteristic of compounds active in this procedure.

TRH given orally produced only a small elevation in the mean number of avoidance responses exhibited by mice during the testing session (mean: 1.6, 2.4, 3.6, and 3.6 for the vehicle EBS, and 1, 10, and 100 mg/kg for the TRH groups, respectively). When compared to the vehicle EBS control values, these differences were not statistically significant, $F(3,16)=1.26$, $p>0.05$.

EXPERIMENT 2

While the results of Experiment 1 indicate that TRH can protect against the EBS-induced memory retrieval deficit, it is unclear whether the effect is the result of TRH's direct action in the central nervous system or whether the protection is mediated through its endocrine activity. TRH is known to affect the thyroid axis beginning with release of thyrotropin stimulating hormone (TSH) from the pituitary gland. Therefore, we chose to compare normal and hypophysectomized mice for TRH protection of retrieval in our procedure. Since there is always the possibility that hypophysectomized animals will not respond to behavioral paradigms in the same way that normal animals do, the second experiment was designed to ascertain whether the use of hypophysectomized animals in this paradigm was feasible. In this experiment, we compared the response of three groups of mice to the behavioral testing described in Experiment 1. The three groups of mice included hypophysectomized mice, mice with a sham operation, and normal unoperated mice of the same strain, age, and weight.

METHOD

Subjects

The subjects were male albino mice, CF1 strain, weighing 19–25 grams. They were housed in groups of 10 for one to two weeks prior to the start of testing. All animals in the three experimental groups were provided with 5% glucose in water in addition to their solid food for the duration of the experiment in order to insure adequate nutrition for the hypophysectomized animals.

Surgery

Hypophysectomies (hypox) and control hypophysectomies were performed by Charles River Inc. The technique employed was a parapharyngeal approach described in detail by Waynforth (40). The animals were given two weeks recovery from surgery before training or testing.

Apparatus and Procedure

The testing apparatus and procedure were as described in Experiment 1, with the exception that no drug or vehicle was given prior to the testing session. There were 10 mice in each group.

Statistical Analysis

In this experiment, two separate analyses were conducted. A one-way analysis of variance as described for Experiment 1 was conducted on the three control groups that received the sham shock. A separate analysis of variance was conducted on the three experimental groups that received the EBS prior to the testing.

RESULTS

The three groups were very similar in response to both behavioral training and testing. The unoperated, hypox, and sham-operated groups, that received the sham shock prior to testing, had mean numbers of avoidance of 6.4, 6.4, and 7.3, respectively. An analysis of variance showed that there were no differences between these groups, $F(1,18)=0.71$, $p>0.05$. The EBS exposure disrupted the avoidance behavior of the three different experimental groups to the same degree, with the mean avoidance for the unoperated, hypox, and sham-operated groups being 2.5, 1.8, and 1.5, respectively. Again, there were no significant differences among the groups, $F(1,18)=0.21$, $p>0.05$.

EXPERIMENT 3

It is evident from the results of Experiment 2 that hypophysectomy does not impair the ability of mice to learn the avoidance task nor does it alter their susceptibility to the disrupting effects of the EBS. Inasmuch as there were no differences between the three experimental groups in Experiment 2, we used unoperated controls instead of sham-operated mice in subsequent experiments.

To establish a time frame for assaying thyroid hormone levels in subsequent experiments, we performed the following time course study of blood level of T_3 and T_4 after a single injection of TRH in normal mice.

METHOD

Subjects

The subjects were male albino mice, CF1 strain, weighing 19–25 grams. They were housed in groups of 10 for one to two weeks prior to the start of testing.

Procedure

Mice were injected intraperitoneally with 3.0 mg/kg TRH dissolved in saline and sacrificed after one of the following time intervals: 0, 15, 30, 45, 60, 90, 120, or 180 minutes. All treat-

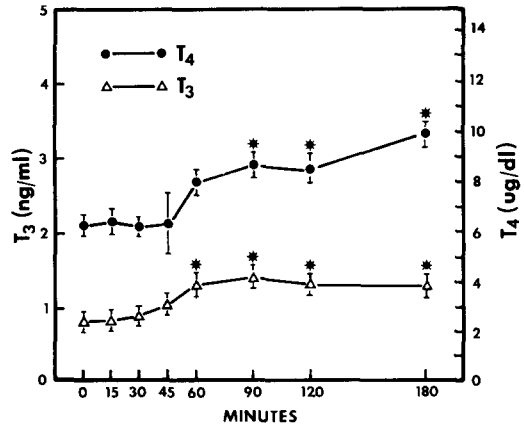

FIG. 2. Mean (±SEM) T_3 and T_4 blood serum concentrations for different groups of mice sampled at various time intervals following administration of TRH (3.0 mg/kg).

ment groups contained 5 animals with the exception of the groups sacrificed at 0, 45 and 120 minutes after TRH administration; these groups contained 4 animals. After the appropriate time elapsed, animals were sacrificed by first rendering them unconscious with CO_2 and then drawing blood directly from the heart through the chest cavity with a 25-gauge needle and syringe. Blood samples were transferred to separator tubes (Microtainer) which were kept on ice until the plasma was separated by centrifugation at 7,000 × g for five minutes. The plasma was stored at −20°C until assayed.

Levels of T_3 and T_4 were determined using commercially available radioimmunoassay kits (Amerlex). Samples and standards were assayed in triplicate. The sensitivity of the assays was 0.1 ng/ml for T_3 and 0.3 μg/100 nl for T_4.

Data Analysis

A separate one-way, repeated-measures analysis of variance was performed on the changes in T_3 and T_4 blood serum concentrations over the time intervals sampled. The mean plasma levels of T_3 and T_4 at the zero time point were compared to each of the other respective time points using a Dunnett *t*-test (41).

RESULTS

Blood plasma T_3 and T_4 concentrations became significantly elevated 60, $F(7,28)=8.56$, $p<0.01$, and 90, $F(7,28)=3.63$, $p<0.01$, minutes, respectively, after TRH administration. As can be seen in Fig. 2, plasma T_3 and T_4 remained at relatively constant baseline levels of 0.8 ng/ml of T_3 and 6.4 μg/dl of T_4 until approximately one hour after TRH injection. Levels of T_4 at approximately 1 hour increased to 8.0 μg/dl, with the last interval sampled at three hours showing a T_4 level of 9.9 μg/dl. In contrast, T_3 levels were significantly elevated at 60 minutes postinjection of TRH (1.1 ng/ml) and remained elevated until the last sampled interval at 3 hours following TRH administration (1.3 ng/ml). A maximum blood plasma concentration of T_3 was reached 90 minutes following injection (1.4 ng/ml). Consequently, in the subsequent studies, mice were sacrificed two hours after TRH administration to maximize the ability to detect any TRH-related endocrine changes.

EXPERIMENT 4

Having determined the time course of blood plasma levels of T_3 and T_4 after TRH injection, we next compared the memory-protective effect of TRH in normal and hypophysectomized mice. Three doses of TRH were evaluated, one of which had been inactive in the first experiment. For both groups of mice, levels of T_3 and T_4 were assayed after behavioral testing.

METHOD

Subjects

The strain and weights of mice used were the same as described in the previous experiments. Ten groups of animals were used, with each group containing 10 mice. One-half of the mice (5 groups) had undergone hypophysectomy as described in Experiment 2. The 50 remaining mice served as unoperated controls.

Apparatus and Procedure

The apparatus and procedure used in this experiment were identical to that described in Experiment 1, with the exception that only three doses of TRH (0.03, 0.3, and 3.0 mg/kg, IP) were used. Seventy-five minutes after the termination of the behavioral testing (corresponding to 120 minutes after TRH administration), the animals were rendered unconscious, blood plasma samples were collected, and levels of T_3 and T_4 were assessed as described in Experiment 3.

Statistical Analysis

A separate one-way analysis of variance was performed on the number of avoidance responses for the four groups of hypophysectomized mice and for the four unoperated groups receiving EBS and vehicle or TRH (sham control animals were not included). Since both of these analyses resulted in significant F values, Dunnett *t*-test (as described above) post hoc comparisons were used to assess the effects of the different doses of TRH.

In order to evaluate the performance of the mice during the 10-trial testing session, a separate analysis was performed on the number of trials to the first avoidance response. If an animal failed to make any avoidances during testing, it was assigned a score of 11, assuming that it might have avoided on the 11th trial if it had occurred.

A one-way analysis of variance was performed on the T_3 and T_4 blood plasma concentrations for the five treatment groups. Significant differences in main effects were analyzed using Dunnett *t*-tests.

RESULTS

The effects of TRH on EBS-induced disruption of memory retrieval are shown in Fig. 3A, B. TRH produced significant protection against EBS in both hypox and unoperated control groups. In unoperated control mice, TRH was shown to have protected against the memory-disrupting effects of EBS relative to vehicle-treated animals, $F(3,36)=7.28$, $p<0.01$. The doses of 0.3 and 3.0 mg/kg IP of TRH were active in unoperated controls ($p<0.01$ and $p<0.05$, respectively), whereas the dose of 0.03 mg/kg was inactive ($p>0.05$). In the hypox mice, there was also a protective effect of TRH against the EBS-induced memory disruption, $F(3,36)=8.14$, $p<0.01$. As was the case in the unoperated control mice, post hoc Dunnett *t*-test analysis indicated that the dose of 0.03 had no effect on the avoidance be-

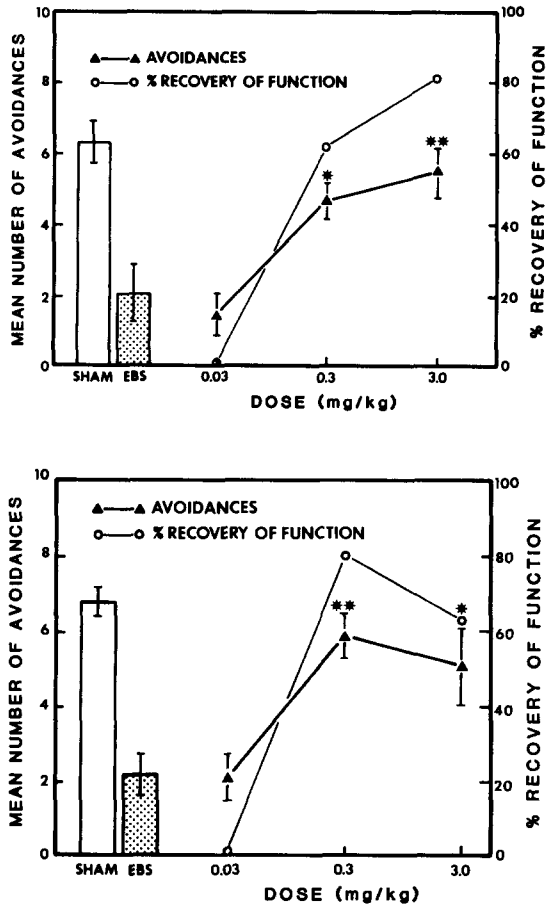

FIG. 3. (A) Mean (±SEM) number of avoidances for nonhypophysectomized mice receiving treatments of sham EBS, vehicle plus EBS, or one of the three doses of TRH and EBS. The percent recovery of function is shown for each group administered TRH. (B) Mean (±SEM) number of avoidances for hypophysectomized mice given sham EBS, vehicle plus EBS, or one of three doses of TRH.

havior, whereas the doses of 0.3 and 3.0 significantly elevated the number of avoidances compared to the vehicle controls ($p<0.05$ and $p<0.01$, respectively). Thus both the behavioral effects of EBS and their reversal of TRH were equivalent in normal and hypophysectomized mice.

The results from analyzing the number of trials to the first avoidance response for all animals (including sham-control mice) in the unoperated and hypophysectomized groups are summarized in Table 1. In both groups, EBS significantly increased the number of trials for animals to make the first avoidance in comparison to sham-treated mice [unoperated: $F(4,45)=4.45$, $p<0.01$; hypox: $F(4,45)=6.31$, $p<0.01$]. Therefore, mice receiving EBS, without TRH treatment, are capable of avoiding footshock. However, the avoidance responses are found to occur later in the 10-trial testing session. The increase in the number of trials to avoid footshock in EBS-treated mice was attenuated by TRH at doses 0.3 and 3.0 mg/kg in unoperated and hypox mice. The lowest tested dose of 0.03 mg/kg was inactive on this behavioral measure in these two treatment groups.

The T_3 and T_4 blood plasma levels for sham EBS, EBS and TRH groups for hypophysectomized and unoperated mice are

TABLE 1

EXPERIMENT IV: NUMBER OF TRIALS TO 1ST AVOIDANCE RESPONSE IN UNOPERATED AND HYPOPHYSECTOMIZED MICE

Treatment/Dose (mg/kg)	N	Mean Number Trials	±SEM
Unoperated			
Sham	10	2.0	0.37
EBS	10	6.1†	0.98
EBS+TRH			
0.03	10	6.1†	1.12
0.3	10	2.9	0.48
3.0	10	4.4	1.13
Hypophysectomized			
Sham	10	2.3	0.37
EBS	10	7.3†	1.40
EBS+TRH			
0.03	10	7.6†	1.20
0.3	10	4.3	0.65
3.0	10	3.4	0.69

*$p<0.05$; † $p<0.01$, compared to sham-EBS group.

shown in Fig. 4A, B. Vehicle-treated control groups had equivalent blood plasma levels of T_3 and T_4 hormones, regardless of whether they had received EBS or sham EBS, indicating that EBS alone does not alter levels of these hormones [T_3: $F(4,45)=1.05$, $p>0.05$; T_4: $F(4,45)=0.82$, $p>0.05$]. In addition, levels of T_3 and T_4 in the hypophysectomized group of mice were unchanged by TRH administration (0.03, 0.3 or 3.0 mg/kg, IP). In contrast, TRH administration to nonhypophysectomized animals resulted in a significant elevation of blood plasma levels of T_3 and T_4 relative to the sham EBS and EBS-treated controls [T_3: $F(4,45)=13.88$, $p<0.01$; T_4: $F(4,45)=17.41$, $p<0.01$]. These data are illustrated in Fig. 4B. Post hoc analysis revealed that all differences were at a level of significance of $p<0.01$, with the exception of the comparison of T_3 values between sham EBS mice and mice given 0.03 mg/kg TRH, which resulted in a significance level of $p<0.05$.

The overall evaluation of behavioral and endocrine data would suggest that the memory-protective effects of TRH can be observed in the absence of changes in T_3 and T_4 levels. Moreover, the elevated T_3 and T_4 levels seen in the unoperated controls receiving 0.03 mg/kg of TRH were not sufficient to result in any behaviorally protective effect. Thus the behavioral properties of TRH in this paradigm are independent of the effect of TRH on levels of T_3 and T_4 thyroid hormones.

EXPERIMENT 5

Although the intensity of EBS used in the previous studies did not induce convulsions, it was still possible that the protective effect of TRH against EBS-induced memory disruption was mediated by a mild anticonvulsant action (22). This could possibly explain the protective effect of TRH obtained in the previous experiments. Anticonvulsive activity is a potential mechanism of action for some but not all compounds (38). To rule out this possibility, we evaluated the effect of TRH on tonic and clonic convulsions produced by maximal electroshock. The procedure employed here is similar to that described by Swinyard (39) and has been shown to be a reliable screening method for anticonvulsant drugs (20).

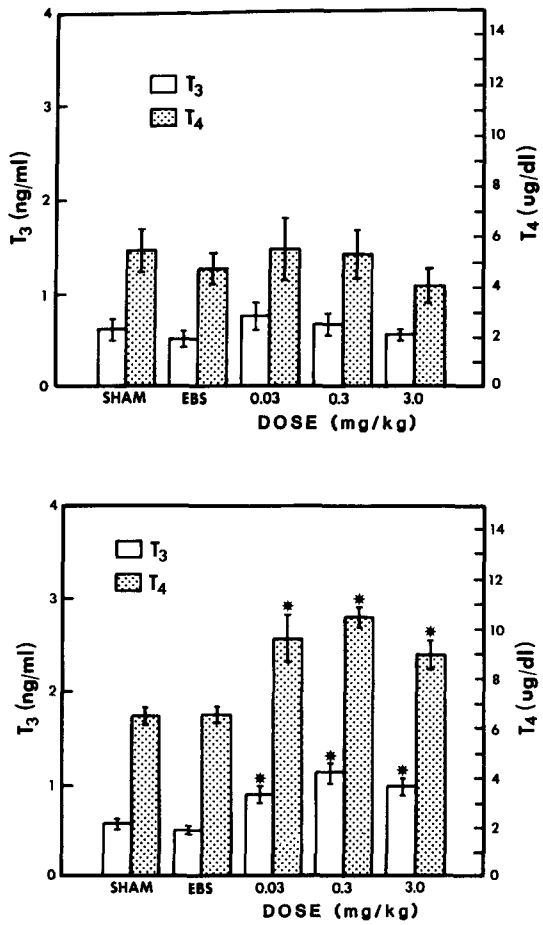

FIG. 4. (A) Mean (±SEM) T_3 and T_4 blood serum concentrations for hypophysectomized mice treated as sham EBS controls, given EBS plus vehicle, or given one of the doses of TRH and EBS. (B) Mean (±SEM) T_3 and T_4 blood serum concentrations for nonhypophysectomized mice treated as sham controls, EBS plus vehicle, or one of three doses of TRH plus EBS.

METHOD

Subjects

The subjects were as described for Experiment 1.

Apparatus and Procedure

The transcorneal shock apparatus described in Experiment I was again used, with the exception that the shock level was increased to 25 mAmps. Doses of 0.03, 0.3, 3.0 and 30 mg/kg of TRH were administered intraperitoneally 30 minutes prior to exposure of the animals to this maximal electroshock. A vehicle-treated control group was also included. Ten mice were evaluated in each treatment condition. Mice were observed for the presence of tonic and clonic convulsions, and the percentage of mice protected was determined.

RESULTS

TRH did not protect at the doses tested against electroshock induction of tonic and clonic convulsions. All mice, regardless of treatment condition, exhibited tonic and clonic convulsions immediately following the administration of EBS.

DISCUSSION

Although the mechanism of action by which EBS produces its effects is not known, the EBS-induced retrieval deficit procedure seems to represent a reliable model of the phenomenon of memory retrieval loss. An animal model such as the one used here is of particular relevance given the fact that retrieval of information is the most commonly reported problem in the elderly (6) and a major factor in cognitive memory loss in Alzheimer's disease (10). Moreover, the protection against EBS-induced memory loss has been shown to be relatively specific to nootropic and other antiamnestic agents (11, 13, 14).

TRH and some of its analogs have been claimed to produce significant improvement against spinocerebellar degeneration (36) and a variety of organic brain dysfunctions (30). The data presented here would suggest that TRH also has a beneficial effect on memory processes, as indicated by its protection against the EBS-induced retrieval deficit. Orally administered TRH produced no protection against the EBS-induced retrieval deficit, most likely due to degradation of the molecule by gut peptidases. However, over a wide dose range, intraperitoneally administered TRH produced a significant protection against EBS-induced disruption of retrieval of a learned avoidance response in mice. It was demonstrated that this protective effect was not mediated through an anticonvulsive mechanism. Additional support for this was given by observation that TRH failed to block the induction of the Straub tail that is normally seen immediately following the application of EBS in this procedure. In contrast, low doses of the anticonvulsant phenobarbital, which do not impair performance in this procedure, do block the induction of Straub tail (unpublished finding). This finding further supported our conclusion that the activity of TRH was not mediated by an anticonvulsive mechanism. We have also shown the protective effect of TRH in hypophysectomized mice; therefore, its activity is not dependent on a pituitary-mediated hormonal response. This conclusion is also supported by the finding that both hypophysectomized and nonhypophysectomized mice showed equivalent memory retrieval protection when administered TRH, even though T_3 and T_4 plasma levels were found to be elevated in the nonhypophysectomized mice. Conversely, although the dose of 0.03 mg/kg of TRH significantly elevated both T_3 and T_4 levels in nonhypophysectomized mice, it did not protect against the EBS-induced retrieval deficit, which further demonstrates that the protective effect of TRH is nonhormonally mediated.

TRH has been characterized as an "ergotropic" or CNS-activating substance functioning either as a neurotransmitter or as a facilitative neuromodulator (24). This characterization is based primarily on the peptide's analeptic properties and ability to reverse the sedation and hypothermia induced by pentobarbital, ethanol, and diazepam (7,8). One possible mechanism of TRH's regulatory effect may be to increase the quantity of neurotransmitter released at the synaptic cleft (23). Evidence for this claim has been obtained in the peripheral nervous system, where it has been shown that TRH is able to induce contraction of the guinea pig ileum through release of acetylcholine from neurons in the myenteric plexus (12). Furthermore, direct neuroexcitatory effects of TRH in the frog spinal cord have been reported by Nicoll (29). These properties of neurotransmitter regulation and modulation of excitability shown by TRH might be of clinical importance in various CNS disease states characterized by synaptic transmission failure (46), and merit further pharmacological and clinical attention.

REFERENCES

1. Adolfsson, R.; Gottfries, C. G.; Roos, B. E.; Winblad, B. Changes in the brain catecholamines in patients with dementia of the Alzheimer's type. Br. J. Psychiatry 135:216–223; 1979.
2. Arai, H.; Kosaka, K.; Iizuka, R. Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. J. Neurochem. 43:388–393; 1984.
3. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
4. Bartus, R. T.; Dean, R. L.; Pontecorvo, M. J.; Flicker, C. The cholinergic hypothesis: A historical overview, current perspective and future directions. In: Olton, D.; Gamzu, E.; Corkin, S., eds. Memory dysfunction: An integration of animal and human research from preclinical perspectives. New York: The New York Academy of Sciences; 1985:332–358. (Ann. NY Acad. Sci., vol. 444.)
5. Braitman, D. J.; Auker, C. R.; Carpenter, D. O. Thyrotropin-releasing hormone has multiple actions in cortex. Brain Res. 194:244–248; 1980.
6. Branconnier, R. J.; DeVitt, D. R. Early detection of incipient Alzheimer's disease: Some methodological considerations on computerized diagnosis. In: Reisberg, B., ed. Alzheimer's disease. New York: Free Press; 1983.
7. Breese, G. R.; Cott, J. M.; Cooper, B. R.; Prange, A. J.; Lipton, M. A. Antagonism of ethanol narcosis by thyrotropin releasing hormone. Life Sci. 14:1053–1063; 1974.
8. Breese, G. R.; Cott, J. M.; Cooper, B. R.; Prange, A. J.; Lipton, M. A.; Plotnikoff, N. P. Effects of thyrotropin-releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. J. Pharmacol. Exp. Ther. 193:11–22; 1975.
9. Carlsson, A. Changes in neurotransmitter systems in the aging brain and in Alzheimer's disease. In: Reisberg, B., ed. Alzheimer's disease. New York: Free Press; 1983.
10. Corkin, S.; Growdon, J. H.; Wissen, M. J.; Haff, F. J.; Freed, D. M.; Sagan, H. J. Recent advances in the neuropsychological study of Alzheimer's disease. In: Wurtman, R. J.; Corkin, S. H.; Growden, J. H., eds. Alzheimer's disease: Advances in basic research and therapies. Cambridge, MA: Center for Brain Sciences and Metabolism; 1984.
11. Cumin, R.; Bandle, E.; Gamzu, E.; Haefely, W. E. Effects of the novel compound (Ro 13-5057) upon impaired learning and memory in rodents. Psychopharmacology (Berlin) 78:104–111; 1982.
12. Furukawa, K.; Nomoto, T.; Tonoue, T. Effects of thyrotropin-releasing hormone (TRH) on the isolated small intestine and taenia coli of the guinea pig. Eur. J. Pharmacol. 64:279–287; 1980.
13. Gamzu, E. Animal models in the discovery of compounds to treat memory dysfunction. In: Olton, D.; Gamzu, E.; Corkin, S., eds. Memory dysfunction: An integration of animal and human research from clinical and preclinical perspectives. New York: The New York Academy of Sciences; 1985:370–393. (Ann. NY Acad. Sci., vol. 444.)
14. Gamzu, E.; Vincent, G.; Verderese, A. Pharmacological protection against memory retrieval deficits as a method of discovering new therapeutic agents. In: Fisher, A.; Hanin, I.; Lachman, C., eds. Alzheimer and Parkinsons disease: Strategies in research and development. New York: Plenum Press; 1986:375–391. (Adv. Behav. Biol., vol. 29.)
15. Giovanni, M. G.; Casamenti, F.; Nistri, A.; Paoli, F.; Pepeu, G. Effect of thyrotropin releasing hormone (TRH) on acetylcholine release from different brain areas investigated by microdialysis. Br. J. Pharmacol. 102:363–368; 1991.
16. Goldman-Rakic, P. S.; Arnstem, A. F. T. Catecholamines and cognitive decline in aged nonhuman primates. In: Olton, D.; Gamzu, E.; Corkin, S., eds. Memory dysfunction: An integration of animal and human research from preclinical and clinical perspectives. New York: The New York Academy of Sciences; 1985:218–234. (Ann. NY Acad. Sci., vol. 444.)
17. Horita, A.; Carino, M. A.; Lai, H. Preliminary studies on the effects of a TRH analog, MK-771, in an animal model of Alzheimer's disease. Proc. West. Pharmacol. Soc. 30:57–58; 1987.
18. Hutson, P. H.; Semark, J. E.; Middlemiss, D. N. The TRH analogue MK-771, increased acetylcholine release in hippocampus but not striatum of the conscious rat. Neurosci. Lett. 116:149–155; 1990.
19. Keller, H. H.; Bartholini, G.; Pletscher, A. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. Nature 248:528–529; 1974.
20. Koella, W. P. Animal experimental methods in the study of antiepileptic drugs. In: Frey, H. H.; Janz, D., eds. Antiepileptic drugs. New York: Springer-Verlag; 74:283–339; 1985.
21. Lotti, V. J.; Yarbrough, G. G.; Clineschmidt, B. V. Investigations on the interaction of thyrotropin-releasing hormone (TRH) and MK-771 with central noradrenergic mechanisms. Psychopharmacology (Berlin) 70:145–148; 1980.
22. Matsuishi, T.; Yano, E.; Inanaga, K.; Terasawa, K.; Ishihara, O.; Shiotsuki, Y.; Katafuchi, Y.; Aoki, N.; Yamashita, F. A pilot study of the anticonvulsive effects of a thyrotropin-releasing hormone analog in intractable epilepsy. Brain Dev. 5:421–428; 1983.
23. Metcalf, G. Regulatory peptides as a source of new drugs—the clinical prospects for analogues of TRH which are resistant to metabolic degradation. Brain Res. 257:389–408; 1982.
24. Metcalf, G.; Dettmar, P. W. Is thyrotropin releasing hormone an endogenous ergotropic substance in the brain? Lancet 1:586–588; 1981.
25. Miyamoto, M.; Nagai, Y.; Narumi, S.; Saji, Y.; Nagawa, Y. TRH and its novel analog (DN-1417): Antipentobarbital action and involvement of cholinergic mechanisms. Pharmacol. Biochem. Behav. 17:797–806; 1982.
26. Miyamoto, M.; Narumi, S.; Nagai, Y.; Saji, Y.; Nagawa, Y. A TRH analog (DN-1417): Motor stimulation with rearing related to catecholaminergic mechanisms in rats. Neuropharmacology 23:61–72; 1984.
27. Miyamoto, M.; Yamazaki, N.; Nagaoka, A.; Nigawa, Y. Effects of TRH and its analogue, DN-1417, on memory impairment in animal models. In: Metcalf, G.; Jackson, I. M. D., eds. Thyrotropin-releasing hormone: Biomedical significance. New York: The New York Academy of Sciences; 1989:508–510. (Ann. NY Acad. Sci., vol. 553.)
28. Narumi, S.; Nagai, Y.; Miyamoto, M.; Nagawa, Y. Thyrotropin-releasing hormone (TRH) and its analog (DN-1417): Interaction with pentobarbital in choline uptake and acetylcholine synthesis of rat brain slices. Life Sci. 32:1637–1645; 1983.
29. Nicoll, R. A. The action of thyrotropin-releasing hormone, Substance P and related peptides on frog spinal motoneurons. J. Pharmacol. Exp. Ther. 207:817–824; 1978.
30. Ogashiwa, M.; Takeuchi, K. Clinical studies of thyrotropin-releasing hormone tartrate (TRH-T) as a direct stimulant to the central nervous system. Int. J. Clin. Pharmacol. Biopharm. 17:145–151; 1979.
31. Okada, M. Effect of a new thyrotropin releasing hormone analogue, YM-14673, on the in vivo release of acetylcholine as measured by intracerebral dialysis in rats. J. Neurochem. 56:1544–1547; 1991.
32. Pagano, R. R. Understanding statistics in the behavioral sciences. New York: West Publishing; 1981.
33. Rastogi, R. B.; Singhal, R. L.; Lapierre, Y. D. Effects of MK-771, a novel TRH analog, on brain dopaminergic and serotonergic systems. Eur. J. Pharmacol. 73:307–312; 1981.
34. Reynolds, G. P.; Arnold, L.; Rosser, M. N.; Iversen, L. L.; Mountjoy, C. Q.; Roth, M. Reduced binding of [^{3}H]ketanserin to cortical 5-HT$_2$ receptors in senile dementia of the Alzheimer type. Neurosci. Lett. 44:47–51; 1984.
35. Sawaguchi, T.; Goldman-Rakic, P. S. D1 dopamine receptors in prefrontal cortex: Involvement in working memory. Science 251: 947–950; 1991.
36. Sobue, I.; Yamamoto, H.; Konagaya, M.; Iida, M.; Takayanagi, T. Effect of thyrotropin-releasing hormone on ataxia of spinocerebellar degeneration. Lancet February 23:418–419; 1980.
37. Stone, T. W. Actions of TRH and cyclo-(his-pro) on spontaneous and evoked activity of cortical neurons. Eur. J. Pharmacol. 92:113–118; 1983.
38. Stwertka, S. A.; MacNeil, D. A.; Gamzu, E. Effects of aniracetam (Ro 13-5057) and diphenylhydantoin on hippocampal after discharge in rats. Soc. Neurosci. Abstr. 9:824; 1983.

39. Swinyard, E. A. Laboratory evaluation of antiepileptic drugs. Epilepsia 10:107–119; 1969.
40. Waynforth, H. B. Experimental and surgical technique in the rat. New York: Academic Press; 1980.
41. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.
42. Yamada, K.; Demarset, K. T.; Moore, K. E. Effects of behaviorally active doses of thyrotropin-releasing hormone and its analog MK-771 on dopaminergic neuronal systems in the brain of the rat. Neuropharmacology 23:735–739; 1984.
43. Yamamoto, M.; Iwai, A.; Ozawa, Y. Effects of a new TRH analogue, YM-14673, on disturbance of passive avoidance in senescence-accelerated mice. Pharmacol. Biochem. Behav. 35:727–729; 1990.
44. Yarbrough, C. G. TRH potentiates excitatory actions of acetylcholine on cerebral cortical neurons. Nature 263:523–524; 1976.
45. Yarbrough, G. Thyrotropin releasing hormone and CNS cholinergic neurons. Life Sci. 33:111–118; 1983.
46. Yarbrough, G. G.; Pomara, M. The therapeutic potential of thyrotropin releasing hormone (TRH) in Alzheimer's disease (AD). Prog. Neuro-Psychopharmacol. Biol. Psychiatry 9:285–289; 1985.
47. Yates, C. M.; Simpson, J.; Gordon, A.; Maloney, A. F. J.; Allison, Y.; Ritchie, I. M.; Urquhart, A. Catecholamines and cholinergic enzymes in pre-senile and senile Alzheimer-type dementia and Down's syndrome. Brain Res. 280:119–126; 1983.
48. Zornetzer, S. F. Catecholamine system involvement in age related memory dysfunction. In: Olton, D.; Gamzu, E.; Corkin, S., eds. Memory dysfunction: An integration of animal and human research from preclinical and clinical perspectives. New York: The New York Academy of Sciences; 1985:242–254. (Ann. NY Acad. Sci., vol. 444.)