ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol Exp 2007, 67: 53–62

Influence of thyrotropin-releasing hormone (TRH) dialyzed into the hippocampus on memory processes in rabbit

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Abstract. There are conflicting reports on the influence of thyrotropin-releasing hormone (TRH) on the process of learning. We decided to study this problem using the paradigm of classical eyeblink conditioning as the animal model of learning processes. During the extinction training TRH in its natural form of pGluTRH was applied into the rabbit hippocampus through a chronically implanted microdialysis probe. A Glu^{1}TRH, analog with less biological potency than TRH as the control of specificity for TRH and a 0.9% NaCl solution as the control for both substances were applied by the same way as pGluTRH. We found that pGluTRH extended the process of extinction and enhanced the further acquisition of the reflex. The analog of TRH, Glu^{1}TRH, was ineffective. Finally, it may be stated that TRH acting in the hippocampus prolonged process of forgetting and improved succeeding learning. The effect was specific and long lasting.

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Key words: microdialysis of the hippocampus, thyrotropin-releasing hormone, conditioned eyelid reflex, rabbit

INTRODUCTION

Thyrotropin-releasing hormon (TRH) apart from its hypophysiotropic function has a neurotransmitter/neuromodulator activity in the central nervous system (CNS). It is widely distributed in the CNS of mammals (Parker and Porter 1983, Yamauchi 1980), and its receptors are present in many brain structures (Parker and Capdevila 1984, Sharif and Burt 1985). So far, two receptors for TRH have been cloned for mammals: TRH-R1 and TRH-R2. They are distributed differently in the brain, and they present some differences in binding affinities and characteristics of internalization (Sun et al. 2003).

A number of studies exist that report on the ability of TRH and its analogs to enhance memory impairments in a variety of diseases in humans and drug or lesion paradigms in animals (Bennett et al. 1997). This hormone crosses the blood-brain barrier, and therefore has the convenience of peripheral administration (Zlokovic et al. 1988). With administration of TRH, improvement in memory was observed in patients with Alzheimer's disease (Mellow et al. 1989), with chronic schizophrenia (Brambilla et al. 1986), and in alcoholics (Khan et al. 1993). TRH also improved learning and memory in mice treated with scopolamine or a cholinergic neurotoxin (Miyamoto et al. 1993) and in senescence-accelerated mice (Miyamoto et al. 1994).

Extensive experimental evidence links variety of human illnesses with TRH neuronal systems. Some clinical studies indicate beneficial effects of TRH in the treatment of status epilepticus (Tanaka et al. 1998) and depression (Sattin 1999). Some authors speculate that TRH and its analogs have protective effects in seizure (Przewłocka et al. 1997). Gary and coauthors (2003) suggest the inclusion of TRH and related peptides into therapeutic application in variety of human illnesses such as Alzheimer's disease, epilepsy, and depression. The authors propose that the TRH neuronal system acts as a homeostatic modulator of the central nervous system, and its effects are dependent on the state of the organism at the time when TRH is activated or administered (Gary at al. 2003).

Some TRH analogs may be more effective than TRH in the correction of memory processes. In experiments of Yamamura and colleagues an analog of TRH named TA-0910 improved impaired memory in rats and mice while TRH was not effective (Yamamura et al. 1991). Similarly, Drago and others observed that an analog named RGH 2202 was more effective than TRH in all behavioral tests done in aged or amnesic rats (Drago et al. 1991).

There are also some conflicting reports on the influence of TRH on the process of learning in animals. In rats with cholinergic lesion as an animal model of Alzheimer's disease, treatment with TRH failed to reverse lesion-induced learning deficit (Santucci and Perez 2002). Tamaki and Kameyama (1982) observed enhancement of acquisition of shuttlebox-avoidance behavior, and no effect on extinction of the avoidance response after intraperitoneal injection of TRH in rats. In experiments of Andrews and Sahgal (1983), TRH applied intracerebroventricularly, retarded acquisition of food-rewarded lever pressing. Moreover, TRH administered this way, had no effect on acquisition of conditioned responses in a two-way shuttle box test (Thompson and Rosen 2000).

In the face of conflicting data on the influence of TRH on learning and memory, we decided to study this problem using classical eyeblink conditioning as the animal model of learning processes. There are many behavioral tests applied in animals for studying influence of neuropeptides on memory processes: active avoidance, passive avoidance, social recognition, Morris water maze, radial arm maze, T-maze, visual go/no go discrimination task, as examples. Classical eyeblink conditioning is a form of associative learning, and the eyelid conditioned reflex is known as a classical or Pavlovian conditioned reflex. We have for the first time used this paradigm to study the influence of vasopressin (AVP) (Orlowska-Majdak et al. 2001b) and oxytocin (OXT) (Orlowska-Majdak et al. 2003) on learning in rabbits.

As the control compound for TRH, chemically named pGlu-His-Pro-NH_2, we applied its analog Glu^{1}TRH, chemically Glu-His-Pro-NH_2 (Fig. 1). This analog seems to have less potency than its maternal molecule. It was shown that Glu^{1}TRH has less affinity than TRH for the central nervous system (CNS) and pituitary TRH receptors in mammals (Sharif at al. 1991), and for pituitary TRH receptors in birds (Harvey and Baidwan 1989). Moreover, monoclonal and polyclonal antibodies raised against TRH had very low cross-reactivity with Glu^{1}TRH (Klootwijk at al. 1995). Therefore, we hypothesized that Glu^{1}TRH may be a very good control compound for TRH in our study.

The chemicals mentioned above were applied into the hippocampus using a microdialysis method – a technique preferred in behavioral research (Orlowska-Majdak 2004).

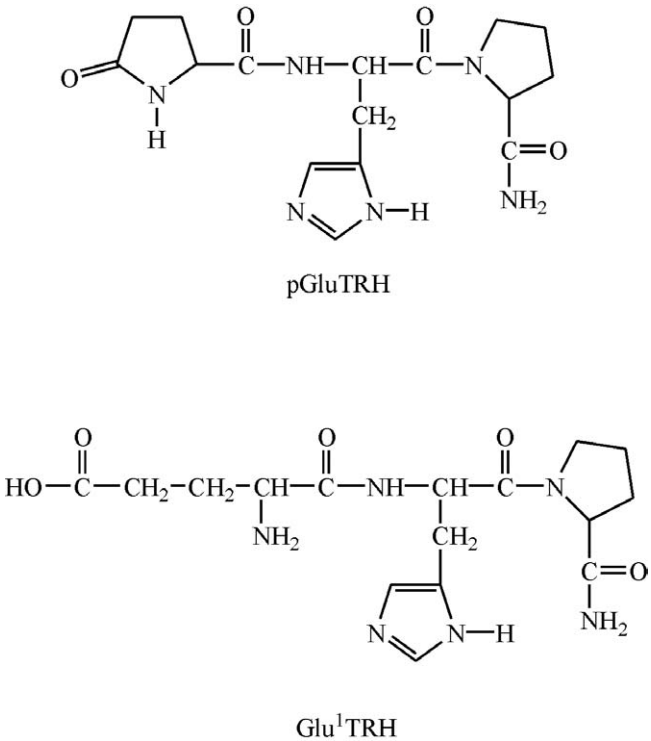

Fig. 1. Structures of TRH in its natural form of pGluTRH and of Glu[1]TRH – an analog of TRH

METHODS

Animals

The experiments were carried out in 25 adult male white New Zealand rabbits, at least four months old, weighing over 3 kg. Animals were kept in separate cages in light (lights on at 06:00 A.M. and off at 08:00 P.M.) and temperature regulated room. They had free access to food and water. Rabbits were stereotaxically implanted with microdialysis probes into the hippocampi. The probes were perfused with the solution of TRH in 15 animals and with the solution of Glu[1]TRH in 10 animals.

All experiments were carried out in accordance with the NIH guide for the care and use of the laboratory animals.

Implantation of the headpiece with guide cannulae for hippocampus

Surgical procedure was carried out under pentobarbital anesthesia (Vetbutal, Biowet-Pulawy, 30 mg/kg). After reaching deep surgical anesthesia the animal was mounted on the stereotaxic frame (Sawyer 1954) and the Plexiglas headpiece with two stainless steel guide cannulae leading to the right and to the left hippocampus was implanted. Typical position of the probes' tips in the hippocampus was shown earlier (Orlowska-Majdak et al. 2001b). An additional cannula inserted into the headpiece leading to the 3rd cerebral ventricle was used as a reference for the other two. It was positioned in the region of Bregma and its tip was positioned 10 mm deep from the surface of the dura mater. After introducing the cannula into the 3rd ventricle, the cerebrospinal fluid meniscus respiratory movements were observed under the operation microscope. This observation confirmed the correct position of the 3rd ventricle cannula, and at the same time two other cannulae. Guide cannulae leading to the hippocampi were positioned 8 mm laterally on each side of the sagittal zero plane. All cannulae were filled with stainless steel stilettes. The headpiece was fastened to rabbit's skull bones with dental cement (Duracril, Spofa). After surgery rabbits received intramuscular injections of 100 000 IU of benzylpenicillin potassium (Polfa-Tarchomin) and 0.5 g of streptomycin (Polfa-Tarchomin) daily for five consecutive days. The animals were left alone to recover after surgery for one month.

Behavioral training

After postoperative recovery the rabbits were accustomed to a special experimental box, which enabled partial immobilization of the animal. Accustomed procedure was continued for one week before the beginning of the behavioral training. For the period of training and during the whole experiment, a rabbit in the box was placed in a noise-attenuated, ventilated and illuminated cage.

Behavioral training was performed with the aid of phonopneumatic stimulator which generated periodic air puffs and tones and controlled the recorder, as described previously (Orlowska-Majdak et al. 2001a). Rabbits were trained on simple delay classical conditioning. The unconditioned stimulus (US) was a 100-ms corneal air puff exerting a pressure of 0.2 kg/cm^2, and conditioned stimulus (CS) was a 70 dB, 450 ms, 1 kHz tone, that began 350 ms prior to the air puff onset, and finished simultaneously with it. The intertrial interval averaged 18 s. Each daily session of acquisition (A) consisted of 120 trials. Conditioned responses were calculated as a percentage of all 120 conditioned stimuli applied during one session. The rabbits learned to a cri-

terion of 80% conditioned responses, and were overtrained to 5 days. After the acquisition training rabbits underwent 5 days of extinction sessions (E). During the extinction only 120 conditioned stimuli (tones) were applied. Such pattern of training was repeated twice.

Rabbits' lid movements were detected with an optoelectronic sensor, based on an infrared light emitting diode (LED). A photoelectric transducer converted the eyelid movements into electric signals. An emitter of infrared light, a receiver of reflected light and air-puff nozzle were mounted with one common holder on the rabbit's headpiece, 2 cm from the corneal surface. The amplitude of the response was proportional to the degree of closure of the eyelid. When the eye was completely closed, maximum light was reflected and the maximal amplitude of the response was observed.

Microdialysis of the hippocampus

TRH and Glu^{1}TRH (Peninsula) solutions in 0.9% NaCl were applied into the hippocampus by microdialysis method. We used microdialysis probes CMA/Microdialysis AB, Stockholm, Sweden (Cat No 8309504). Their polycarbonate dialysis membrane had the length of 4 mm, and a molecular cut off of approximately 20 000 Daltons. A microdialysis probe was implanted into the hippocampus through one of the two guide cannulae inserted on the rabbit's head. On the day of implantation the rabbits received an i.v. infusion of a 20% solution of mannitol (600 mg/kg b.w.). The microdialysis probe was filled and continuously perfused with degassed 0.9% NaCl solution at a rate of 1 µl/min, and was inserted into the guide cannulae under the operation microscope, and fixed in the headpiece by screws. A syringe pump for simultaneous multiple infusions (World Precision Instruments) was used for perfusion of the probes and finally, the hippocampus with the appropriate solution: 0.9% NaCl, TRH or Glu^{1}TRH dissolved in 0.9% NaCl solution. Regular microdialysis experiments were started not earlier then 24 h after the implantation procedure.

Experimental protocol

Rabbits with implanted headpieces underwent the preliminary behavioral training (see Table I). After that the microdialysis probe was inserted into the hippocampus, and the control microdialysis with 0.9% NaCl

Table I

The course of proceeding during microdialysis of the rabbit hippocampus (HP) with TRH (pGluTRH or Glu^{1}TRH)

day of the experiment	procedure (group of variables)
1– 5	acquisition
6–10	extinction
11–15	acquisition
16–20	extinction
21	implantation of the microdialysis probe
22–26	acquisition and dialysis of HP with 0.9% NaCl
27–31	extinction and dialysis of HP with 0.9% NaCl (control E), Fig. 2
32–36	acquisition and dialysis of HP with 0.9% NaCl (control A), Fig. 3
37–41	extinction and dialysis of HP with TRH in concentration of 0.05 mg/ml, Fig.2
42–46	acquisition and dialysis of HP with 0.9% NaCl (NaCl-1), Fig. 3
47–51	extinction and dialysis of HP with TRH in concentration of 0.5 mg/ml, Fig. 2
52–56	acquisition and dialysis of HP with 0.9% NaCl (NaCl-2), Fig. 3
57–61	extinction and dialysis of HP with TRH in concentration of 5.0 mg/ml, Fig. 2
62–66	acquisition and dialysis of HP with 0.9% NaCl (NaCl-3), Fig. 3
67–71	extinction and dialysis of HP with TRH in concentration of 50.0 mg/ml, Fig. 2

The results of the experiment, as the mean percentage of conditioned responses, are shown in Fig. 2 for extinction procedure, and in Fig. 3 for acquisition one. The upper graphs in the both figures represent the percentages of responses during/after pGluTRH and the lower graphs during/after Glu^{1}TRH microdialysis.

solution was performed simultaneously with acquisition (control A) and extinction (control E) training (see Table I). Then, during the extinction TRH or Glu[1]TRH solution was perfused through the probe. Four concentrations of the peptides were applied: 0.05; 0.5; 5.0 and 50 μg/ml. After each extinction procedure with peptide solution dialyzed into the hippocampus, acquisition procedure was done with 0.9% NaCl solution, but not after the 50.0 μg/ml of TRH. The effect of TRH was studied in 15 animals and the effect of Glu[1]TRH in 10 ones.

The probes position in the brain was marked with 10% Ianus Green, which was applied as perfusion fluid at the end of experiments in each rabbit. The animals were sacrificed with a lethal dose of Vetbutal, their heads were cut off and brains were fixed with 10% formalin solution, frozen with solid CO_2, and cut parallel to the frontal plane. Positioning of the microdialysis probe in the brain was checked under the stereomicroscope.

Data analysis

Percentages of conditioned responses were subjected to an analysis of variance (ANOVA) involving the factors of group and day with repeated measures on the day factor. For statistical analysis the percentage were transformed to arcsine values according to the formula: $2 \times \arcsin\sqrt{p}$. The least significance difference (LSD) and Newman-Keuls *post hoc* test was used following a significant ANOVA. Differences were considered significant for $P<0.05$.

RESULTS

Influence of the TRH on the conditioning procedure

Analysis of extinction data yielded a significant effect of group ($F_{4,52}=4.48$, $P<0.01$), day ($F_{4,208}=42.99$, $P<0.001$) and day by group interaction ($F_{16,208}=1.84$, $P<0.05$). Figure 2 upper graph shows the course of extinction in the control and TRH groups of variables. Almost all mean percentages of conditioned responses observed during TRH dialysis into the hippocampus had greater values in comparison with the control data, but significant effect appeared only on the 3rd 4th and 5th day of extinction. The doses of 5 and 50 μg/ml were the most effective on all mentioned days, and additionally the dose of 0.5 μg/ml on the 3rd day of extinction. As it is seen in the Fig. 2, the effect was directly proportional to the

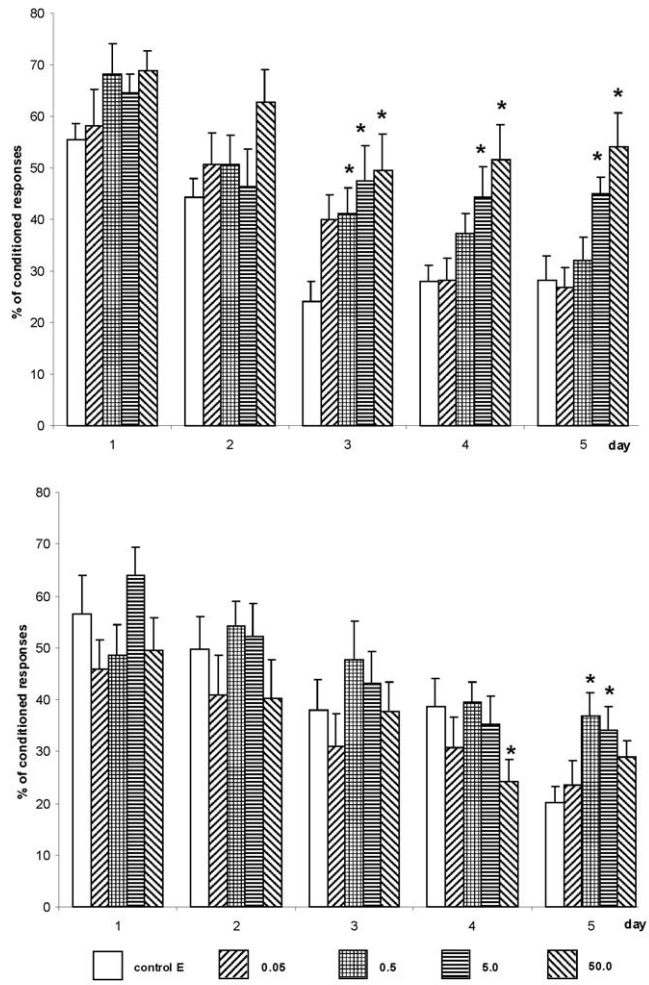

Fig. 2. The course of the extinction of the conditioned eyelid reflex during five days of training in rabbits simultaneously with TRH (upper graph) or Glu[1]TRH (lower graph) dialysis into the hippocampus (means ± SE). Bars represent % of conditioned responses. Four concentrations of the chemicals were applied: 0.05, 0.5, 5.0, and 50 μg/ml on each day of experiment (hatching bars) and the effects were compared with the control effects, when the solvent – 0.9% NaCl solution was applied (empty bars). Asterisks indicate the statistical significance.

applied peptide dose: the highest increase in the value of percentage of responses was observed when TRH was applied into the hippocampus in the dose of 50 μg/ml, $P<0.001$ on the 3rd, 4th and 5th day of the experiment.

The mean percentage of responses on the test day of acquisition after the TRH application is illustrated in Fig. 3, upper graph. ANOVA revealed a statistically significant effect of group ($F_{3,43}=10.42$, $P<0.001$) and day ($F_{4,172}=8.05$, $P<0.001$). The effect of group by day interaction was not significant. Mean values of responses

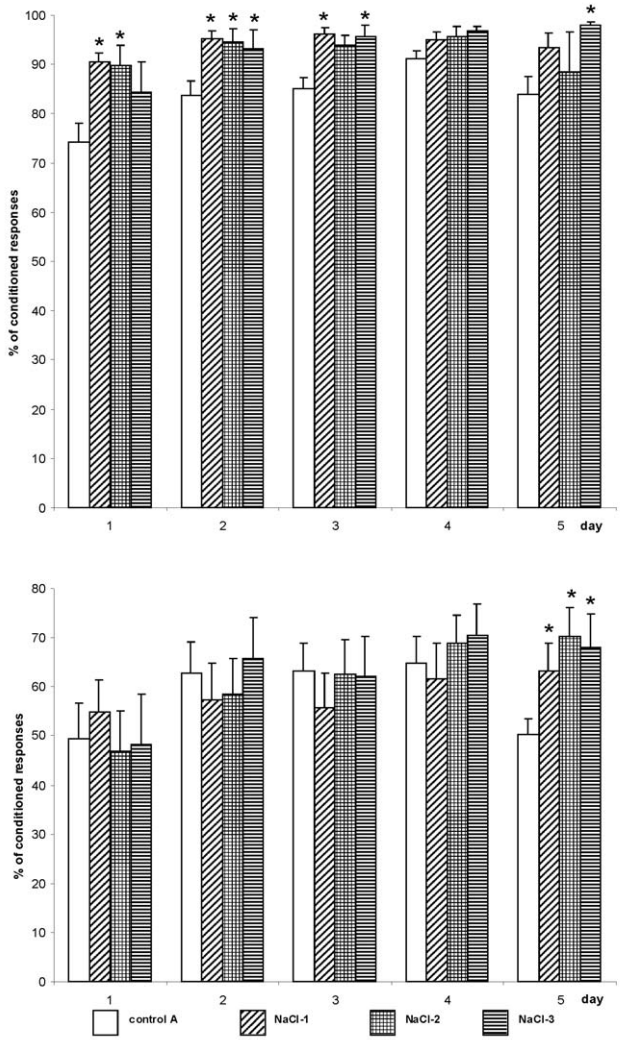

Fig. 3. The course of the acquisition of the conditioned eyelid reflex during five days of training in rabbits pre-dialyzed into the hippocampus with TRH (upper graph) or Glu[1]TRH (lower graph) (means ± SE). Hatching bars represent % of conditioned responses after the chemicals applied in concentrations of 0.05 (NaCl-1), 0.5 (NaCl-2), and 5.0 (NaCl-3) μg/ml and empty bars represent % of control conditioned responses, when 0.9% NaCl was used as pre-dialyzing solution. Asterisks indicate the statistical significance.

observed during acquisition procedure after the TRH application into the hippocampus were higher in relation to control values. The greatest differences were seen during the first three days of acquisition. Asterisks mark the statistically significant differences in relation to controls. Significance of differences observed during the acquisition procedure did not achieve the high value of $P<0.001$ as did during the extinction procedure, but $P<0.01$ at the most on the 1st day of acquisition.

Influence of the Glu[1]TRH on the conditioning procedure

Analysis of extinction and acquisition data have shown only a significant effect of day ($F_{4,43}=36.21$, $P<0.001$ for extinction and $F_{4,156}=5.48$, $P<0.001$ for acquisition). Both effects of group and day by group interaction were not significant either for the extinction or for the acquisition procedure. Mean values of percentages of responses ± SE are visible in Fig. 2, lower graph (extinction procedure) and in Fig. 3, lower graph (acquisition procedure). Only on the 5th day of extinction and on the 5th day of acquisition values of percentage responses were significantly higher in relation to control. Curiously enough, sometimes the tendency to decrease values of percentage of responses was observed during the extinction procedure, when Glu[1]TRH was applied into the hippocampus and during the acquisition, i.e. after the peptide was applied. This effect of diminished value was even statistically significant on the 4th day of extinction when the highest dose of peptide – 50 μg/ml was applied into the hippocampus ($P<0.05$).

DISCUSSION

We found that TRH dialyzed into the hippocampus of the rabbit during the extinction of the conditioned eyelid reflex restrained this process, and improved the acquisition of the reflex. The extinction of the conditioned reflex corresponds to forgetting of the acquired association of the stimuli, and the acquisition is equivalent to learning. Finally, it may be stated that TRH acting in the hippocampus extended the process of forgetting and enhanced further learning.

It is commonly known that the hippocampus is involved in memory consolidation and hippocampal or fornix/fimbria system contributes to cognitive-spatial learning (Petri and Mishkin 1994). In the present experiment classical conditioning of the rabbit eyelid reflex was used as the model of learning, and TRH was applied directly into the hippocampus. The hippocampus plays a modulatory role in this paradigm of learning (Berger and Thompson 1978), because consistent eyelid conditioning-specific hippocampal changes were shown (Sanchez-Andres and Alkon 1991). We think that TRH effect observed in our experiments depends on direct administration of the peptide into the hippocampus. In experiments men-

tioned in the Introduction, which brought conflicting effects, TRH had been applied intraperitoneally (Santucci and Perez 2002, Tamaki and Kameyama 1982) or intracerebroventricularly (Andrews and Sahgal 1983, Thompson and Rosen 2000) but not intrahippocampally.

Our conclusion is correct if TRH receptors are present in the rabbit hippocampus. It was shown that distribution of TRH binding sites was highly heterogeneous in the brains of rat and guinea pig. They were present in high concentrations in the rat, but not in the guinea pig septohippocampal area (Pazos et al. 1985). Moreover, in the rat hippocampal formation both TRH specific receptors TRH-R1 and TRH-R2 were found (Sun at al. 2003). Nothing is known about the distribution of the TRH binding sites in the rabbit brain besides that they are present there but in much smaller quantities in comparison with the brains of rat, dog, and guinea pig (Sharif at al. 1991). The results of our experiment suggest the existence of TRH receptors in the rabbit hippocampus in quantities sufficient to enable TRH action.

It is necessary to emphasize that TRH was effective both in the extinction and acquisition stage of training although it was applied only during the extinction. Statistically significant longer time of forgetting, as the result of TRH infusion was seen during the extinction on the 3rd, 4th, and 5th day of training. In these days the greatest disappearance of the acquired conditioned reflex was observed in the control (Fig. 2, upper graph). TRH applied during the extinction additionally significantly improved learning during the first 3 days of following acquisition training (Fig. 3, upper graph). This phenomenon points to the long lasting effect of TRH. Moreover, the effect was found to be directly proportional to the dose of TRH applied. It indicates specificity of TRH action.

These effects of TRH are to a certain degree different than the effects of AVP in our earlier experiments when AVP applied during the extinction had changed only the course of this stage of training without any effect on the acquisition. The effects were inversely proportional to the dose (Orlowska-Majdak at al. 2001b). It is worth mentioning that TRH and AVP were dialyzed into the hippocampus in the same quantities and in the same way in our both experiments, and the values of mean recovery for both peptides should be similar. Kendrick (1991) calculated values of mean *in vitro* recovery as 18.3% for AVP and 19.4% for TRH. Finally, probably similar quantities of AVP and TRH reached neurons of the hippocampus during our both experiments. So it may be stated basing on the comparison of both peptides action, that in memory processes TRH acts stronger, longer and more specifically than AVP.

Unlike TRH, its analog Glu^{1}TRH had a very weak influence on learning processes in our experimental model. Natural TRH is chemically pGlu-His-Pro-NH_2, distinct from Glu^{1}TRH that is Glu-His-Pro-NH_2. The molecule of Glu^{1}TRH possesses in its structure N-terminal glutamine in a chain form, while in the molecule of the natural TRH, N-terminal glutamine appears in a cyclic form (Fig. 1). The N-terminal amino acid of many proteins, hormones and neurotransmitter peptides, like TRH, is pyroglutamic acid (pGlu, <Glu). Only two proteins with N-terminal glutaminyl residues and 81 with pyroglutamyl were found in a computer search with the Protein Sequence Database (Fischer and Spiess 1987). The prefix “pyro” indicates that water has been removed from the N-terminal glutamine to form a cyclic amide structure. This structure of the N-terminus protects the molecule from enzymatic degradation and prolongs the half-life of TRH. In physiological conditions the posttranslational processing of hormonal precursors to pyroglutamyl peptides may be spontaneous and enzymatically driven, however the enzymatic reaction was found to be about 70 times faster than the nonenzymatic one (Fischer and Spiess 1987). It is believed that glutaminyl cyclase may play a key role in posttranslational modification of most if not all pGlu-containing hormones (Schilling et al. 2003).

Modification of N-terminal structure in Glu^{1}TRH mentioned above probably decreases the affinity of this analog for the hippocampal TRH receptors or this structure is more sensitive to enzymatic degradation than pGluTRH and a smaller number of molecules of Glu^{1}TRH than pGluTRH are finally effective in hippocampus. Moreover, nothing is known about the permeability of Glu^{1}TRH through dialysis membrane. In the comparative studies of the binding affinities of some TRH analogs for the TRH receptors Glu^{1}TRH revealed much lower potency than native TRH (Harvey and Baidwan 1989, Sharif at al. 1991). Glu^{1}TRH small binding affinity for TRH receptors was the reason why we chose this analog for comparison with TRH. The result of this comparison obtained in

the present experiment is consistent with our expectations and it proves specificity of native TRH effect on memory processes.

The mechanism by which TRH enhances memory processes is unknown. Influence on the long-term potentiation (LTP) in the hippocampus must be taken into consideration (for review in Polish see Kolodziejski 1999). Some papers report that TRH and its analogs may intensify LTP (Ishihara at al. 1991, Mormoto and Goddard 1985, Stocca and Nistri 1995). The phenomenon of LTP, first described by Bliss and Lomo (1973) in the rabbit hippocampal formation, consists in a long-term increase in synaptic strength resulting from the pairing of presynaptic activity with postsynaptic depolarization or from high frequency stimulation of presynaptic afferents. LTP manifests itself as enhancement of the amplitude of the population excitatory postsynaptic potential (EPSP) or as population spike (PS) when the stimulation is sufficiently strong. The effects maintain for hours or days. LTP is widely accepted as a cellular model of plasticity in CNS and supporting evidences strongly suggest that it plays a direct causal role in learning and memory formation. Long lasting effect of TRH disclosed in the present observation points to this way of action. Such effect of TRH action may be a result of upregulation of stimulatory processes by NMDA receptors (Stocca and Nistri 1995) or a result of downregulation of inhibitory processes by GABA receptors (Stocca and Nistri 1996).

In action of TRH and its analogs in memory processes, some other transmitters may be involved. In humans (Molchan at al. 1990) and animals (Ogasawara at al. 1996, Toide at al. 1993) TRH and its analogs may enhance the activity of the central cholinergic system, possibly *via* facilitation of the release of its transmitters. Enhancement of the catecholaminergic system and its transmitters should be taken into consideration as well (Mora at al. 1980, Ogasawara at al. 1996). TRH may also cooperate with AVP acting in memory processes because TRH has been noted to stimulate the release of AVP in the rabbit (Weitzman at al. 1979) and in the rat (Ciosek and Stempniak 1997).

Additionally, it must be stated that the effect of TRH observed in our experiment may be explained not by action of TRH itself but by action of its metabolites. TRH is degraded by pyroglutamyl aminopeptidase to free acid and histidyl-proline-diketopiperazine (cyclo[His-Pro]). The last compound is the main metabolite of TRH and its existence was shown in CNS of the rat with the hippocampus among other structures (Mori at al. 1982). Peptide cyclo (His-Pro) belongs to the great family of cyclic dipeptides possessing some physiological and/or pharmacological activities (Prasad 1995). Many biological effects of cyclo (His-Pro) seem to be mediated through a dopaminergic mechanism (Jikihara at al. 1993). Moreover, this dipeptide and other similar dipeptides have neuroprotective activity (Faden at al. 2004). It seems possible that cyclo (His-Pro) derived from exogenous TRH might change the course of conditioning in our experiment.

In face of the present data there is no doubt that TRH or its metabolites enhance memory processes. The mechanism of such their action needs elucidation.

CONCLUSION

TRH applied directly into the hippocampus improved learning and strengthened memory trace in rabbits. Its effects were specific and long term. We postulate that TRH-based drugs may be effective as adjunctive therapy in Alzheimer's disease and other dementias with cognition deficits.

ACKNOWLEDGEMENTS

TRH was biosynthesized by Professor Gotfryd Kupryszewski, Department of Bioorganic Chemistry, University of Gdansk, Poland. This work was supported by the Medical University of Lodz, Grant no. 502-11-557 and 503-8079-1. We thank Mrs. Krystyna Sadzinska and Mrs. Anna Kliszko for their help with surgery and animals care.

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Received 24 August 2006, accepted 22 December 2006