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Research Article

$ACTH_{6-9}$-PGP improves memory consolidation processes in rats

Svetlana A. Dodonova[1], Igor' I. Bobyntsev[1], Andrey E. Belykh[1], Anton O. Vorvul'[1]

1 *Kursk State Medical University (KSMU), 3 K. Marx St., Kursk, Kursk region 305041, Russian Federation*

Corresponding author: Svetlana A. Dodonova (dodonovasveta@mail.ru)

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Abstract

Introduction: The His-Phe-Arg-Trp sequence corresponding to the 6-9th amino acid residue of the adrenocorticotropic hormone molecule ($ACTH_{6-9}$) is the critical pharmacophore of all endogenous melanocortin receptor agonists. In order to effects prolongation it may be stabilized by the addition of the amino acid sequence Pro-Gly-Pro (PGP) to the C-terminus. The aim of this work was to study the effect of $ACTH_{6-9}$-PGP (HFRWPGP) on the processes of memory consolidation in the model of passive avoidance conditioning in comparison with $ACTH_{4-7}$-PGP effects.

Materials and methods: The study was carried out on the model of passive avoidance conditioning. The effects of $ACTH_{6-9}$-PGP were studied after its intraperitoneal injection to male Wistar rats at doses of 0.5, 5, 50, 150, and 450 μg/kg 15 minutes before the experiment, whereas the effects of $ACTH_{4-7}$-PGP– under the similar conditions at doses of 50, 150, and 450 μg/kg.

Results and discussion: It was found that $ACTH_{6-9}$-PGP had a pronounced stimulating effect on the memory consolidation process in the dose range from 0.5 μg/kg to 150 μg/kg, significantly increasing the latent period of an animal entering the dark chamber. Administration of $ACTH_{4-7}$-PGP led to an improvement in the consolidation processes of the acquired conditioned reflex at the doses of 50 μg/kg and 450 μg/kg.

Conclusion: The range of effective doses of $ACTH_{6-9}$-PGP is lower than that of $ACTH_{4-7}$-PGP, which indicates the greater activity of HFRWPGP sequence in relation to memory consolidation processes and allows considering this peptide as a promising molecule for creating nootropic pharmacological drugs.

Keywords

synthetic analog of ACTH, melanocortins, memory, passive avoidance conditioning.

Introduction

Nootropic drugs are a special group of neuropsychotropic drugs, the specific effect of which is determined by their ability to improve learning and memory processes, cognitive and intellectual functions of healthy individuals, and in case of various diseases. They are used for the treatment of the psychoorganic syndromes of neurodegenerative or vascular genesis (e.g. senile dementia, including Alzheimer's disease), craniocerebral traumas, acute and chronic disorders of cerebral circulation, including strokes, etc. Nootropic drugs administration is also recommended for healthy people when they need to improve their mental performance,

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concentration, planning, and decision-making abilities (Mironov 2012).

One of the classes of regulatory peptides, currently actively studied, is melanocortins, which include adrenocorticotropic hormone (ACTH), α-, β-, and γ-melanocyte-stimulating hormones (Catania 2008; Catania et al. 2010; Eves and Haycock 2010). An important aspect of the neurotropic activity of melanocortins is their adaptive action through the effect on memory, learning, and attention. ACTH fragments, as other melanocortin peptides, have a nootropic effect and stimulate the above processes (Catania et al. 2010; Umnov et al. 2013; Clark et al. 2016; Koroleva and Myasoedov 2018; Levitskaya et al. 2019). Moreover, the His-Phe-Arg-Trp sequence corresponding to the $ACTH_{6-9}$ region is required for the activation of all types of melanocortin receptors (Wikberg et al. 2000; Hill and Faulkner 2017; Fridmanis et al. 2017; Palmer et al. 2017). His-Phe-Arg-Trp is known to be a critical pharmacophore (Levitskaya and Kamensky 2009; Fridmanis et al. 2017; Palmer et al. 2017; Todorovic et al. 2018) for all endogenous melanocortin receptor (MCR) agonists, i.e. it represents the structure which is necessary to ensure optimal supramolecular interactions with an appropriate biological target (Eves and Haycock 2010; Palmer et al. 2017, Todorovic et al. 2018). The structurally modified molecule (by the attachment of Pro-Gly-Pro (PGP) tripeptide sequence to its C-terminus in order to increase resistance to the action of carboxypeptidases) of this fragment also has a neurotropic effect (Levitskaya et al. 2019). However, the effect of the $ACTH_{6-9}$-PGP peptide on various memory phases in a wide dose range has not yet been studied.

At the same time, the structurally close synthetic fragment $ACTH_{4-7}$-PGP, which is the active substance of the pharmacological drug Semax ($ACTH_{6-9}$-PGP), has the similar effects (Dolotov et al. 2006, Koroleva and Myasoedov 2018, Yasenyavskaya et al. 2019). Therefore, for the purpose of structural and functional analysis of the N-terminal fragments of ACTH, it seemed necessary to study this type of neurotropic activity of the $ACTH_{6-9}$-PGP molecule.

The aim of this work was to study the effects of $ACTH_{6-9}$-PGP on the processes of memory consolidation in the model of passive avoidance conditioning (PAC) in comparison with the effects of $ACTH_{4-7}$-PGP.

Materials and methods

Experimental animals

The studies were carried out on male Wistar rats, weighing 300–350 g, obtained from AL'KONDI Nursery (Moscow). The rats were kept under the standard vivarium conditions, with a 12-hour light regime (12 h light – 12 h darkness cycle) and controlled temperature (22±2 °C); the animals had free access to standard pelleted food and water. The study was approved by the Ethics Committee of Kursk State Medical University (Minutes No. 3 dated October 27, 2015). The conditions for keeping animals and working with them were in accordance with the principles of *Directive 2010/63/EU* of the European Parliament and of the Council the European Union of 22 September 2010 on the protection of animals used for scientific purposes, *Rules of Laboratory Practice in the Russian Federation*, approved by the Ministry of Health of the Russian Federation (Order of No. 708n dated August 23, 2010).

Study substances

The $ACTH_{6-9}$-PGP peptide with the formula His-Phe-Arg-Trp-Pro-Gly-Pro (HFRWPGP) was used. The reference peptide was the structurally and functionally similar $ACTH_{4-7}$-PGP peptide with the amino acid sequence Met-Glu-His-Phe-Pro-Gly-Pro (MEHFPGP). The peptides were synthesized at the Institute of Molecular Genetics of Russian National Research Centre Kurchatov Institute. The purity of the substances used was 98.9% for $ACTH_{6-9}$-PGP and 98.7% for $ACTH_{4-7}$-PGP, according to a chromatographic study. $ACTH_{6-9}$-PGP was dissolved in normal saline (0.9% sodium chloride solution) and injected once intraperitoneally 15 min before the start of the experiment. $ACTH_{4-7}$-PGP was also dissolved in normal saline and administered in a similar manner. The choice of doses was based on literature data on an $ACTH_{4-7}$-PGP effective dose range (Dolotov et al. 2006, Koroleva and Myasoedov 2018). The control animals were injected with the equivalent volumes of normal saline at the rate of 1 ml per 1 kg of body weight.

Study design

The animals were divided into groups of 15 animals each, depending on a type and dose of the peptide obtained: 1 – control (normal saline of 0.9% sodium chloride solution); 2–0.5 μg/kg of $ACTH_{6-9}$-PGP; 3–5 μg/kg of $ACTH_{6-9}$-PGP; 4–50 μg/kg of $ACTH_{6-9}$-PGP; 5–150 μg/kg of $ACTH_{6-9}$-PGP; 6–450 μg/kg of $ACTH_{6-9}$-PGP; 7–50 μg/ kg of $ACTH_{4-7}$-PGP; 8–150 μg/kg of $ACTH_{4-7}$-PGP; and 9–450 μg/kg of $ACTH_{4-7}$-PGP. All the studies were carried out from 9 am to 3 pm.

Test of passive avoidance conditioning (PAC)

During the experiment, the animals were placed in a shuttle box experimental unit (PanLab Harvard Apparatus, Spain), consisting of two chambers 25×25×28 cm in size, separated by an automatic guillotine door (8×10 cm). One compartment was brightly lit, whereas the other was dark, with an electric grid floor. An animal was placed in the illuminated compartment and allowed to examine it for 30 seconds, with the guillotine door closed. At the end of the familiarization time, the guillotine door between the compartments was automatically lifted, and after that, a latent period (LP) of the animal's transition from the light compartment into the dark compartment was recorded. After

the animal entering the dark compartment, the guillotine door was closed, and the animal, after a short 2-second delay, was exposed to an electric current (0.8 mA) for 10 seconds (Trabace et al. 2000; Mironov 2012). The trained animals were considered to be the rats, which never again entered the dark compartment after having been exposed to an electric current. Untrained rats were excluded from the experiment. The total time spent by the rats in the shuttle box was 180 sec. The studied peptides were injected immediately after training in order to assess their effect on the processes of memory consolidation.

Twenty-four hours after exposure to the electric current, the animal was again placed in the lit compartment, and the LP of the animal's re-entry into the dark compartment (avoidance delay) was fixed. The test for memory consolidation lasted for 180 seconds, after which the animal was removed from the experimental unit (Trabace et al. 2000; Mironov 2012).

Statistical analysis

A statistical data analysis was performed using the Microsoft Excel 2016 software (Microsoft, USA), Statistica 13.3 software (TIBCO Software Inc., USA), and R Foundation for Statistical Computing (Vienna, Austria). The type of sample data distribution was determined using the Shapiro-Wilk test; the homogeneity of variance was verified using the Levene's test (lawstat package). The results obtained were reported as the median (Me), lower (25) and upper (75) quartiles (Q1 and Q3). The significance of the data differences was assessed using a nonparametric one-way analysis of variance by means of the Kruskal-Wallis test; the Mann-Whitney test (U-test) with Benjamini-Hochberg correction procedure was used to identify intergroup differences as a post-hoc analysis. Fisher's exact test was used to compare qualitative characteristics in the independent groups. The significance level (p-value) when testing statistical hypotheses was taken equal to 0.05.

Results and discussion

In the course of the experiment, when evenly distributing the trained animals within the experimental groups, the retention of avoidance conditioning varied in the animals 24 hours after the training, depending on the dose and a type of administered peptide (Table 1). Wherein the greatest avoidance conditioning retention was observed after the administration of $ACTH_{6-9}$-PGP at a dose of 0.5 μg/ kg and $ACTH_{4-7}$-PGP at a dose of 50 μg/kg. These values exceeded those of the control animals by 2 times, and the differences were statistically significant. However, it is worth noting that the dose of $ACTH_{6-9}$-PGP was 100 times less than that of the reference peptide. At other doses, the intensity of $ACTH_{6-9}$-PGP effects was comparable to that of $ACTH_{4-7}$-PGP at a dose of 450 μg/kg. The proportion of rats with a retained avoidance conditioning was actually

Table 1. Indicators of Memory Trace Formation and Retention in Rats in PAC Test.

Dose, μg/kg	Animals, total	Number of trained animals		Retention of conditioning after 24 hours	
		abs	%	abs	%
control	**15**	**11**	**73.3**	**5**	**45.5**
$ACTH_{6-9}$-PGP					
0.5	15	11	73.3	10	90.9*
5	15	11	73.3	7	63.6
50	15	11	73.3	8	72.7
150	15	11	73.3	8	72.7
450	15	11	73.3	7	63.6
$ACTH_{4-7}$-PGP					
50	15	11	73.3	10	90.9*
150	15	10	66.7	5	50.0
450	15	10	66.7	7	70.0

Note: * – p<0.05-0.01 (according to the Fisher's exact test).

at the level of the control group when $ACTH_{4-7}$-PGP was applied at a dose of 150 μg/kg.

It was found that $ACTH_{6-9}$-PGP at doses from 0.5 μg/ kg to 150 μg/kg had a pronounced effect on the consolidation of the avoidance conditioning by significantly increasing the latent period of the animal entering the dark chamber (Table 2). For example, the introduction of the peptide at a dose of 0.5 μg/kg led to a statistically significant increase in this indicator by 9 times ($p = 0.004$). Increasing in an $ACTH_{6-9}$-PGP dose to 5 μg/kg was accompanied by retaining the obtained effect, and the latent period of entering the dark chamber increased by 7 times ($p = 0.05$). At doses of 50 μg/kg and 150 μg/kg, this indicator assessing the memory consolidation processes also significantly differed from that in the control group animals, which was reflected in a 9-fold increase in the latent period of entering the dark chamber ($p = 0.03$). However, the subsequent increase in an $ACTH_{6-9}$-PGP dose to 450 μg/kg was accompanied by the leveling of the previously revealed effect. Against the background of a tendency towards an increase in latent period duration, no statistically significant differences from the control group of animals were found ($p = 0.1$).

Table 2. Influence of $ACTH_{6-9}$-PGP and $ACTH_{4-7}$-PGP on Latent Period of Animal Entering the Dark Chamber (Me [Q1; Q3]).

Indicator	Latent period, sec	
Dose, μg/kg	1st day	2nd day
Control	**14.6 [5.9; 37.1]**	**31.7 [17.5 180]**
$ACTH_{6-9}$-PGP		
0.5	11.3 [10.2; 17.4]	180.0 [180; 180]*
5	21.5 [10.4; 39.7]	180.0 [35.2; 180]*
50	15.1[8.2; 17.4]	180.0 [26.0; 180]*
150	6.0 [3.6; 14.2]	180.0 [15.8; 180]*
450	9.8 [5.8; 21.2]	180.0 [36.3; 180]
$ACTH_{4-7}$-PGP		
50	16.0 [7.9; 24.9]	180.0 [180; 180]*
150	16.2 [9.3; 22.8]	48.6 [19.6; 180]
450	13.0 [8.1; 14]	180.0 [180; 180]*

Note: * – p<0.05-0.01.

The administration of $ACTH_{4-7}$-PGP also led to significant changes in the consolidation processes of the avoidance conditioning. For example, a statistically significant 9-time increase in the latent period of an animal entering

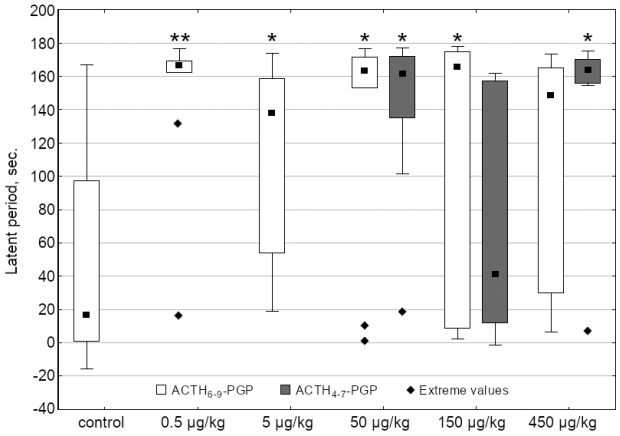

Figure 1. Influence of $ACTH_{6-9}$-PGP and $ACTH_{4-7}$-PGP on the difference between the latent period of entering the dark chamber before administering the peptides and the latent period of entering the dark chamber 24 hours after the administration of the peptides to the trained rats, Me [Q1-Q3]. **Note:** ** – significant differences ($p \leq 0.005$); * – significant differences ($p \leq 0.05$) in comparison with the control group indicators.

the dark chamber was recorded at doses of 50 µg/ kg and 450 µg/kg ($p = 0.006$ and $p=0.01$, respectively). Wherein no significant effects of the peptide were revealed at a dose of 150 µg/kg.

Our study showed that $ACTH_{6-9}$-PGP has a pronounced stimulating effect on the consolidation of the passive avoidance conditioning in rats in the dose range from 0.5 µg/kg to 150 µg/kg. Wherein there are literature data on the peptide positive effect on other learning and memory processes (fixation and reproduction of a memory trace in the PAC test) at only one dose – 50 µg/kg (Levitskaya et al. 2019). Taken together, these literature data and the results of our work indicate the $ACTH_{6-9}$-PGP effect on various phases of memory. We also showed that the reference peptide $ACTH_{4-7}$-PGP has a stimulating effect on learning processes at doses of 50 µg/kg and 450 µg/ kg, which is consistent with the literature data (Koroleva and Myasoedov 2018, Levitskaya et al. 2019). However, a number of studies showed that $ACTH_{4-7}$-PGP at doses below 15 µg/kg with the intraperitoneal administration loses its ability to exert a nootropic effect (Koroleva and Myasoedov 2018). Therefore, high efficiency of $ACTH_{6-9}$-PGP on the consolidation of the avoidance conditioning at doses much lower than 15 µg/kg indicates its greater activity in relation to this process.

A wide range of effective doses is important for the development of pharmacological drugs based on regulatory peptides. It is known that the recommended method for their administration (e.g. for Semax) is intranasal. The amount of peptide entering the body using such a mode of administration can vary significantly due to a number of different circumstances. Under the experimental conditions, it was shown that the drug losses could be up to 85% during the intranasal administration (Makarenko et al. 2009; Wang-Fischer 2009). Therefore, it can be assumed that the pharmacological effects of a peptide with a wide range of low effective doses will be more pronounced and stable, and the $ACTH_{6-9}$-PGP peptide appears to may be a more effective molecule than $ACTH_{4-7}$-PGP for influencing the processes of memory trace consolidation.

A higher intensity of the effects of $ACTH_{6-9}$-PGP in comparison with that of $ACTH_{4-7}$-PGP was also found in the study of its influence on temperature pain sensitivity (Dodonova et al. 2020), antidepressant activity (Dodonova et al. 2019), an anxiety level in the punished and unpunished behavior (Dodonova et al. 2020). Therefore, the data obtained in this work are consistent with the previously obtained results indicating the polyfunctional character of the neurotropic action of $ACTH_{6-9}$-PGP and its greater intensity in comparison with $ACTH_{4-7}$-PGP.

One of the mechanisms of the established $ACTH_{6-9}$- PGP nootropic action may be an increase in the content of neurotrophic factors in the brain. These factors are known to affect the growth and differentiation of nerve cells, as wel as to stimulate the synthesis of various biologically active substances. In addition, neurotrophic factors, mainly BDNF (brain-derived neurotrophic factor), are involved in learning and the formation of a memory trace in the mammalian brain (Dolotov et al. 2006; Dmitrieva et al. 2010; Koroleva and Myasoedov 2018; Korokin et al. 2019). It is important to note that the BDNF level significantly increases only 3 hours after the administration of ACTH analogs, in particular, $ACTH_{4-7}$-PGP, which indicates its participation only in delayed mechanisms of memory formation, including the consolidation of the memory trace.

It should also be noted that the functional activity of the biogenic amines system increases after the introduction of ACTH fragments. In particular, there is evidence in the literature about the effect of $ACTH_{4-7}$-PGP on serotonin and dopamine metabolisms, which can improve attention, release significant stimuli, improve motivaton, and accelerate learning (Koroleva and Myasoedov 2018). However, in the literature, there are currently no experimental data either on the effect of $ACTH_{6-9}$-PGP on the state of the brain neurotransmitter systems or on the content of neurotrophic factors.

At the same time currently there are no data on the participation of the known MCRs in the implementation of the nootropic effect (Hoogduijn et al. 2002, Hruby et al. 2011). Therefore, to date, the question of the action of ACTH analogs through MCRs is still open, and it is assumed that there is at least one more undescribed subtype of receptors, through binding to which their nootropic effects can be realized (Dolotov et al. 2004).

Conclusion

The administration $ACTH_{6-9}$-PGP in the dose range from 0.5 µg/kg to 150 µg/kg has a pronounced stimulating effect on the processes of memory consolidation in the PAC test. The range of effective doses of $ACTH_{6-9}$-PGP

is narrower than that of $ACTH_{4-7}$-PGP, which indicates its greater activity in relation to this process. Therefore, based on the results obtained, $ACTH_{6-9}$-PGP can be considered as a promising molecule for creating pharmacological drugs with a nootropic effect.

The study results also expand the data on the biological effects of the N-terminal analogs of ACTH and may serve as a theoretical basis for the development of new pharmacological drugs with the nootropic action.

Conflict of interests

The authors declare no conflict of interests.

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Author contributions

- **Svetlana A. Dodonova**, Assistant lecturer of the Department of Pathophysiology, e-mail: dodonovasveta@mail.ru, **ORCID ID** https://orcid.org/0000-0001-8491-3082. The author collected processed and analyzed the data, and wrote this manuscript.
- **Igor' I. Bobyntsev**, Doctor Habil. of Medicine, Professor, Head of the Department of Pathophysiology, e-mail: bobig@mail.ru, **ORCID ID** https://orcid.org/0000-0001-7745-2599. The author conceived and designed the study and wrote this manuscript.
- **Andrey E. Belykh**, PhD, Associate Professor of the Department of Pathophysiology, e-mail: a.e.belykh@gmail.com, **ORCID ID** https://orcid.org/0000-0001-9766-2104. The author collected, processed and analyzed the data, and wrote this manuscript.
- **Anton O. Vorvul'**, Junior researcher of the Research Institute of General Pathology, PhD student of the Department of Pathophysiology, e-mail: vorvul1996@mail.ru, **ORCID ID** https://orcid.org/0000-0002-1529-6014. The author collected, processed and analyzed the data, and wrote this manuscript.