CHANGES IN THE NOCICEPTIVE RESPONSE TO THERMAL STIMULATION IN RATS FOLLOWING ADMINISTRATION OF N-TERMINAL ANALOGS OF THE ADRENOCORTICOTROPIC HORMONE

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Melanocortins (MCs) are an increasingly studied class of regulatory peptides exerting a wide range of biological effects. All naturally occurring MCs share a His-Phe-Arg-Trp fragment (HFRW) corresponding to the sequence of amino acid residues 6–9 of the adrenocorticotropic hormone (ACTH). This is also a central active component of ACTH. Attaching the Pro-Gly-Pro (PGP) sequence to the C-end of the peptide extends the duration of the peptide’s effect. The aim of this study was to investigate the effects of ACTH_{1–6}PGP (HFRWPGP) on the spinal and supraspinal mechanisms involved in the nociceptive response in rats and to compare them with those of the structural analog ACTH_{1–5}PGP (MEHFPGP). ACTH_{1–5}PGP effects were studied following the intraperitoneal administration of the peptide at doses 0.5, 1.5, 5, 15, 50, or 450 μg/kg 15 minutes before the hot plate and tail flick tests. ACTH_{1–5}PGP effects were studied under the same conditions at the following doses: 50, 150 and 450 μg/kg. We found that ACTH_{1–6}PGP administered intraperitoneally at 5 or 150 μg/kg induced a pronounced reduction in pain sensitivity 15 and 45 minutes after the injection (p = 0.04); this effect was implemented via supraspinal mechanisms. In the tail flick test, 150 μg/kg ACTH_{1–6}PGP increased pain sensitivity, with the participation of segmental spinal mechanisms (p = 0.04). ACTH_{1–5}PGP did not have any effect on the studied mechanisms of pain sensitivity. Thus, unlike ACTH_{1–5}PGP, ACTH_{1–6}PGP can both increase pain sensitivity and exert an analgesic effect.

Keywords: regulatory peptide, ACTH, pain, hot plate, tail flick

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Author contribution: Dodonova SA, Belykh AE — collected, processed, and analyzed the data; Bobyntsev II, Andreeva LA, Myasoedov NF — conceived and designed the study; Dodonova SA, Belykh AE, Bobyntsev II — wrote this manuscript.

Compliance with ethical standards: the study was approved by the Ethics Committee of Kursk State Medical University (Protocol № 3 dated October 27, 2015). The animals were treated in strict compliance with the Declaration of Helsinki, Directive 2010/63/EU of the European Parliament and the Council (September 22, 2010) on the protection of animals used for scientific purposes, and Good Laboratory Practice guidelines established by the Order 708n of the Ministry of Healthcare of the Russian Federation (August 23, 2010).

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COMPARATIVE EFFECTS OF VARIOUS N-TERMINAL ANALOGS OF THE ADRENOCORTICOTROPIC HORMONE IN RATHS


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Melanocortins (MC) are a class of regulatory peptides that elicit a variety of biological effects. They share a common fragment: His-Phe-Arg-Trp (HFRW) corresponding to residues 6–9 of the adrenocorticotropic hormone (ACTH). The HFRW fragment is centrally involved in ACTH activity. Attaching the Pro-Gly-Pro (PGP) sequence to the C-end of the peptide prolongs the duration of the peptide’s effect. The aim of this study was to investigate the effects of ACTH_{1–6}PGP (HFRWPGP) on the spinal and supraspinal mechanisms involved in the nociceptive response in rats and to compare them with those of the structural analog ACTH_{1–5}PGP (MEHFPGP). ACTH_{1–5}PGP effects were studied following the intraperitoneal administration of the peptide at doses 0.5, 1.5, 5, 15, 50, or 450 μg/kg 15 minutes before the hot plate and tail flick tests. ACTH_{1–5}PGP effects were studied under the same conditions at the following doses: 50, 150 and 450 μg/kg. We found that ACTH_{1–6}PGP administered intraperitoneally at 5 or 150 μg/kg induced a pronounced reduction in pain sensitivity 15 and 45 minutes after the injection (p = 0.04); this effect was implemented via supraspinal mechanisms. In the tail flick test, 150 μg/kg ACTH_{1–6}PGP increased pain sensitivity, with the participation of segmental spinal mechanisms (p = 0.04). ACTH_{1–5}PGP did not have any effect on the studied mechanisms of pain sensitivity. Thus, unlike ACTH_{1–5}PGP, ACTH_{1–6}PGP can both increase pain sensitivity and exert an analgesic effect.

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Regulatory melanocortin peptides and their fragments exert a wide range of biological effects. This phenomenon has inspired the creation of synthetic melanocortin analogs for structure-function analysis and therapeutic applications [1, 2]. It is known that N-terminal regions of the adrenocorticotrophic hormone (ACTH) have a neurotropic effect and affect the body’s response to pain [3]. The His-Phe-Arg-Trp sequence found at ACTH1–24 is required for the activation of all melanocortin receptor types [4]. Some of ACTH1–24 effects are retained after the Pro-Gly-Pro (PGP) tripeptide is attached to its C-terminus so as to enhance resistance to carboxypeptidases [5]. However, the impact of the ACTH1–24-PGP peptide on sensitivity to pain has not been studied so far. At the same time, a synthetic fragment ACTH1–5-PGP, which is structurally close to ACTH1–24-PGP and is also an active ingredient of a pharmaceutical drug Semax, has similar effects [3]. Therefore, it is important to investigate the neurotropic action of ACTH1–5-PGP as part of the structure-function analysis of N-terminal ACTH fragments.

The aim of this work was to study the effect of the ACTH1–5-PGP peptide on the spinal and supraspinal mechanisms of nociceptive response in rats and to compare them to the effects of ACTH1–24-PGP.

METHODS

Our experiments were carried out in 121 male Wistar rats weighing 150–190 g. The animals were kept in cages (10 rats per cage) in standard housing conditions at a 12/12 light/dark cycle at controlled ambient temperature (22 ± 2 °C). The rats were fed a standard pellet diet and had free access to water. The experiments were carried out between 9 am and 15 pm.

Ten experimental and one control group were formed consisting of 11 rats each. Each experimental group received a single dose of either ACTH1–24-PGP or ACTH1–5-PGP (Semax) from the dose range specified below. The peptides used in the experiment had been synthesized in advance at the Institute of Molecular Genetics (RAS). ACTH1–24-PGP was dissolved in 0.9% normal saline. Fifteen minutes before the experiment, 7 experimental groups received a single intraperitoneal injection of ACTH1–24-PGP at one of the following doses: 0.5, 1.5, 5, 15, 50, 150, or 450 μg/kg. ACTH1–5-PGP was also dissolved in normal saline and administered intraperitoneally to the animals from the 3 remaining experimental groups at a single dose of 50, 150 or 450 μg/kg 15 min before the tests. The control group received equivalent amounts of normal saline (1 ml per 1 kg body weight).

Pain response to thermal stimulation was assessed using the hot plate and the tail flick tests [6] and the corresponding equipment: a hot plate (model LE7106) and a tail-flick meter (model LE7106) (PanLab Harvard Apparatus; Spain). The hot plate test consisted of 4 trials separated by 15-min breaks and conducted at 53 °C. In this test, the nociceptive threshold was measured once before the injection and 3 times after the injection. Each animal was placed on a hot plate and the latency to the first behavioral response (hind paw licking, jumping) to the nociceptive stimulus was measured. In the tail flick test, the middle section of the tail was exposed to the heat stimulus of fixed intensity (set to 50 units) and the latency to tail flicking was recorded. In this test, 5 measurements were taken at 15-min intervals: 2 pretreatment measurements of the baseline nociceptive threshold (averaged) and 3 measurements of the nociceptive threshold after the injection. The maximum possible effect (MPE) was calculated using the following formula: [6]:

\[
MPE = \frac{LP_{\text{posttest latency}} - LP_{\text{pretreatment latency}}}{CO_{\text{cut-off time}}} \times 100\%.
\]

where LP is posttreatment latency, LP is pretreatment latency, and CO is cut-off time (45 s in the hot plate test and 9 s in the tail flick test).

Statistical analysis was carried out in MS Excel 2016 (Microsoft; USA), Statistica 13.3 (StatSoft; USA) and the R environment (The R Foundation for Statistical Computing; Austria). Normality of data distribution was assessed using the Shapiro Wilk test. The equality of variances was tested using Levene’s test (lawstat). The obtained results were expressed as a median (Me), lower (25) and upper (75) quartiles (Q1 and Q3). Statistical significance was assessed using the nonparametric univariate Kruskal–Wallis test; post-hoc intergroup comparisons were done using the Mann–Whitney U test and the Benjamini–Hochberg procedure. The results were considered significant at \( p < 0.05 \).

RESULTS

A single injection of the ACTH1–5-PGP peptide produced a dose-dependent effect on the MPE value in the hot plate test (see Table). The analgesic effect was the most pronounced for the 5 μg/kg dose 15 and 45 min after the injection, with a significant 2.5-fold (\( p = 0.04 \)) and 7-fold (\( p = 0.02 \)) elevation of the nociceptive threshold, respectively. At a lower dose of 1.5 μg/kg, the peptide did not have any considerable impact on pain sensitivity. However, further dose reduction to 0.5 μg/kg changed the trend: 30 min after the ACTH1–5-PGP injection, the MPE value dropped by 193% (\( p = 0.15 \)) and remained at this low level for at least 45 min (\( p = 0.07 \)).

Increasing the ACTH1–5-PGP dose to 15 μg/kg did not have any significant effect. However, the peptide tended to increase sensitivity to pain 45 min after the injection when administered at 50 μg/kg (\( p = 0.1 \)). Further dose increase to 150 μg/kg was accompanied by a significant elevation of the nociceptive threshold 15 min (\( p = 0.04 \)) and 45 min (\( p = 0.04 \)) after the injection. When administered at 450 μg/kg, ACTH1–5-PGP did not have any significant effect on the studied parameters.

No statistically significant differences in the studied parameters were observed between the rats in the experimental groups following the injection of ACTH1–5-PGP at all studied doses in comparison with the controls.

On the basis of the results of the tail flick test, no reliable changes in the nociceptive threshold were observed in the rats injected with 0.5, 1.5, 15, 50, and 450 μg/kg of ACTH1–24-PGP (see Table). Although no significant effect of ACTH1–24-PGP administered at 5 and 150 μg/kg was observed in the hot plate test 30 min after the injection, the peptide did affect the rats’ sensitivity to heat in the tail flick test 30 min after its administration: at 150 μg/kg the nociceptive threshold was significantly lower (\( p = 0.04 \)) and at 5 μg/kg the peptide tended to increase pain sensitivity (\( p = 0.1 \)).

The tail flick test did not reveal any significant changes in pain sensitivity in the animals treated with ACTH1–5-PGP.

DISCUSSION

Biological effects of melanocortin peptides are implemented via a variety of melanocortin receptors (MCRs). Specifically, MCR1 is expressed in the neurons of the periaqueductal gray, MCR3 is expressed in the cortex and the thalamus, whereas MCR4, in the brain stem. The activation of these brain structures plays an important role in nociception by reducing pain progression [7, 8]. Considering that supraspinal structures are involved in the nociceptive response to thermal stimulation in the hot plate test [8], the interaction between ACTH1–24-PGP and...
Effects of ACTH on nociception in rats. The table presents the percentage of maximal possible effect (MPE) for ACTH and ACTH analogs in the hot plate and tail flick tests. The data are presented as Me (Q1; Q3).

<table>
<thead>
<tr>
<th>Group</th>
<th>15 min Hot plate test</th>
<th>30 min Hot plate test</th>
<th>45 min Hot plate test</th>
<th>15 min Tail flick test</th>
<th>30 min Tail flick test</th>
<th>45 min Tail flick test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.92 (-6.05; 26.83)</td>
<td>1.9 (-9.63; 5.84)</td>
<td>3.58 (-3.18; 9.53)</td>
<td>15.53 (-22.91; 29.78)</td>
<td>15.69 (-5.07; 28.69)</td>
<td>-17.81 (-46.16; 25.12)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 0.5 μg/kg (n = 11)</td>
<td>4.41 (-16.20; 7.73)</td>
<td>-18.11 (-38.62; -0.72)</td>
<td>-18.73 (-31.94; -7.92)</td>
<td>17.08 (8.06; 50.00)</td>
<td>0.05 (30.59; 23.43)</td>
<td>-14.31 (-41.03; 5.79)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 1.5 μg/kg (n = 11)</td>
<td>8.98 (-0.59; 19.74)</td>
<td>16.28 (8.43; 23.68)</td>
<td>-9.05 (-26.00; 11.33)</td>
<td>11.68 (8.08; 38.79)</td>
<td>22.42 (-3.37; 54.11)</td>
<td>-5.95 (-7.96; 19.13)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 5 μg/kg (n = 11)</td>
<td>45.44* (23.88; 54.69)</td>
<td>28.05 (-1.46; 42.62)</td>
<td>31.11* (27.57; 36.58)</td>
<td>-7.73 (-28.63; 16.98)</td>
<td>-8.76 (-19.43; 12.29)</td>
<td>8.6 (-8.97; 41.44)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 15 μg/kg (n = 11)</td>
<td>18.48 (11.57; 48.20)</td>
<td>11.75 (-0.76; 29.79)</td>
<td>10.83 (-11.48; 46.64)</td>
<td>0.62 (-8.11; 22.87)</td>
<td>-6.11 (-17.38; 12.50)</td>
<td>1.96 (-3.77; 22.24)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 50 μg/kg (n = 11)</td>
<td>3.57 (-9.45; 23.48)</td>
<td>-4.09 (-32.97; 7.96)</td>
<td>-10.42 (-23.20; 2.20)</td>
<td>11.65 (-13.77; 40.37)</td>
<td>19.97 (9.45; 40.40)</td>
<td>15.73 (-5.16; 20.34)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 150 μg/kg (n = 11)</td>
<td>41.02* (16.37; 86.96)</td>
<td>10.06 (2.88; 29.73)</td>
<td>30.40* (19.13; 37.31)</td>
<td>-18.04 (-44.05; 6.37)</td>
<td>-31.67* (-84.45; 21.85)</td>
<td>-20.85 (-43.43; 8.58)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 450 μg/kg (n = 11)</td>
<td>24.15 (9.36; 26.39)</td>
<td>6.61 (-7.00; 17.15)</td>
<td>4.56 (-2.13; 6.92)</td>
<td>3.77 (-21.28; 23.96)</td>
<td>11.16 (-28.31; 40.84)</td>
<td>-5.78 (-22.03; 38.22)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 50 μg/kg (n = 11)</td>
<td>0.45 (-14.1; 37.27)</td>
<td>11.01 (-18.57; 23.17)</td>
<td>10.23 (-0.03; 42.15)</td>
<td>1.21 (-18.85; 38.97)</td>
<td>22.7 (-58.53; 32.46)</td>
<td>-4.63 (-47.24; 25.36)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 150 μg/kg (n = 11)</td>
<td>12.07 (-5.06; 34.59)</td>
<td>12.48 (0.64; 31.16)</td>
<td>1.52 (-5.75; 16.44)</td>
<td>10 (-43.69; 26.44)</td>
<td>0.1 (-44.11; 27.93)</td>
<td>-3.91 (-40.36; 21.57)</td>
</tr>
<tr>
<td>ACTH&lt;sub&gt;1-24&lt;/sub&gt;-PGP 450 μg/kg (n = 11)</td>
<td>20.37 (8.63; 27.69)</td>
<td>12.19 (-9.18; 32.12)</td>
<td>21.9 (-9.18; 55.83)</td>
<td>25.78 (11.54; 80.88)</td>
<td>-16.71 (35.19; 25.67)</td>
<td>11.83 (-27.97; 28.38)</td>
</tr>
</tbody>
</table>

Note: * = differences are significant (p < 0.05) in comparison with the control group at the same time points (according to the Mann-Whitney U test and the Benjamini–Hochberg procedure).

CONCLUSIONS

In the hot plate test, a reduction in pain sensitivity was observed 15 and 45 minutes after the intraperitoneal injection of 5 and 150 μg/kg ACTH<sub>1-24</sub>-PGP; this reduction was mediated by supraspinal mechanisms. Other studied doses of the peptide did not produce any effect on the nociceptive response. In the tail flick test, the injection of 150 μg/kg ACTH<sub>1-24</sub>-PGP increased pain threshold, with the participation of segmental spinal mechanisms. No effect on pain response was observed for ACTH<sub>1-24</sub>-PGP. Our findings broaden the knowledge of physiological effects of N-terminal ACTH analogs and can provide a theoretical rationale for developing neurotropic pharmaceutical agents based on these compounds.
References


