# Comparative Study of Analgesic Potency of $ACTH_{4-10}$ Fragment and Its Analog Semax

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The effects of $ACTH_{4-10}$ fragment and its analog semax on nociception were examined on various animal models. $ACTH_{4-10}$ in a dose of 0.5 mg/kg decreased nociception in rats during hindpaw compression test and in mice subjected to acetic acid writhing test. Lower doses of $ACTH_{4-10}$ produced no analgesic effect. Semax (0.015-0.500 mg/kg) decreased pain sensitivity in all experimental models. Hence, the substitution of three C-terminal amino acid residues in $ACTH_{4-10}$ for Pro-Gly-Pro sequence augmented the analgesic potency of the peptide after its peripheral injection.

**Key Words:** *adrenocorticotropic hormone; analogs; semax; analgesia*

Melanocortins (MC), a class of peptide regulators, are now extensively studied. The MC family includes ACTH, α-, β-, and γ-melanocyte-stimulating hormones (MSH), their fragments and synthetic analogs. Apart from hormonal effects, the peptides of this class demonstrate a wide range of physiological activities. MC produce nootropic and neuroprotective effects, regulate sexual and feeding behavior, exhibit antiinflammatory and antipyretic activities, modulate pain threshold (PT), and affect the cardiovascular system [12]. The involvement of MC in the regulation of nociception remains little studied. The effect of various MC on PT depends on their structure, dose, and administration route. Systemic administration of ACTH increases PT in animals [10] and humans [9]. Intracerebral injection of ACTH can both increase and decrease PT depending on the site of injection. Injection of ACTH or α-MSH into the region of periaqueductal gray or posterior arcuate nucleus elevates PT [10,13]. Intracerebroventricular injection of MC induced hyperalgesia in the majority of studies [8].

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A broad spectrum of physiological activities of MC widens prospects of clinical use of MC peptides. However, the obstacle for wide use of natural MC is their poor bioavailability after systemic administration. For example, duration of neurotropic action of $ACTH_{4-10}$ fragment (MEHFRWG) injected intraperitoneally is 30-60 min [1]. Heptapeptide semax (MEHFPGP, $ACTH_{4-10}$ analog) produces a prolonged nootropic effect during 20-24 h [7]. In addition, this peptide demonstrates neuroprotective, neurotrophic [3,6], antihypoxic, and antihemorrhagic [5] effects. Semax is used in medical practice as a nootropic and neuroprotective agent [1]. Experiments on animals showed that intraperitoneal injection of 0.05 and 0.50 mg/kg semax produced an antinociceptove effect [4].

It is difficult to compare the analgesic doses of semax and other MC, because in most studies the effects of ACTH-like peptides on PT in animals were examined after intraventricular injection [8,10, 13]. Experiments with systemic administration of ACTH showed that the hormone decreased PT in doses of 0.50 or 0.25 mg/kg and was ineffective in a dose of 0.125 mg/kg [13]. However, it should be taken into account that corticosteroids released in response to ACTH also possess analgesic activity

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[2], hence the antinociceptive effect of ACTH results from intrinsic analgesic activity of ACTH and analgesic activity of corticosteroids.

Our aim was to study the effect of peripherally injected $ACTH_{4-10}$ on PT in animals and to compare its analgesic effects with those of semax.

## MATERIALS AND METHODS

The experiments were carried out on male random-bred albino rats weighing 200-250 g and on male random-bred mice weighing 25-30 g. The animals were maintained under standard vivarium conditions. All experiments were performed during the period from 11:00 to 18:00. Each experimental group consisted of 10-15 animals.

$ACTH_{4-10}$ (Sigma) and heptapeptide semax (synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences) were used. The test solutions were prepared immediately before the experiments. The peptides were injected intraperitoneally in doses of 0.015, 0.050, and 0.500 mg/kg in a volume of 1 ml/kg. The control animals received an equivalent volume of the solvent according to the same scheme.

For evaluation of PT we used hindpaw compression test, hot plate test, and acetic acid writhing test (to assess visceral nociception).

In the hindpaw compression test, the squeezing pressure increased linearly, and PT was determined as the pressure attained to the moment of paw withdrawal. The measurements were performed with an Ugo Basile Analgesymeter. Pressure was measured in relative units (1 rel. unit corresponded to 20 g/cm$^2$). The maximum pressure applied to the paw was 25 rel. unit. PT was measured 3 times before (initial PT) and 4 times after peptide injection with an interval of 15 min.

In the hot plate test (Ugo Basile), the animal was placed on a metal plate heated to 53°C. The latency of reaction to noxious stimulus (hindpaw licking) was recorded. The maximum duration of noxious stimulus was 30 sec. PT was measured 3 times before (initial PT) and 3 times after peptide injection with an interval of 15 min.

To analyze the data obtained in both tests (hindpaw compression and hot plate) the initial values were averaged to obtain the baseline PT. The analgesic effect was evaluated as percent of maximum action. The relative change in PT was calculated for each animal in each test by the formula:

$$(p_i-p_0)/(p_{max}-p_0)\times 100,$$

where $p_i$ is experimental PT, $p_0$ baseline PT, $p_{max}$ is the maximum pressure applied to the paw or maximum duration of the noxious stimulus.

In the acetic acid writhing test, the mice receiving ACTH (group 1), semax (group 2), or solvent (control group) were intraperitoneally injected with 0.6% acetic acid in a volume of 0.1 ml/10 g, and after 5 min the number of writhings (reflex contractions of the abdominal wall) was calculated during 2 successive 10-min intervals.

The data were analyzed statistically using Statistica 5.0 software (ANOVA and Fisher exact test) at $p<0.05$.

## RESULTS

Injection of $ACTH_{4-10}$ (0.5 mg/kg) to the rats significantly decreased PT in the hindpaw compression test during postinjection minutes 15-60 ($F_{1.35}$=5.84; $p<0.03$, Fig. 1). $ACTH_{4-10}$ in a dose of 0.05 mg/kg produced no significant changes in PT (Fig. 1). Injection of semax in a dose of 0.5 mg/kg significantly increased PT in this test during postinjection minutes 15-90 compared to control values ($F_{1.59}$=10.32; $p<0.005$, Fig. 2). Lower doses of semax also produced the analgesic effect, but it became less pronounced. Semax in doses of 0.05 and 0.015 mg/kg significantly decreased PT during postinjection minutes 45-90 ($F_{1.43}$=4.39; $p<0.05$) and 30-45 ($F_{1.45}$=4.89; $p<0.05$, Fig. 2), respectively.

In experiments on rats, $ACTH_{4-10}$ in doses of 0.05 and 0.50 mg/kg produced no significant changes in PT in the hot plate test (Fig. 3, *a*). However, in animals receiving $ACTH_{4-10}$ in a dose of 0.5 mg/kg PT tended to increase in comparison with the control value ($F_{1.38}$=3.04; $p<0.10$). Semax (0.5 mg/kg) signi-

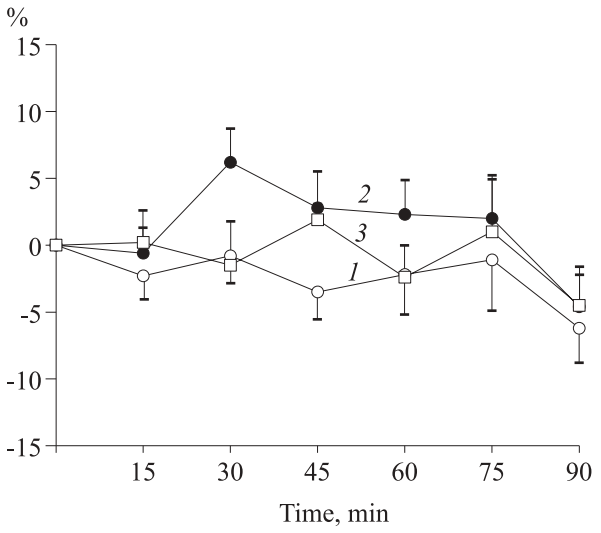


**Fig. 1**. Effect of $ACTH_{4-10}$ on PT in hindpaw compression test in rats. *1*) control; *2*) $ACTH_{4-10}$, 0.50 mg/kg; *3*) $ACTH_{4-10}$, 0.05 mg/kg. Here and in Figs. 2, 3: the data are presented as mean±SEM; ordinate: changes of PT relatively the baseline value in percent from the maximum possible effect.

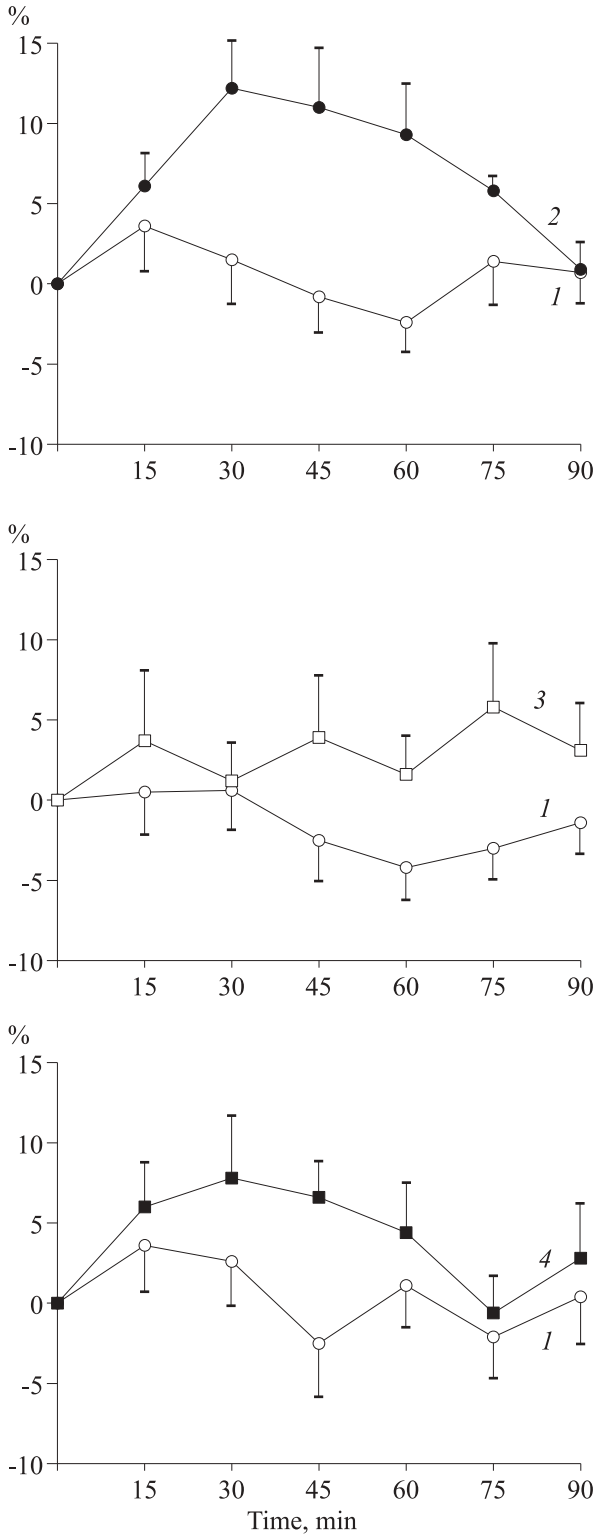


**Fig. 2**. Effect of semax on rat PT in hindpaw compression test. *1*) control; *2*) semax, 0.50 mg/kg; *3*) semax, 0.05 mg/kg; *4*) semax, 0.015 mg/kg.

ficantly decreased PT in the hot plate test in comparison with the control level during postinjection minutes 15-45 ($F_{1.24}$=8.89; $p$<0.01, Fig. 3, *b*). When semax was injected in a lower dose (0.05 mg/kg), it significantly increased PT during the postinjection minutes 30-45 ($F_{1.24}$=4.90; $p$<0.05).

In the writhing test, $ACTH_{4-10}$ injected a dose of 0.5 mg/kg 35 min before injection of acetic acid significantly decreased the number of writhings in comparison with the control during the entire period of recording (Table 1). In mice injected with 0.05 mg/kg $ACTH_{4-10}$, the number of writhing also decreased, but this effect was insignificant. Injection of semax in both tested doses significantly decreased the number of writhings on minutes 40-60 postinjection (Table 1).

These findings suggest that peptide fragment $ACTH_{4-10}$ injected intraperitoneally in a dose of 0.5 mg/kg decreases PT in rats and mice in various nociceptive tests. The changes of PT in the hindpaw compression and hot plate tests were 6.5-7.5% of the maximum possible effect; the duration of this effect did not exceed 60 min. Decreasing the injected dose of the peptide fragment to 0.05 mg/kg eliminated its analgesic action. Semax administered intraperitoneally produced the analgesic effect in a dose range of 0.015-0.500 mg/kg. Changes in PT recorded after injection of the peptide in a dose of 0.5 mg/kg in paw compression and hot plate tests was 12-23% of the maximum possible effect, and these changes were significant in comparison with the control values during postinjection minutes 75-90.

Comparison of the analgesic effects of semax and its natural prototype $ACTH_{4-10}$ showed that substitution of three amino acid residues in $ACTH_{4-10}$ for Pro-Gly-Pro sequence increased the analgesic potency of the peptide after its peripheral injection. Comparative study of nootropic effects of $ACTH_{4-10}$ and semax showed that this modification of the natural peptide prolongs its effect on learning in

**TABLE 1**. Effect of $ACTH_{4-10}$ and Semax on PT in Acetic Acid Writhing Test (number of writhings; $M \pm m$)

| Group | Postinjection time, min | |
|---|---|---|
| | 40-50 | 50-60 |
| Group 1 | | |
| control | 22.0±3.6 | 11.4±3.5 |
| $ACTH_{4-10}$, 0.05 mg/kg | 15.5±2.4 | 7.8±2.3 |
| $ACTH_{4-10}$, 0.50 mg/kg | 10.7±3.0* | 5.0±1.5* |
| Group 2 | | |
| control | 31.6±2.6 | 20.3±2.4 |
| semax, 0.05 mg/kg | 26.0±3.0* | 15.0±2.4* |
| semax, 0.50 mg/kg | 25.9±2.3* | 13.6±1.7** |

**Note.** *$p$<0.05, **$p$<0.01 compared to the control.

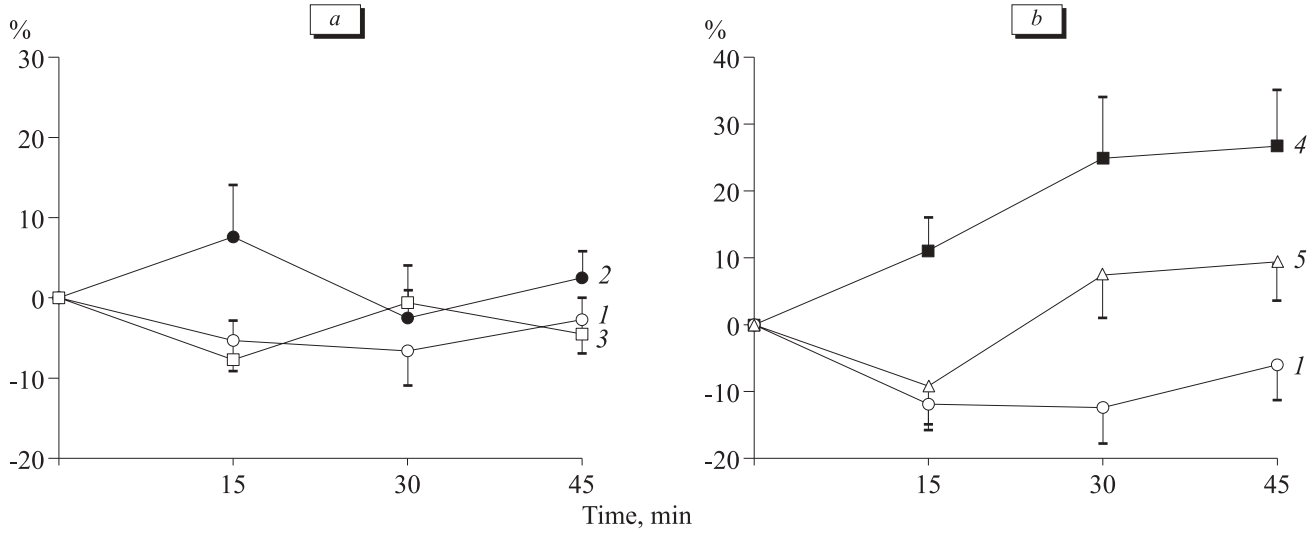


**Fig. 3**. Effect of $ACTH_{4-10}$ (*a*) and semax (*b*) on rat PT in hot plate test. *1*) control; *2*) $ACTH_{4-10}$, 0.50 mg/kg; *3*) $ACTH_{4-10}$, 0.05 mg/kg; *4*) semax, 0.50 mg/kg; *5*) semax, 0.05 mg/kg.

animals. When administered in equal doses, both agents exerted similar effects on learning and memory [7]. In case of the analgesic action of the examined peptides, insignificant prolongation of the effects was accompanied by an increase of analgesic potency and increase in effective doses of semax in comparison with those of $ACTH_{4-10}$. Higher activity of semax probably results from its higher resistance to proteases [11], which explains its higher bioavailability after peripheral administration.

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