ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 1, 8 SECTIO DDD 2011

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Behavioral impairment induced by dexamethasone in mice. Effect of $ACTH_{4-9}$

Zaburzenie zachowania wywołane deksametazonem u myszy. Efekt $ACTH_{4-9}$

INTRODUCTION

In recent years there have been reports that long-term treatment with glucocorticoids (GCs) or prolonged stress can induce neuronal damage of the brain [1]. The hypersecrection of GCs occurs in several human pathologies, including Huntington`s disease, dementia of Alzheimer`s type, depression or psychosis [4, 25].

The elevated levels of glucocorticosteroids are known to be toxic especially to the CA1 and CA3 subfields of the hippocampus – a structure of brain containing the highest concentration of GCs receptors and which plays an important role in memory, mood and behaviour [14, 19]. Moreover, the administration of GCs following ischemic insult exacerbates the neuronal damage, while the adrenalectomy occuring 24 h after ischemia seems to have a protective effect [1].

A similar alteration was also observed following the administration of dexamethasone (DEX- a synthetic GCs receptor agonist) that can also induce mood disorders, including psychosis or depression in some patients [7 8,14].

It has also been reported that ACTH plays a role in stress, motivation, learning and memory [10].

$ACTH_{4-9}$ – synthetic adrenocorticotropin-(4-9) analogue, HMet(O_2)-Glu-His-Phe-D-Lys-Phe-OH (ORG 2766) is a centrally active peptide fragment of ACTH with minimal corticotropic activity. It has been reported that $ACTH_{4-9}$ has trophic effect on neuronal tissue [16]. Several earlier studies showed its neuroprotective effect in some models of central and peripheral neuronal degeneration [15]. Other authors have shown that $ACTH_{4-9}$ prevented damage of hippocampal neurons induced by dexamethasone administered chronically [20] and damage of neurons in different insults e.g. ischemia [17] or in taxol-induced neuropathy [13] or in peripheral neurotoxicity after anticancer drug used e.g cisplatin [21].

MATERIALS AND METHODS

All procedures were conducted according to NIH Animal Care and Use Committee guidelines and approved by the Ethics Committee of the Medical University of Lublin.

Male Albino Swiss mice (initial weight 22–26 g) were used in the experiments. They were housed fifteen per cage at the temperature of 20°C ± 2°C under natural light-dark cycle. The animals were allowed free access to standard laboratory feed (LSM, Motycz, Poland) and tap water. All procedures were performed between 8.00 and 14.00 h.

Drugs. The following drugs were used: dexamethasone (DEX) (Jelfa, Poland) and $ACTH_{4-9}$ – synthetic adrenocorticotropin-(4-9) analogue, HMet(O_2)-Glu-His-Phe-D-Lys-Phe-OH, (ORG 2766), (Sigma, USA). DEX was administered ip, at the doses: 8 or 16 mg/kg/day, as commercially available solution for injection (Dexaven). $ACTH_{4-9}$ was injected at the dose of 50 µg/kg, twice a week, 30 min before DEX. The drugs were administered during 14 days for assessing motor performance in „chimney" test, locomotor activity, and passive avoidance acquisition and retention testing, or 28 days for recording the body weight or lethality of mice. The behavioural tests were performed 24 or 48 h after the last injection of drugs.

"Chimney" test. The effects of the chronic treatment with DEX alone or combined with $ACTH_{4-9}$ on motor performance were evaluated with the "chimney" test [3]. The animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of mice to climb backwards up the tube within 60 s. The mice were pretrained 24 h before the treatment and those unable to perform the test were rejected from experimental groups.

The locomotor activity. Locomotor activity of mice was registered with the DIGISCAN Optical Animal Activity Monitoring System. The DIGISCAN monitors animal locomotor activity via a grid of invisible infrared light beams. A number of equally spaced beams traverse the animal cage from front to back and an equal number of beams traverse the same cage from left to right. The body of animal placed within the DIGISCAN will make some of these beams break thus revealing its position in the horizontal or vertical plane. The DIGISCAN analyser collects the beam status information from the Activity Monitor and subjects it to rapid analysis. Each time it receives the beam status, it is able to determine the position of animal. Since it determines the animal position 100 times per second, the analyser can effectively develop a dynamic picture of the animal activity. The dynamic picture reveals whether the animal is resting, ambulating or rearing. In our experiment ambulation (locomotion) of mice was monitored each 15 min during the first 60 minutes.

Passive avoidance acquisition and retention testing. The step-through passive avoidance task is regarded as a measure of long-term memory acquisition [24]. The mice were placed in an illuminated box (10 x 13 x 15 cm) connected to a larger (25 x 20 x 15 cm) dark compartment equipped with an electric grid floor. In this test, entry into the dark compartment was punished by an electric footshock (0.6 mA for 2 s) for facilitation of acquisition. The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the following day (24 h later), the same animals were again placed in the illuminated box and those avoiding the dark compartment for longer than 180 s were regarded as remembering the task. Retention was evaluated as the mean time (in seconds) required to enter the dark compartment.

Body weight and lethality. Body weight and lethality were controlled every day of the experiment. The number of dead mice was recorded during the experiment.

Statistical analysis. The results of the experiments are expressed as the mean ± SEM. The data of behavioral tests were analysed by Kruskal-Wallis Nonparametric Anova and Dunn`s post test, while body weight data were assessed by one-way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons post test, and lethality with Fisher`s Exact Test for 2x2. Statistically significant differences were designated by $P<0.05$.

RESULTS

DEX, administered for 14 days, caused the significant impairment ($p<0.05$) of the motor coordination in "chimney" test (about 100%) (Fig. 1), of the locomotor activity during 60 min of observation (about 60%) (Fig. 2) and also decreased memory acquisition in the step-through passive avoidance test (about 25%) especially at the dose of 16 mg/kg/day (Table1). Moreover, DEX given for 30 days, at either dose, evoked the significant lethality (Table 2) and decreased the body weight (about 30%) (Fig. 3).

Table 1. The effect of $ACTH_{4-9}$ on the long-term memory acquisition impaired by dexamethasone (DEX)

Drugs (dose/24h)	Retention time (S) Means ± SEM
Vehicle	155.0 ± 11.9
$ACTH_{4-9}$ 50 g/kg	158.5 ± 10.2
DEX 8 mg/kg	120.2 ± 24.4
DEX 16 mg/kg	108.2± 20.5[a]
$ACTH_{4-9}$ 50 g/kg + DEX 8 mg/kg	173. 3 ± 6.6[b]
$ACTH_{4-9}$ 50 µg/kg + DEX 16 mg/kg	177.0 ± 3.0[b]

DEX (8 or 16 mg/kg) was administered for 14 days, the last dose 48 h before the test.
$ACTH_{4-9}$ was given twice a week, 30 min before the injection of DEX. [a]$p<0.05$ vs vehicle, [b]$p<0.05$ vs DEX alone treated mice. Kruskal-Wallis Nonparametric Anova and Dunn`s post test. N=12

Table 2. The effect of $ACTH_{4-9}$ on the lethality of mice treated chronically with dexamethasone (DEX)

Drugs (dose/24 h)	The number of dead mice			
	days of experiment			
	7	14	21	28
Vehicle	0/15	0/15	0/15	0/15
$ACTH_{4-9}$ 50 µg/kg	0/15	0/15	0/15	0/15
DEX 8 mg/kg	0/15	0/15	4/15	6/15[b]
DEX 16 mg/kg	0/15	2/15	5/15[a]	9/15[b]
$ACTH_{4-9}$ 50 µg/kg + DEX 8 mg/kg	0/15	1/15	3/15	9/15
$ACTH_{4-9}$ 50 µg/kg + DEX 16 mg/kg	0/15	0/15	3/15	8/15

DEX was administered for 28 days. $ACTH_{4-9}$ was given twice a week, 30 min before the injection of DEX. [a]$p<0.05$ vs vehicle; [b]$p<0.05$ vs DEX alone treated mice. Fisher`s Exact test for 2x2. N=15

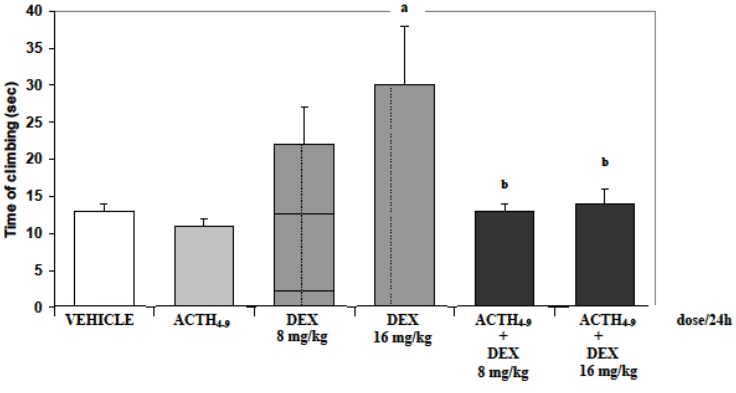

Fig. 1. The effect of prolonged treatment with $ACTH_{4-9}$ on the motor impairment of mice induced by dexamethasone (DEX) ("chimney" test).DEX (8 or 16 mg/kg/24h) was administered for 14 days, the last dose 24 h before the test. $ACTH_{4-9}$ (50 µg/kg) was injected twice a week, for 14 days, 30 min before DEX. [a]p< 0.05 vs vehicle, [b]p<0.05 vs DEX alone treated mice. Kruskal-Wallis Nonparametric Anova and Dunn`s post test. N=9–12

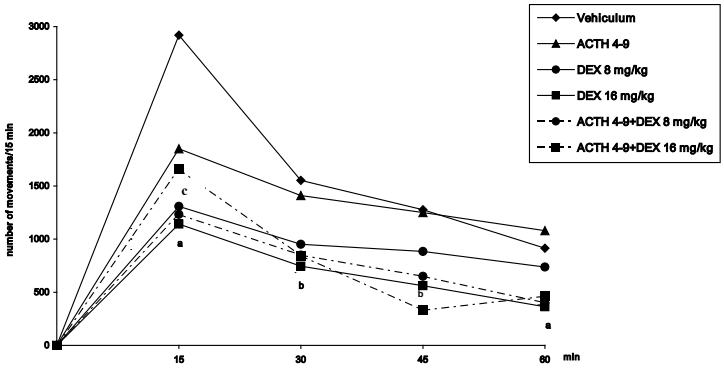

Fig. 2. The effect of prolonged treatment with $ACTH_{4-9}$ on the locomotor activity impairment of mice induced by dexamethasone (DEX). DEX (8 or 16 mg/kg/24 h) was administered for 14 days, the last dose 24 h before the test. $ACTH_{4-9}$ (50 µg/kg) was injected twice a week, for 14 days, 30 min before DEX. [a]p<0.01, [b]p<0.05 vs vehicle, [c]p<0.05 vs DEX alone treated mice. Kruskal-Wallis Nonparametric Anova and Dunn`s post test. N=9–12

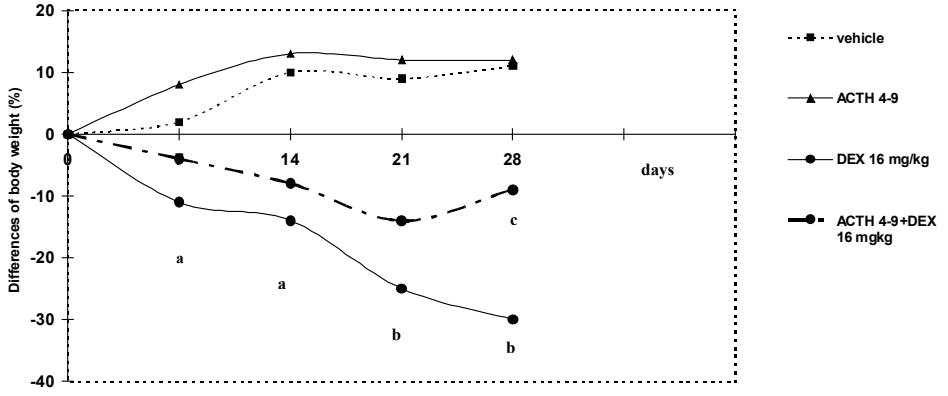

Fig. 3. The effect of prolonged treatment with $ACTH_{4-9}$ on the body weight of mice induced by dexamethasone (DEX). DEX (16 mg/kg/24 h) was administered for 28 days. $ACTH_{4-9}$ (50 µg/kg) was given twice a week, for 28 days, 30 min before DEX. $^{a}p<0.05$, $^{b}p<0.001$ vs vehicle; $^{c}p<0.05$ vs DEX alone treated mice. One-way analysis of variance (ANOVA) and Tukey-Kramer post test. N=9–12

$ACTH_{4-9}$, given alone at the dose 50 µg/kg twice a week, neither changed all the parameters of the behavioral tests nor the body weight and the lethality in comparison to the control group of mice (Fig. 1–3, Tab.1,2).

Instead, $ACTH_{4-9}$ significantly counteracted ($p<0.05$) the climbing time prolongation

(Fig. 1), improved the locomotor activity (Fig. 2) and also improved the attenuated long-term memory ($p<0.05$) in mice treated with DEX at the dose 16 mg/kg/day but not 8 mg/kg/day (Tab. 1). $ACTH_{4-9}$ (given in doses not affecting the body weight gain) diminished body weight loss induced by prolonged treatment with DEX at the dose 16 mg/kg/day (not 8 mg/kg/day) (Fig. 3), but did not prevent the lethality of mice receiving DEX in both doses for 28 days (Tab. 2).

DISCUSSION

The results of this study indicate that chronic (for 14 days) treatment of DEX at the doses of 8 mg/kg/day impaired the retention time in the memory task and also the locomotor activity or the motor coordination in comparison to the control group of mice and significantly decreased the above parameters at the dose of 16 mg/kg/day. Moreover, reduction of the body weight and the significant lethality were observed after 28 days of treatment of DEX in mice.

Our earlier study and studies of other authors indicate that GCs and their preparations, e.g. DEX, impair memory and reduced locomotor activity or motor coordination in animals [6–9]. Moreover, GCs, such as cortisol, are released by adrenal cortex in response to a wide range of stressors. Elevated levels of endogenous GCs can damage the brain, especially the hippocampus which plays an important role in memory, mood and behavior [4,14,25].

Similarly, DEX induces mood disorders [14] and neuronal damage after acute as well as prolonged administration [8,14,20]. Moreover, DEX aggravates ischemic neuronal damage by causing

glutamate to accumulate in the extracellular space [5] by increasing glutamate release, decreasing its uptake and up-regulation of glutamate receptor expression [11]. An activation of NMDA receptors by high concentrations of glutamate may be decisive for induction of degenerative changes in nerve cells under the influence of GCs [18].

In our experiment, we have observed the improvement of behavioral effects after administration of $ACTH_{4-9}$ in mice chronically treated with DEX. $ACTH_{4-9}$ (at the dose 50 μg/kg twice a week, for 28 days) did not influence all parameters of behavioral tests, the body weight and the lethality in comparison to the control group of mice. But, we have observed the significant improvement of the long-term memory acquisition, the motor coordination and the locomotor activity or lower body weight reduction (but not lethality) after prior treatment of $ACTH_{4-9}$ in mice subjected to DEX (at either doses used).

This mechanism of $ACTH_{4-9}$ action the neuroprotective effect may be highly complex and is still under consideration.

Some authors indicate association of $ACTH_{4-9}$ with glutamate system. It has been shown that the increase in locomotor activity after microinjection of NMDA given into ventricle was significantly reduced by a chronic pretreatment of $ACTH_{4-9}$ (given at the dose 1 μg/0.5ml saline), sc, for 7 days, what proves that $ACTH_{4-9}$ exerts its effects on behavior and neural recovery by modulating NMDA receptor activity in the brain [22].

Horváth et al. [16] have shown that early postnatal administration of $ACTH_{4-9}$, in adulthood has trophic effects on neuronal tissue, by decreasing the extent of cholinergic neuronal degeneration after NMDA lesion of the MBN (magnocellular basal nucleus). Moreover, the lower neuronal damage in the postnatally $ACTH_{4-9}$ treated animals may be caused by a reduced sensitivity to NMDA, either through modification of NMDA receptor function or through changes at the level of intracellular cascade mechanisms.

The association between GCs and excitatory amino acids suggests that additional factors and neurotransmitters (e.g. dopamine, acetylcholine or serotonine) may contribute to the pathological consequences of GCs [6,12].

$ACTH_{4-9}$ administered at single doses (25, 50 or 100 μg/kg) potentiated the anti-immobility effect of all used antidepressants and dopamine agonists and also facilitated effect of selegiline administered for 7 days in rats, what suggests a functional interaction of $ACTH_{4-9}$ with serotonergic and dopaminergic brain mechanisms of drugs action [26].

It is well known that GCs can modulate neurodegenerative processes acting through two receptor types, the mineralocorticoid (MR) and glucocorticoid receptor (GR). The occupational ratio of these two receptors can strongly influence the excitability of neurons. $ACTH_{4-9}$ can selectively increase MR expression, not only in adulthood but also after postnatal administration of peptide. An increased MR number could be directly or indirectly responsible for the attenuated neurodegeneration [16]. Moreover, Abrahám et al. [2] have shown that slightly elevated levels of corticosterone, which mainly occupies MR, significantly decrease NMDA neurotoxicity in the MBN.

In the light of studies, the effect of $ACTH_{4-9}$ activity (like many peptides) is connected with the compensatory mechanism which relies mainly on enhanced non-selective attention by activation of limbic structures and also modulating efficacy on NMDA-receptor [23].

The results of our study suggest that $ACTH_{4-9}$ administered chronically at a low dose, counteracted the behavioral deficits and the body weight reduction induced by chronic treatment with DEX. The above findings confirm the neuroprotective properties of $ACTH_{4-9.}$

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SUMMARY

Long-term treatment with glucocorticoids (GCs) and their preparations, e.g. dexamethasone (DEX) can induce neuronal damage of the brain, especially hippocampus (with high concentration of GCs receptors) that plays an important role in memory, mood and behavior.

Several earlier studies showed neuroprotective effect of $ACTH_{4-9}$ -(-synthetic adrenocorticotropin-4-9-analog) in some models of central and peripheral neuronal degeneration. The aim of the present study was to investigate influence of $ACTH_{4-9}$ (at the dose of 50 g/kg/twice of week) on impairment of behavioral effects of the chronic treatment with DEX in mice. The results of present study showed that DEX (8 or 16 mg/kg/day for 14 days) impaired the motor coordination ("chimney" test), the locomotor activity during 60 min of observation and also decreased memory acquisition in the step-through passive avoidance test (especially at the dose of 16 mg/kg/day). Moreover, DEX, at either doses, evoked the significant lethality and decreased the body weight of mice during 28 days of experiment. $ACTH_{4-9}$ improved the behavioral effects but not diminished of the lethality of mice. The above findings confirm the neuroprotective properties of $ACTH_{4-9.}$

Keywords: glucocorticoids, dexamethasone, $ACTH_{4-9}$, behavioral tests, neurodegeneration, neuroprotection, mice

STRESZCZENIE

Długotrwałe stosowanie glikokortykosteroidów (GCs) i ich preparatów, takich jak: deksametazon, może wywoływać neuronalne uszkodzenia mózgu, szczególnie hipokampa (struktury bogatej w receptory dla GCs), odgrywającej ważną rolę w pamięci, nastroju i zachowaniach. Wcześniejsze badania wskazują na neuroprotekcyjne działanie $ACTH_{4-9}$ –(syntetyczny adrenokortykotropowy-4-9-analog) w ośrodkowych i obwodowych modelach neuronalnej degeneracji. Celem tego eksperymentu było zbadanie wpływu $ACTH_{4-9}$ (w dawce 50 μg/kg stosowanej 2 razy w tygodniu) na zaburzone efekty zachowań myszy poddanych przewlekłemu działaniu DEX. Wyniki tych badań wykazały, że DEX (w dawkach 8 lub 16 mg/kg/dobę przez 14 dni) zaburzał koordynację motoryczną (w teście „komina"), aktywność lokomotoryczną podczas 60 min obserwacji, jak również zmniejszał nabywanie pamięci w teście biernego unikania (szczególnie w dawce 16 mg/kg/dobę). Ponadto, DEX, w każdej dawce, wywoływał znaczną śmiertelność i zmniejszał ciężar ciała myszy podczas 28 dniowego eksperymentu. $ACTH_{4-9}$ poprawiało parametry w testach zachowań ale nie zmniejszało śmiertelności myszy. Powyższe dane potwierdzają neuroprotekcyjne właściwości $ACTH_{4-9.}$

Słowa kluczowe: glikokortykosteroidy, deksametazon, $ACTH_{4-9}$, testy zachowań, neurodegeneracja, neuroprotekcja, myszy