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Effects of Acute Administration of Adipokinetic Hormone on Depression, Anxiety, Pain, Locomotion and Memory in Mice

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Abstract

The neurosecretory cells in the corpus cardiacum of insects synthesize a set of hormones that are called adipokinetic, hypertrehalosemic or hyperprolinemic depending on the insect in question. They are the Adipokinetic Hormone/Red Pigment-Concentrating Hormone (AKH/RPCH) family of peptides. The present study investigated the effects of acute administration of *Locusta* Migratoria (Locmi-AKH-II) and *Anax* Imperator (Anaim-AKH) on depression, anxiety, pain (analgesy), locomotion and memory in mice in forced swimming (FST), elevated plus maze (EPM), hot plate, locomotor activity and passive avoidance tests. Both Locmi-AKH-II (4 mg/kg) and Anaim-AKH (0.25 and 0.50 mg/kg) decreased immobility time (in sec, s) in the FST test. Anaim-AKH (0.5 and 1 mg/kg) increased the percentage of time spent in open arms/total time spent and the percentage of the number of open arm/total arm entries in the EPM test. Anaim-AKH (1 and 2 mg/kg) significantly increased latency (s) (initial time passed) for mice to lick their hind paws or jumping in the hot plate test. Anaim-AKH (4 mg/kg) significantly decreased the total distance (cm) moved, or the speed (cm/s) of movement of the animals in the locomotor activity test. Neither Locmi-AKH-II nor Anaim-AKH altered the retention latency (s) in the passive avoidance test. Both Locmi-AKH-II and Anaim-AKH exerted antidepressant effects, while only Anaim-AKH had anxiolytic and analgesic effects when administered acutely. Anaim-AKH diminished locomotion at higher doses while Locmi-AKH-II had no such effects. Neither Locmi-AKH-II nor Anaim-AKH disturbed learning and memory when acutely administered. Data of our studies suggest clinical potentials of AKH to be used in depression, anxiety and pain without disturbing memory.

Key Words: acute effect, adipokinetic hormone, affective disorders, animal behavior, memory, mice

Introduction

The neurosecretory cells in the corpus cardiacum of insects synthesize a set of hormones that are called adipokinetic (lipid mobilizing), hypertrehalosemic (increase in the level of the haemolymph sugar, trehalose) or hyperprolinemic (increase of haemolymph proline levels) depending on the insect (5). They are the Adipokinetic Hormone (AKH)/Red Pigment-Concentrating Hormone (AKH/RPCH) family of peptides. The AKH/RPCH family of peptides exert multiple physiological effects in various insect model systems, although they primarily act on the metabolic status of the fat body (7). Most physiological research focuses on the functions of adipokinetic hormones in locusts during flight (7). These hormones have a direct effect on the mobilization of carbohydrates and lipids and/or the utilization of such substrates by flight muscles, but the hormones also

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have additional indirect effects on the transport of lipids, as lipoproteins, to flight muscles; there are also further effects on the enzyme system of the lipoprotein lipase in the flight muscles (4). Lipoprotein lipase is responsible for the unloading of diacylglycerol from lipoproteins, thus making it ultimately available for oxidation to power the contraction of the flight muscle (4).

Adipokinetic and hypertrehalosemic peptides act as hormones and are thus neurohormones, which are especially needed when the oxidative metabolism of insects is very high. For example, when flight muscles maximally contract, particularly over a relatively long period of flight time, insects need large amounts of energy that eventually has to be mobilized from stores in the fat body (4, 5).

Studies using transgenic manipulations of the Drosophila adipokinetic hormone gene have demonstrated that AKH induces both hypertrehalosemia and hyperlipemia (4). Similar to other neuropeptides, AKHs are multifunctional, and are involved in cardioacceleration in cockroaches and migration of tegumentary and retinal distal pigments in crustaceans (3). AKH peptides also have excitatory effects on motor neurons (10). Evidence from studies in other insects supports the central role of AKH for locomotion (19).

The structural organization of the insect corpora cardiaca is similar to the hypothalamus-neurohypophysis of the vertebrate endocrine system (4, 5, 7). In a recent study, adipokinetic neuropeptides from the corpora cardiaca of the major families of all three suborders of the Odonata were identified (8). Interestingly, the insect AKH receptors are structurally and evolutionarily related to the gonadotropin-releasing hormone receptors in vertebrates (21). Combined genetic, physiological, and biochemical analyses provide *in vivo* evidence that AKHR is as important for chronic accumulation and acute mobilization of storage fat as is the Brummer lipase, the homolog of mammalian adipose triglyceride lipase (ATGL). Simultaneous loss of the Brummer lipase and AKHR causes extreme obesity and blocks acute storage-fat mobilization in flies (9). One of the most pronounced effect of the AKH-injections was a significant reduction of ovary mass due to delayed maturation of the oocytes, from which a significantly lower number of terminal oocytes was produced. It is understood that AKH indirectly inhibits egg production through interference with the formation of energy stores in the fat body, which are mobilized for fuel egg production (11).

Using heterologous (in locusts and cockroaches) and homologous bioassays, the neuropeptide pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-NH_2 was isolated from extracts of corpora cardiaca of the Emperor dragonfly, *Anax* Imperator. Low concentrations of this synthetic peptide injected into the Emperor dragonfly increased the hemolymph lipid concentration, suggesting a possible role of the peptide in lipid homeostasis during flight. Therefore, the synthetic peptide is named Anaim-AKH, an abbreviation for *Anax* Imperator adipokinetic hormone (6). There are three *Locusta* Migratoria, namely Locmi-AKH-I, Locmi-AKH-II, Locmi-AKH-III. Each one has a different amino acid sequence. In our study, Lom-AKH-II was used. Its amino acid sequence is as follows: pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2.

As a result of a literature search, we found that acute effects of the AKH/RPCH family of peptides on animal behavior in a mouse model have not been investigated before. In our recent study (13), antidepressant, anxiolytic, analgesic and locomotion-enhancing effects of the *Anax* Imperator homeopathic remedy were shown. Also shown in our other previous study (12) were effects of chronic administration of *Anax* Imperator adipokinetic hormone, *Libellula* Auripennis adipokinetic hormone and *Phormia Terranovae* hypertrehalosaemic hormone on depression, anxiety, pain (analgesy) and locomotion. The present study aimed to relate to the acute effects of *Locusta* Migratoria adipokinetic hormone (Locmi-AKH-II) and *Anax* Imperator adipokinetic hormone (Anaim-AKH) on depression, anxiety, pain, locomotion and memory in mice in forced swimming (FST), elevated plus maze (EPM), hot plate, locomotor activity and passive avoidance tests.

Materials and Methods

Animals

Male, inbred BALB/c ByJ mice (Uludag University, Bursa, Turkey), aged 7-8 weeks and weighing 20-25 g upon arrival to the laboratory, were used in this study. The mice were kept in the laboratory for two weeks before the experiments began. The mice were maintained under standard laboratory conditions (12-h light:12-h dark cycle, lights on 07:00 h, 21 ± 1°C). All animals received food and water *ad libitum*. All procedures were conducted in accordance with the European Community Council's Directive for ethical treatment of animals (86/609/EEC) and with the approval of the Kocaeli University Medical Faculty (7/3/2014).

Experimental Groups and Drug Administration

Anaim-AKH was obtained from SciLight Biotechnology, China, and Locmi-AKH-II was obtained from Bachem, Switzerland. Both Anaim-AKH and Locmi-AKH-II were dissolved in saline with 1% DMSO. Fluoxetine (15 mg/kg) (n = 10), diazepam (2 mg/kg) (n = 10) and metamizole sodium (500 mg/kg) (n = 10) were tested as reference antidepressant, anxiolytic and analgesic drugs, respectively. Fluoxetine was obtained from Sigma Chermicals (St. Louise, MO,

USA) and was dissolved in 0.9% saline. Pharmaceutical products (ampoules) were used for diazepam and metamizole sodium and were diluted with 0.9% saline. Prior to each forced swim test (FST), elevated plus-maze test (EPM), hot plate, passive avoidance and locomotor activity tests, the animals were acutely treated intraperitoneally (i.p.) with Locmi-AKH-II (2 and 4 mg/kg) (n = 10 or 15), Anaim-AKH (0,25-4 mg/kg) (n = 10), or vehicle (saline with DMSO 1%) (n = 10 or 15) 60 min before the test in a volume of 0.1 ml/10 g body weight. A separate group of animals was used in each test.

Forced Swim Test (FST)

The FST was conducted in a manner similar to a previously described method (15). Briefly, the mice were individually placed in Plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23-25°C, where they were left for 6 min. Because this is a situation from which they cannot escape, the animals rapidly become immobile, that is, floating in an upright position and making only small movements to keep their heads above the water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

Elevated Plus-Maze Test (EPM)

Anxiety-related behavior was measured by the elevated plus-maze test. Experiments were conducted in a dimly lit, semi-soundproof room, illuminated by a table lamp (80 lux). The maze was made of wood and consisted of two open (29 cm long × 5 cm wide) and closed arms (29 cm × 5 cm with 15 cm high walls), forming a square cross with a 5-cm square center piece. To avoid falls, the open arms were surrounded by a short (1 cm) plexiglass edge. The maze was elevated 40 cm above the floor. Each mouse was placed at the center of the maze, facing one of the open arms, and was allowed to explore the maze. The open-arm activity was evaluated as follows: 1. time spent in the open arms relative to the total time spent in the plus maze (300 s), and 2. the number of entries into the open arms relative to the total number of entries into both the open and closed arms. These values were expressed as percentages and accepted as indexes of anxiety in mice.

Hot Plate Test

The hot-plate test was used to measure pain reaction latencies. Animals were placed into a glass square on a hot plate maintained at 55 ± 0.1°C. The latency of licking the hind paws or jumping was recorded as an index of pain reaction. A cutoff time of 60 s was used.

Locomotor Activity Test

Locomotor activity was measured using an open field test in a square arena of a 40 × 40 × 40 cm box. The animal was placed in the center of the apparatus, and its behaviors were recorded for a period of 5 min using the Ethovision-XT video tracking system. The locomotor activity was evaluated by measuring the total distance traveled (cm) in the apparatus as well as the speed (cm/s) of the animals.

Passive Avoidance Test

Animals were trained in a one-trial step-through passive avoidance (PA) apparatus to evaluate memory based on contextual fear conditioning and instrumental learning (14). A decrease in retention latency indicates memory impairment in the PA task. The apparatus consists of a box with an illuminated area (L 7 × 12.5 × h 14 cm) and a dark area (L 24 × 12.5 × h 14 cm), both equipped with a floor grid composed of steel bars (0.3-cm diameter) spaced 0.9 cm apart. The inhibitory avoidance task consisted of two trials. On the first day of training, the mice were placed individually into the light compartment and were allowed to explore the boxes. The intercompartment door was opened after a 60 s acclimation period. In the acquisition trial, each mouse was placed in the illuminated compartment, which was lit by a bright bulb (2,000 lux). The animals received drugs prior to the acquisition training. If the mouse stepped into the dark compartment (in which 2/3 of the tail was inside), the door was closed and an inescapable foot shock (0.25 mA/1 s) was delivered through the grid floor of the dark compartment. A cutoff time of 5 min was selected. The time taken to enter the dark compartment (training latency) was recorded. Immediately after the shock, the mouse was returned to the home cage. The retention trial started 24 h after the end of the acquisition trial. Each mouse was placed in the illuminated compartment in the same manner as the training trial. The door was opened after a 30 s acclimation period. The step-through latency in the retention trial (with a maximum 300 s cutoff time) was used as the index of retention of the learned experience. No shock was applied during the retention trial.

Statistical Evaluation

The results of the FST, EPM, hot plate, passive avoidance and locomotor activity tests were evaluated by one-way ANOVA followed with Dunnett's *post-hoc* test when significant differences were detected. The data are expressed as the mean values ± SEM. The differences were considered to be statistically significant when P was less than 0.05.

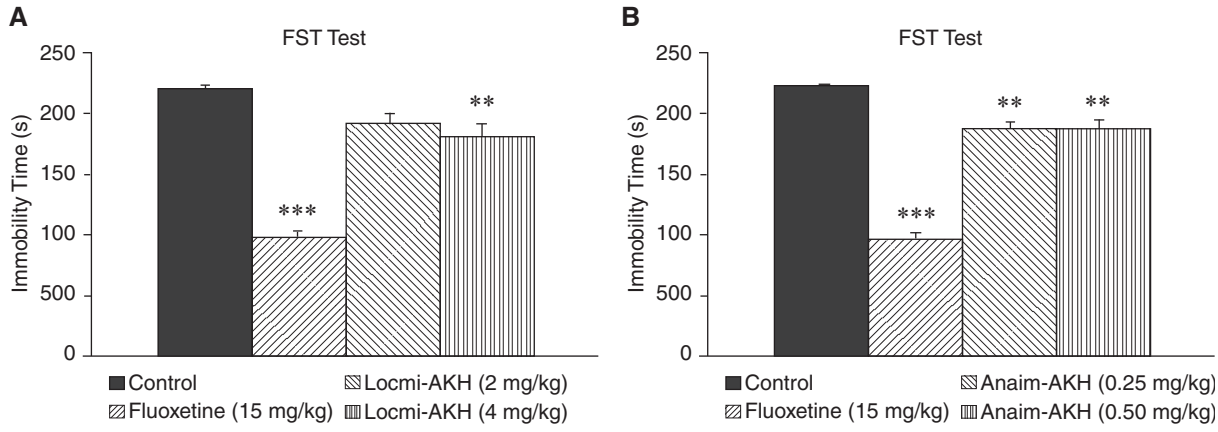

Fig. 1. Effects of fluoxetine (15 mg/kg) (n = 10), Locmi-AKH-II (2 and 4 mg/kg) (n = 10) (A) and Anaim-AKH (0.25 and 0.50 mg/kg) (n = 10) (B) given acutely on immobility time (s) in the FST in mice. Data are means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* the control group.

Results

Effects of Locmi-AKH-II and Anaim-AKH on Depression in the Forced Swimming Test

In the FST test, fluoxetine (15 mg/kg) ($P < 0.001$) and Locmi-AKH-II (4 mg/kg) ($P < 0.01$) significantly diminished immobility time compared to the control group [F(3,36)=41.50; $P < 0.0001$; Fig. 1A]. Anaim-AKH (0.25 and 0.5 mg/kg) ($P < 0.01$) also significantly diminished immobility time compared to that of the control group [F(3,36)=75.34; $P < 0.0001$; Fig. 1B]. Effect of Locmi-AKH-II (4 mg/kg) was 18% lower than the control while effects of Anaim-AKH (0.25 and 0.5 mg/kg) were 16% lower than the control.

Effects of Locmi-AKH-II and Anaim-AKH on Anxiety in the Elevated Plus Maze Test

In the EPM test, diazepam (2 mg/kg) significantly increased the percentage of time spent in the open arms ($P < 0.05$; Fig. 2A) and the percentage of open arm/total arm entries ($P < 0.05$; Fig. 2B) while Locmi-AKH-II (2 and 4 mg/kg) did not alter the percentage of time spent in the open arms [F(3,36)= 2.86; $P = 0.05$; Fig. 2A] and the percentage of open arm/total arm entries [F(3,36)=2.91; $P = 0.04$; Fig. 2B] when the drug was administered acutely.

In the EPM test, diazepam (2 mg/kg) significantly increased the percentage of time spent in the open arms/total time spent ($P < 0.01$; Fig. 2C) and the percentage of open arm/total arm entries ($P < 0.05$; Fig. 2D) and Anaim-AKH (0.5 and 1 mg/kg) ($P < 0.05$) also significantly increased the percentage of time spent in the open arms [F(3,36)=4.62; $P = 0.0078$; Fig. 2C], while Anaim-AKH (0.5 and 1 mg/kg) ($P < 0.05$, $P < 0.01$; respectively) significantly increased the percentage of open arm/total arm entries [F(3,36)=4.51; $P = 0.0087$; Fig. 2D]. Effects of Anaim-AKH (0.5 and 1 mg/kg) were about 2 times higher than that of the control.

Effects of Locmi-AKH-II and Anaim-AKH on Pain (Analgesy) in the Hot Plate Test

In the hot plate test, metamizole sodium (500 mg/kg) ($P < 0.01$) significantly increased the latency for the first time the mice licked their hind paw while Locmi-AKH-II (2 and 4 mg/kg) ($P > 0.05$) did not alter the latency for the first time that the mice licked their hind paws compared to the controls [F(3,36)= 3.70; $P = 0.02$; Fig. 3A]. In the hot plate test, Anaim-AKH (1 and 2 mg/kg) ($P < 0.05$) significantly increased the latency for the first time the mice licked their hind paws compared to the controls [F(3,36)=8.23; $P = 0.0003$; Fig. 3B]. Effects of Anaim-AKH (1 and 2 mg/kg) were 65% higher than that of the control.

Effects of Locmi-AKH-II and Anaim-AKH on Locomotion in the Locomotor Activity Test

In the locomotor activity test, Locmi-AKH-II (2 and 4 mg/kg) did not alter the total distance moved compared to the control mice [F(2,42)=0.25; $P = 0.77$; Fig. 4A]; additionally, Locmi-AKH-II did not alter the speed of the animals compared to the controls [F(2,42)= 0.23; $P = 0.78$; Fig. 4B]. In the locomotor activity test, Anaim-AKH (4 mg/kg) ($P < 0.05$) decreased the total distance moved [F(2,27)=4.26; $P = 0.02$; Fig. 4C] and decreased the speed of the animals [F(2,27)=4.30; $P = 0.02$; Fig. 4D]. Effect of Anaim-AKH (4 mg/kg) was about 40% lower than in the control for both total distance moved and speed of the animals.

Effects of Locmi-AKH-II and Anaim-AKH on Learning and Memory in the Passive Avoidance Test

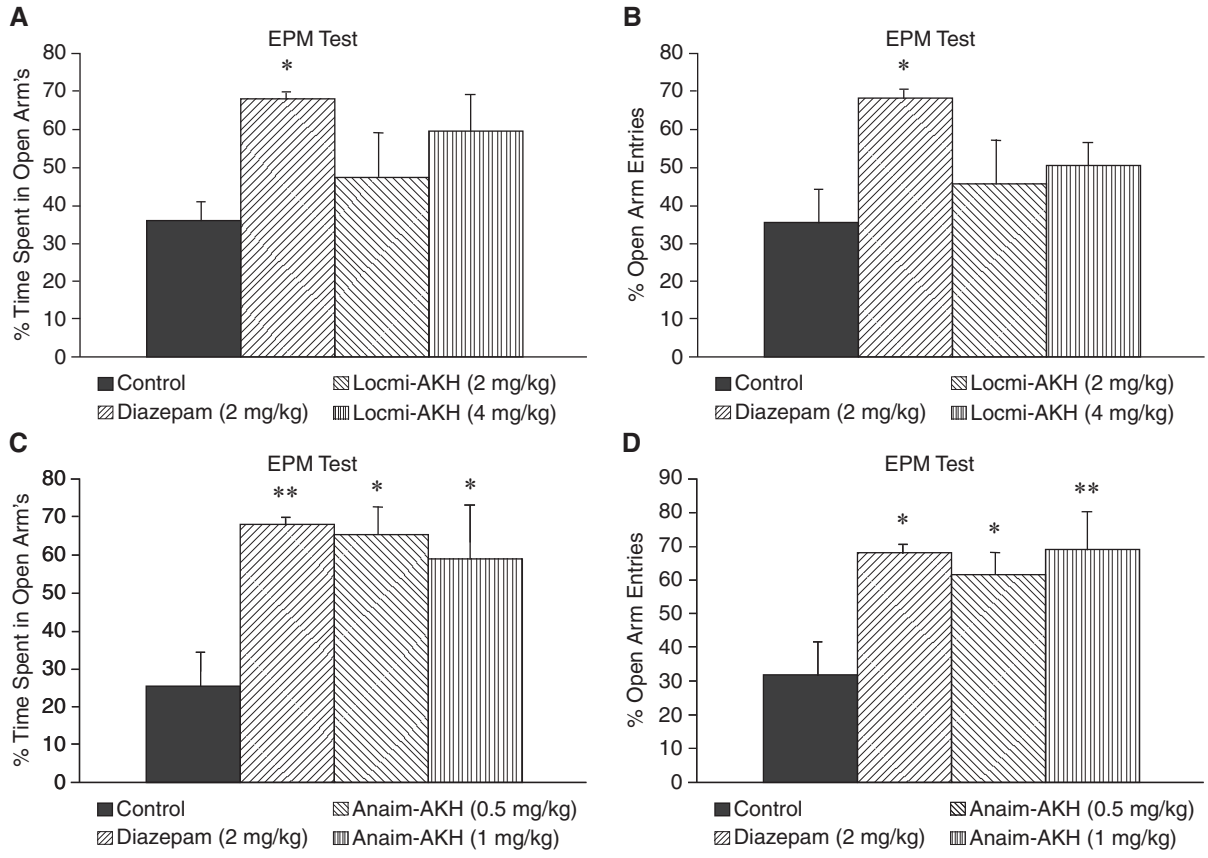

Fig. 2. Effects of diazepam (2 mg/kg) (n = 10), Locmi-AKH-II (2 and 4 mg/kg) (n = 10) (A, B) and Anaim-AKH (0.5 and 1 mg/kg) (n = 10) (C, D) given acutely on percentage time spent in the open arms/total time spent in the plus maze and on percentage open arm/total arm number of entries in the EPM test in mice. *$P < 0.05$, **$P < 0.01$ *vs*. the control group.

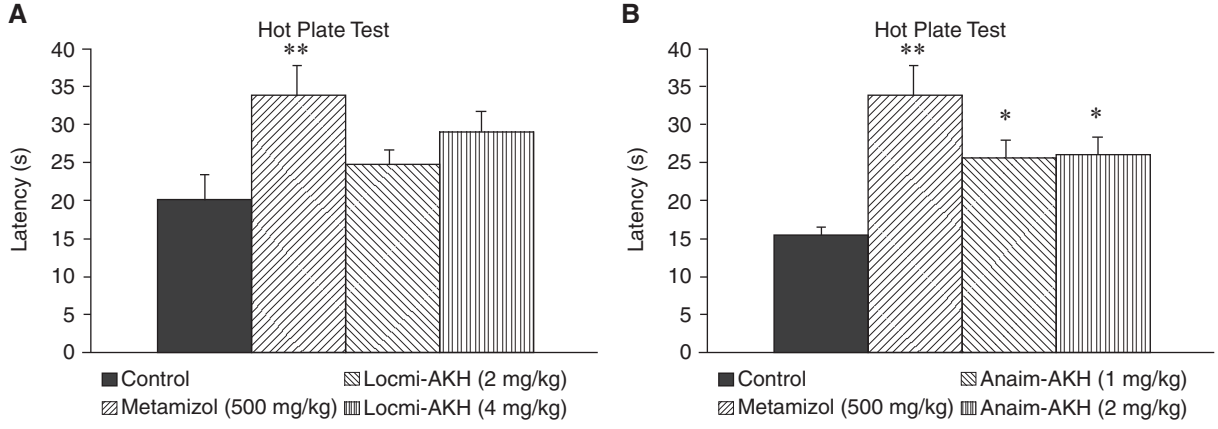

Fig. 3. Effects of metamizol sodyum (500 mg/kg) (n = 10), Locmi-AKH-II (2 and 4 mg/kg) (n = 10) (A) and Anaim-AKH (1 and 2 mg/kg) (n = 10) (B) given acutely on latency (s) to lick the hindpaws in the hot plate test in mice. *$P < 0.05$, **$P < 0.01$ *vs*. the control group.

In the passive avoidance test, Locmi-AKH-II (2 and 4 mg/kg) did not alter the first-day latency [$F(2,27)= 2.46$; $P = 0.10$] and nor the retention latency compared to the control mice [$F(2,27)=2.09$; $P = 0.14$; Fig. 5A]. Additionally, Anaim-AKH (0.25 and 0.5 mg/kg) also did not alter the first day latency [$F(2,27)=0.45$; $P = 0.64$] nor the retention latency compared to the controls [$F(2,27)=0.98$; $P = 0.38$; Fig. 5B].

Discussion

There are many studies performed on the insect

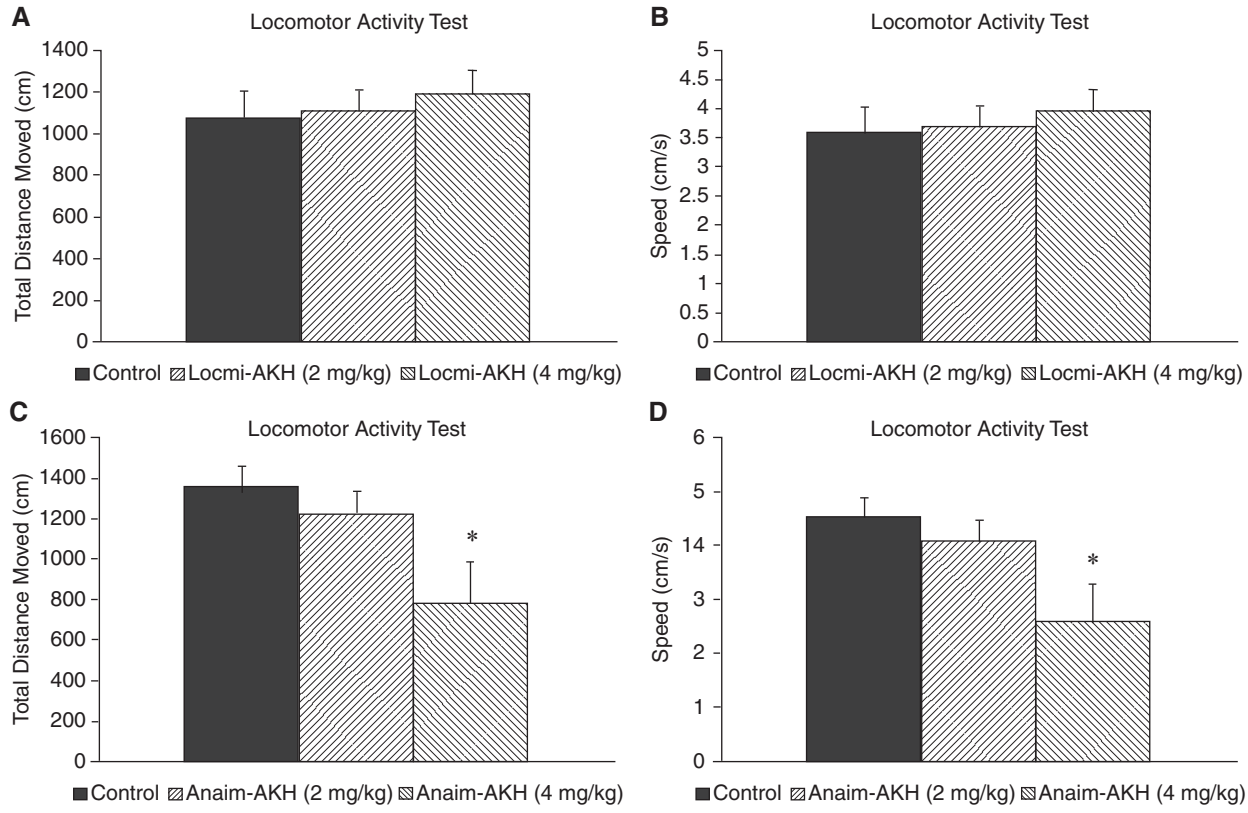

Fig. 4. Effects of Locmi-AKH-II (2 and 4 mg/kg) (n = 15) (A, B) and Anaim-AKH (2 and 4 mg/kg) (n = 10) (C, D) given acutely on total distance moved (cm) (A) and speed (cm/s) (B) in the locomotor activity test. *$P < 0.05$ *vs*. the control group.

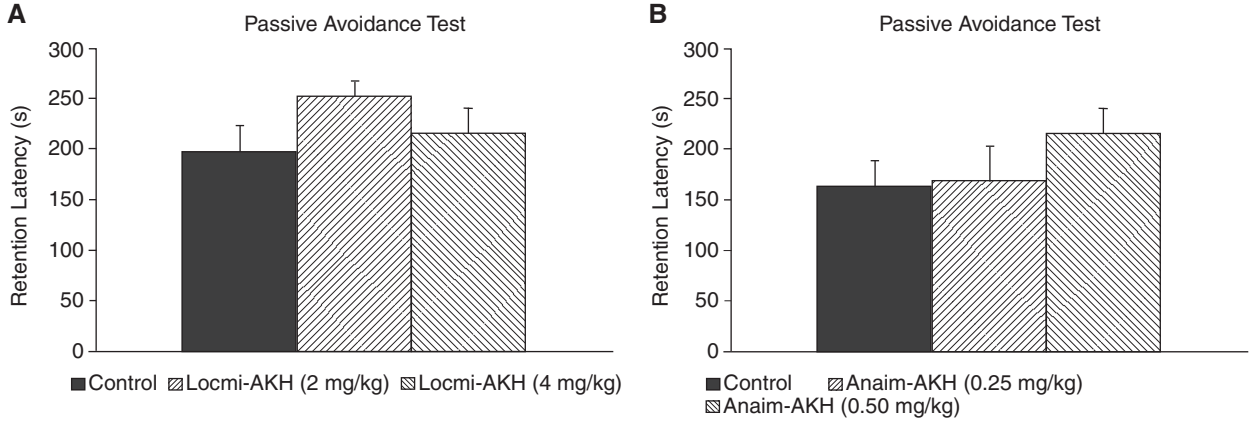

Fig. 5. Effects of Locmi-AKH-II (2 and 4 mg/kg) (n = 10) (A) and Anaim-AKH (0.25 and 0.50 mg/kg) (n = 10) (B) given acutely on retention latency (s) of the passive avoidance test.

neuropeptides for the identification of their structures and influences. The AKH/RPCH family of peptides were our focuses in previous studies (12). In the present study, we investigated the insect peptides Locmi-AKH-II and Anaim-AKH. On acute administration to mice, we found that Locmi-AKH-II and Anaim-AKH both exert antidepressant effects in the FST test. Anaim-AKH had anxiolytic and analgesic effects in the EPM and hot plate tests while Locmi-AKH-II had no such an effect. Locmi-AKH-II did not alter locomotion in the locomotor activity test, while Anaim-AKH decreased locomotion. Both Locmi-AKH-II and Anaim-AKH were not observed to alter retention latency in the passive avoidance test which supports that AKH does not disturb memory when acutely administered.

Antidepressant, anxiolytic and analgesic effects of AKH correlated with our recent study (12) in which the chronic effects of AKH were investigated in animal behavioral studies. We showed that Anaim-AKH, Libellula Auripennis AKH and Phormia Terranovae

hypertrehalosaemic hormone had antidepressant effects in forced swimming test in the mice (12). In the present study, we also found that Locmi-AKH-II and Anaim-AKH had antidepressant effects in forced swimming test, which supported that AKH may possess strong antidepressant-like effects both after acute and chronic injections.

We also showed previously that Libellula Auripennis AKH and Phormia Terranovae hypertrehalosaemic hormone had anxiolytic effects when given chronically in elevated plus maze test while Anaim-AKH had no anxiolytic effect in this test after chronic injection (12). In the present study, Anaim-AKH had anxiolytic effects in the EPM test after acute injection, while Locmi-AKH-II had no significant effect. According to these results, anxiolytic effects of AKH may change due to the different type of insect peptides, amount of given doses and administration type.

We also showed previously that Anaim-AKH and Phormia Terranovae hypertrehalosaemic hormone had antinociceptive effects in hot plate test in male balb-c mice after chronic injections (12). In present study, Anaim-AKH had analgesic effect, while Locmi-AKH-II had no analgesic effect after acute injections. It also seems that the analgesic effects of AKH were related to the different types of insect peptides and the administration modes.

It has been suggested that adipokinetic hormones may contribute to the neuronal function in the human central nervous system (22). Several studies (16-18, 20) have shown the effects of AKH on the central nervous system. The central action of Periplanetin CC-1 (Pea-HrTH) (Glp-Val-Asn-Phe-Ser-Pro-Asn-TrpNH_2), an octapeptide of the insect adipokinetic hormone family (AKH-family) isolated from American cockroach-Periplaneta Americana, was studied in Albino Swiss mice (20-25 g). CC-1 was intracerebroventricularly (i.c.v.) injected in a volume of 5 microliters at a dose of 50 ng/mouse. CC-1 showed a strong analgesic activity in a writhing syndrome test and hot plate test. In addition, Periplanetin CC-1 decreased the threshold for tonic seizures while increasing mortality in pentetrazol-induced seizures, but CC-1 had no influence on electric convulsions (18). There are many other informative publications regarding the AKH Family (1, 2, 23, 24). In our previous published study (12) and in this study, we also showed the central action and analgesic effects of AKH which were correlated with above studies.

In our recent study (12), we showed that chronic administration of AKH/RPCH peptides had antidepressant, anxiolytic, analgesic and locomotor enhanching effects in mice. We also showed that the effect mechanism of the AKH/RPCH family peptides can be related to increase in the expression of neurotrophic factors in the brain and to the potential proliferative and neuroprotective effects of AKH in hippocampal neurogenesis and neurodegeneration in mice (12). Furthermore, chronic administration of AKH enhanced locomotion whereas in the present study acute administration of Anaim-AKH decreased the total distance moved and decreased the speed of the animals while Locmi-AKH-II had no effect. The difference between the two studies and the drugs used could be due to different experimental conditions, pharmacokinetic effects, drug doses and administration periods in acute *vs*. chronic adminstration. AKH also did not disturb memory when acutely administered. This is important because most of the antidepressant, anxiolytic and analgesics have memory disturbing effects in patients. Effects of chronic administration of AKH on learning and memory and the effect mechanism of acute administration of Locmi-AKH-II or Anaim-AKH on animal behaviour should be further investigated. In conclusion, data from this study support the potentials of acute administration of AKH to treat depression, anxiety and pain without disturbing memory, although further studies with different methods, doses and administration routes should be conducted to confirm these conclusions.

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