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Effects of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides on MK-801-induced schizophrenia models

Running title: Adipokinetic hormone schizophrenia

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ABSTRACT

The adipokinetic and red pigment-concentrating hormone (AKH/RPCH) family of peptides controls fat, carbohydrate and protein metabolism in insects. In our previous study, we showed that AKH possesses antidepressant, anxiolytic, and analgesic effects, causes hyperlocomotion and exerts neuroprotective effects and increased brain neurotrophic factors in mice. The aim of this study is to investigate the effects of *Anax imperator* AKH (Ani-AKH), *Libellula auripennis* AKH (Lia-AKH) and *Phormia-Terra* hypertrehalosemic hormone (Pht-HrTH) on MK-801-induced memory deterioration in the active allothetic place avoidance test (AAPA) and MK-801-induced sensorimotor gating deficit in the prepulse inhibition test (PPI). In the AAPA task, Long Evans rats were treated with Ani-AKH (2 mg/kg), Lia-AKH (2 mg/kg), Pht-HrTH (2 mg/kg), MK-801 (0.15 mg/kg) and the combination of MK-801 with the hormones sub-chronically. In the prepulse inhibition test, Wistar-albino rats were treated with Ani-AKH (1 mg/kg), Lia-AKH (1 mg/kg), Pht-HrTH (1 mg/kg), MK-801 (0.1 mg/kg) or the combination of MK-801 with hormones acutely before the test. In our study, Ani-AKH (2 mg/kg), Lia-AKH (2 mg/kg) and Pht-HrTH (2 mg/kg) reversed MK-801 (0.15 mg/kg)-induced cognitive memory impairment effects in the AAPA task. Lia-AKH (1 mg/kg) significantly potentiated the MK-801-induced PPI disruption, while Ani-AKH (1 mg/kg) partially potentiated the impairment caused by MK-801, and Pht-HrTH did not modify the effect of MK-801. In conclusion, AKH had no effect in sensoriomotor gating deficits in the PPI test in schizophrenia model while AKH improved memory in the schizophrenia model of MK-801.

Key words: Adipokinetic hormone; schizophrenia; MK-801; rat

INTRODUCTION

One of the major neuropeptide groups in insects is the adipokinetic and hypertrehalosemic peptide family, which belongs to the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides [1]. Adipokinetic and hypertrehalosemic peptides control fat, carbohydrate and protein metabolism [2]. Approximately 30 different peptides are currently known, rendering this family one of the largest family of peptides. All members are from 8 to 10 amino acids long, N-terminally blocked by a pyroglutamate residue and C-terminally blocked by an amide. These peptide hormones are products of neurosecretory

neurons located in the corpora cardiaca, neuroendocrine glands attached to the brain [3]. Adipokinetic hormones (AKHs) are metabolic neuropeptides, mediating the mobilization of energy substrates from the fat body in many insects. Moreover, it is known that AKH peptides have excitatory effects on motor neurons [4]. AKH receptors are structurally close to gonadotropin-releasing hormone (GnRH) and the vasopressin/oxytocin superfamily of receptors [5]. In a recent study, improvement in the effects of oxytocin on MK-801-induced deficits in the prepulse inhibition test (PPI) and modulation of dopaminergic and glutamatergic system by oxytocin was shown [6].

In previous studies, it was suggested that adipokinetic hormone may contribute to the neuronal function in the human central nervous system [7]. This study revealed that an antiserum raised against locust adipokinetic hormone I can display a considerable quantity of adipokinetic hormone-like immunoreactivity in the human cerebrospinal fluid [7]. In another study, a novel peptidergic system was identified in the rat central nervous system by using an antiserum to locust adipokinetic hormone I [8]. Immunoreactive fibers were present in the hypothalamic median eminence and periventricular nucleus and the spinal cord dorsal horn, intermediolateral cell column and sacral parasympathetic nucleus. Immunoreactive cells were present in the dorsal gray commissure of the lumbosacral spinal cord, hypothalamic periventricular nucleus and cerebral cortex [8]. In our recent study, we showed that the (AKH/RPCH) peptide family possesses antidepressant, anxiolytic, and analgesic effects, causes hyperlocomotion and exerts neuroprotective effects after chronic injection in mice [9].

Some structural and functional differences exist among the various types of insect AKHs. Using a heterologous (in locusts and cockroaches) and a homologous bioassay, the neuropeptide pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-NH_2 was isolated from the extracts of the corpora cardiaca of the Emperor dragonfly, *Anax imperator* (Ani-AKH). The amino acid sequence of *Libellula auripennis* AKH (Lia-AKH) was determined as pGlu-Val-Asn-Phe-Thr-Pro-Ser-Trp-NH_2, while *Phormia-Terra* hypertrehalosemic hormone (Pht-HrTH) showed a sequence of pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH_2 [3]. The adipokinetic or hyperlipemic effect refers to the increase in the hemolymph lipid concentration, while the hypertrehalosemic effect is the increase in hemolymph carbohydrates in insects. Studies using transgenic manipulations of the dAkh gene have demonstrated that AKH plays a role in both

carbohydrate and lipid metabolism, while the hypertrehalosemic hormone mostly plays a role in carbohydrate metabolism in insects [10].

For many years, positive psychotic symptoms were the primary target in the treatment of schizophrenia. However, schizophrenia is also associated with cognitive dysfunction [11]. Cognitive impairment in schizophrenia is common, and it decreases the quality and function of life [12]. Therefore, effective treatment of cognitive deficits in schizophrenic patients may greatly affect the patient's quality of life. MK-801 (dizocilpine) is frequently used non-competitive N-methyl-d-aspartate (NMDA) receptor antagonist in the animal models to mimic psychosis for experimental purposes [13]. Unlike dopaminergic agonists, which mimic only the positive symptoms of schizophrenia, a single injection of MK-801 was also successful in modeling negative symptoms of schizophrenia [14, 15]. Prepulse inhibition (PPI) is a neurological phenomenon in which a weaker prestimulus (prepulse) inhibits the reaction of an organism to a subsequent strong startling stimulus (pulse). Many studies have shown that PPI is disturbed in many diseases and disorders, especially in schizophrenia [16]. MK-801 disturbs PPI and can be used as a pharmacological model of schizophrenia and psychosis [17].

AKH exerts improved effects in depression and anxiety by increasing neurogenesis and brain neurotrophic factors in the central nervous system [9, 18]; therefore, it might also be important to study whether AKH shows improved effects in the model of schizophrenia. The aim of this study was to investigate the effects of three insect peptides which we have found significant behavioural effects in our previous studies [9, 18]; *Anax imperator* AKH (Ani-AKH), *Libellula auripennis* AKH (Lia-AKH) and *Phormia-Terra* hypertrehalosemic hormone (Pht-HrTH) on MK-801-induced memory deterioration in the active allothetic place avoidance task (AAPA) and MK-801-induced psychosis model using the prepulse inhibition test (PPI).

MATERIALS AND METHODS

Animals

Three to four-month-old male Long Evans rats were used for the active allothetic place avoidance task and were obtained from the accredited breeding colony of the Institute of Physiology, Academy of Science, Prague. Male outbred Wistar rats (Velaz, Czech Republic) weighing 180-250 g were used for the PPI test. The rats were acclimatized for 7-10 days prior to testing, during which they were weighed twice and handled four times.

The rats were housed in pairs in transparent plastic cages (30×30×40 cm) in an air-conditioned animal facility with a constant temperature of 21°C ± 2°C and a 12/12 light/dark cycle (lights on at 7:00). All animals received food and water ad libitum. All procedures were in accordance with Czech and European legislation regarding treatment of laboratory animals (Directive 86/609/EEC).

Drugs and treatments

(+)-MK-801 maleate ((5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo(a, d)-cyclo-hepten-5,10-imine maleate, further referred only as MK-801) was obtained from Tocris Bioscience, United Kingdom. Ani-AKH, Lia-AKH and Pht-HrTH were purchased from TRC (Toronto/Canada). MK-801 was dissolved in saline, while AKH was dissolved in saline mixed with 15% DMSO. Vehicle consisting of saline with 15% DMSO was used as the control group.

In the AAPA task, Long Evans rats were treated regularly for 4 days (subchronically) with all the above-specified hormones at a dose of 2 mg/kg, MK-801 (0.15 mg/kg) alone or in combination or with a vehicle before the AAPA task. All treatments were administered at a volume of 2 ml/kg body weight, and the number of animals per group varied between 7 and 8 animals. On the 4^{th} day, the hormones were administered intraperitoneally (i.p.) 60 min before the AAPA task, while MK-801 was administered 40 min before the test.

In the PPI test, the rats were treated with hormones at 1 mg/kg i.p., MK-801 (0.1 mg/kg) alone or in combination or with a vehicle according to this paradigm. All hormones were administered intraperitoneally (i.p.) for 60 min before the PPI test, while MK-801 was

administered 30 min before the test in a volume of 2 ml/kg body weight, and the number of animals per group varied between 12 and 15 animals. Strain of animals used in each test, doses of the drugs and administration period of drugs were chosen according to the previous studies [9, 13, 18, 19, 20]. A separate group of animals was used in each test.

Active allothetic place avoidance task (AAPA)

The active allothetic place avoidance task is a spatial memory test that can determine the ability of animals to organize their behavior efficiently under both normal and pathological conditions. Successful performance of the task requires the rat to solve a conflict between two discordant subsets of spatial stimuli (extramaze and intramaze cues). We believe that successful performance of the active allothetic place avoidance task, in which the rat has to differentiate between relevant and irrelevant stimuli, depends on the mode of information processing disturbed in schizophrenic patients [13]. The active allothetic place avoidance arena was described in detail in previous papers [21]. Briefly, it consists of a smooth metallic circular arena (82 cm in diameter), enclosed by a 30-cm-high transparent Plexiglas wall. The arena was elevated 1 m above the floor of a 4×5-m experimental room containing an abundance of extra-maze landmarks. The arena together with its wall was rotated (1 rpm) by an electric motor placed underneath the arena disc. At the beginning of each training session, a rat was placed in the arena at a location opposite an imperceptible 60° to-be-avoided sector (shock sector), which was defined by the computer-based tracking system (iTrack, Biosignal Group, USA). The arena rotation started immediately after placement of the rat. The location of the shock sector could be determined solely by its spatial relationship to the distal orienting cues located in the room. The rats were wearing a light latex harness, to which an infrared light-emitting diode (LED) was attached between the rat's shoulders. Whenever the rat entered the shock sector for more than 500 ms (milliseconds), the tracking system delivered a mild, constant-current shock (50 Hz, 0.5 s, 0.4-0.7 mA) and counted an entrance. If the rat did not leave the sector, additional shocks were given every 1200 ms, but no additional entrances were counted until the rat left the sector for more than 300 ms. This procedure using shocks has previously been shown to be effective and safe for animals, leading to rapid avoidance behavior [21]. The appropriate shock current (ranging between 0.4 and 0.7 mA) was carefully adjusted for each rat to evoke a rapid escape reaction but prevent freezing. Most animals responded appropriately to a shock intensity of 0.5 mA. The arena surface was carefully cleaned by a detergent solution between rats. For each of the groups, the same control and MK-801-treated rats were used as a reference. Animals were trained in

four consecutive daily sessions in the active allothetic place avoidance task, with the shock sector stretching from the center of the arena to its north circumference. Experimental sessions in the active allothetic place avoidance task lasted 20 min, and each rat had one session every day, carried out during daylight hours.

Prepulse inhibition test (PPI)

The procedure was adopted from our previously published studies [22, 23]. Two ventilated startle chambers (SR-LAB, San Diego Instruments, California, USA), each comprising a sound-attenuated, evenly lit enclosure containing a high-frequency loudspeaker (which produced the discrete broadband acoustic pulses and prepulses) mounted 24 cm above a Plexiglas stabilimeter (8.7 cm in inner diameter). A dynamic calibration system ensured equivalent stabilimeter sensitivity between chambers. A piezoelectric accelerometer detected the average startle amplitudes (AVG) that were digitized and used for subsequent analyses. The sound levels were measured using a RadioShack sound level meter.

Two days before the test, the rats were acclimatized to the startle chamber using a drug-free, 5-minute pre-training procedure consisting of 5 presentations of pulse-alone stimuli (115 dB/20 ms) over background white noise (75 dB). The startle data were not recorded for acclimatization. On the test day, there were 72 trials in total with an inter-trial interval (ITI) of 4-20 s (mean ITI: 12.27 s). The rats were acclimatized for 5 min to the startle chamber in which 75-dB background white noise was continuously presented. Six 125 dB/40 ms-duration pulse-alone trials were then delivered to establish the baseline acoustic startle response (ASR). Thereafter, 60 trials were presented pseudorandomly as follows: (A) pulse alone: 40 ms/125 dB; (B) prepulse-pulse: 20-ms/83-dB or 91-dB prepulse, a variable (30, 60 or 120 ms) inter-stimulus interval (ISI: mean 70 ms), followed by a 40-ms/125-dB pulse; (C) no stimulus for 60-ms. Finally, six pulse-alone trials were delivered. Habituation was calculated by the percentage reduction in ASR from the initial six baseline trials to the final six trials. PPI was calculated as follows: [100 – (mean prepulse – pulse trials/mean pulse-alone trials) $*$ 100]. The mean ASR was derived from the pulse-alone trials.

Statistical Evaluation

The results of the AAPA and PPI tests were evaluated by two-way ANOVA followed by Bonferroni post hoc test when significant differences were detected. The data were expressed as mean values ± SEM. The differences were considered statistically significant when the alpha value was equal to or below 0.05.

Results

Effects of Ani-AKH, Lia-AKH and Pht-HrTH on MK-801-induced memory deterioration in the AAPA test

In the AAPA test, when the Ani-AKH groups were evaluated for the total distance moved (Fig 1a), two-way ANOVA revealed no significant effect of Ani-AKH [$F(1,28)=0.54$; $p=0.46$] while there was a significant effect of MK-801 [$F(1,28)=7.29$; $p=0.01$] and interaction [$F(1,28)=9.53$; $p=0.004$]. In the AAPA test, when the Lia-AKH groups were evaluated (Fig 1b), two-way ANOVA revealed no significant effect of Lia-AKH [$F(1,27)=0.11$; $p=0.73$] while there was a significant effect of MK-801 [$F(1,27)=4.76$; $p=0.03$] and interaction [$F(1,27)=4.98$; $p=0.03$]. In the AAPA test, when the Pht-HrTH groups were evaluated (Fig 1c), two-way ANOVA revealed significant effect of Pht-HrTH [$F(1,27)=4.69$; $p=0.03$] and MK-801 [$F(1,27)=7.95$; $p=0.008$] while there was no significant effect of interaction [$F(1,27)=1.66$; $p=0.20$]. Bonferroni post hoc test found that AKH treatment did not affect locomotion in naive rats. MK-801 significantly increased locomotion ($p<0.05$) compared with that in the control, and this effect was reversed by Ani-AKH 2 mg/kg ($p<0.05$) while Lia-AKH 2 mg/kg had a partial effect. Ani-AKH 2 +MK-801 was statistically different from the MK-801 group ($p<0.05$). Pht-HrTH 2 mg/kg showed no reversing effect on MK-801-induced hyperlocomotion, while the total distance moved was also increased in the Pht-HrTH 2 mg/kg+MK-801 group compared with that in the control group ($p<0.01$).

In the AAPA test, when the Ani-AKH groups were evaluated for the number of entries (Fig 2a), two-way ANOVA revealed no significant effect of MK-801 [$F(1,28)=2.65$; $p=0.11$] while there was a significant effect of Ani-AKH [$F(1,28)=8.53$; $p=0.006$] and interaction [$F(1,28)=12.53$; $p=0.0014$]. In the AAPA test, when the Lia-AKH groups were evaluated (Fig 2b), two-way ANOVA revealed no significant effect of MK-801 [$F(1,27)=2.61$; $p=0.11$]

while there was a significant effect of Lia-AKH [$F(1,27)=4.41$; $p=0.04$] and interaction [$F(1,27)=7.93$; $p=0.009$]. In the AAPA test, when the Pht-HrTH groups were evaluated (Fig 2c), two-way ANOVA revealed no significant effect of Pht-HrTH [$F(1,27)=2.38$; $p=0.13$] and MK-801 [$F(1,27)=2.06$; $p=0.16$] while there was significant effect of interaction [$F(1,27)=8.49$; $p=0.007$]. Bonferroni post hoc test found that MK-801 treatment significantly increased the number of entries ($p<0.01$), while Ani-AKH 2 mg/kg ($p<0.001$), Lia-AKH 2 ($p<0.01$) and Pht-HrTH 2 mg/kg ($p<0.01$) significantly reversed this effect.

In the AAPA test, when the Ani-AKH groups were evaluated for the number of shocks (Fig 3a), two-way ANOVA revealed significant effect of Ani-AKH [$F(1,28)=7.89$; $p=0.008$], MK-801 [$F(1,28)=4.90$; $p=0.03$] and interaction [$F(1,28)=10.29$; $p=0.003$]. In the AAPA test, when the Lia-AKH groups were evaluated (Fig 3b), two-way ANOVA revealed no significant effect of Lia-AKH [$F(1,27)=2.73$; $p=0.10$] and MK-801 [$F(1,27)=1.88$; $p=0.18$] while there was significant effect of interaction [$F(1,27)=7.72$; $p=0.009$]. In the AAPA test, when the Pht-HrTH groups were evaluated (Fig 3c), two-way ANOVA revealed no significant effect of Pht-HrTH [$F(1,27)=3.10$; $p=0.08$] and MK-801 [$F(1,27)=3.13$; $p=0.08$] while there was significant effect of interaction [$F(1,27)=7.60$; $p=0.01$]. Bonferroni post hoc test found that MK-801 treatment significantly increased the number of shocks ($p<0.05$), while Ani-AKH 2 mg/kg ($p<0.001$), Lia-AKH 2 mg/kg ($p<0.01$) and Pht-HrTH 2 mg/kg ($p<0.01$) significantly reversed this effect.

In the AAPA test, when the Ani-AKH groups were evaluated for the maximum time of avoidance (Fig 4a), two-way ANOVA revealed significant effect of Ani-AKH [$F(1,28)=13.21$; $p=0.001$] and interaction [$F(1,28)=15.96$; $p=0.0004$] while there was no significant effect of MK-801 [$F(1,28)=0.13$; $p=0.71$]. In the AAPA test, when the Lia-AKH groups were evaluated (Fig 4b), two-way ANOVA revealed significant effect of Lia-AKH [$F(1,27)=4.75$; $p=0.03$] and interaction [$F(1,27)=5.60$; $p=0.02$] while there was no significant effect of MK-801 [$F(1,27)=2.21$; $p=0.14$]. In the AAPA test, when the Pht-HrTH groups were evaluated (Fig 4c), two-way ANOVA revealed no significant effect of Pht-HrTH [$F(1,27)=1.66$; $p=0.20$] and MK-801 [$F(1,27)=0.60$; $p=0.44$] while there was significant effect of interaction [$F(1,27)=8.80$; $p=0.0062$]. Bonferroni post hoc test found that MK-801 treatment significantly decreased the maximum time of avoidance ($p<0.05$) in the Ani-AKH and Lia-AKH groups, while the effect was not significant in the Pht-HrTH groups. Ani-AKH

2 ($p<0.001$), Lia-AKH 2 ($p<0.01$) and Pht-HrTH 2 mg/kg ($p<0.05$) significantly reversed the MK-801-induced decrease in the maximum time of avoidance.

Effects of Ani-AKH, Lia-AKH and Pht-HrTH on MK-801-induced sensorimotor gating deficits in the PPI test

Two-way ANOVA revealed no significant effect of Ani-AKH [$F(1,47)=0.26$; $p=0.61$], MK-801 [$F(1,47)=3.20$; $p=0.07$] and interaction [$F(1,47)=0.24$; $p=0.62$] on the ASR data. There was also no significant effect of Ani-AKH [$F(1,47)=1.45$; $p=0.23$] and interaction [$F(1,47)=0.44$; $p=0.50$] while there was significant effect of MK-801 [$F(1,47)=4.59$; $p=0.03$] on the habituation data although post hoc tests showed no significant difference between the groups. In the PPI test, when the Ani-AKH groups were evaluated (Fig 5), two-way ANOVA revealed significant effect of Ani-AKH [$F(1,47)=5.92$; $p=0.01$] and MK-801 [$F(1,47)=20.67$; $p<0.0001$] while there was no significant effect of interaction [$F(1,47)=0.90$; $p=0.34$]. Bonferroni post hoc test found that %PPI was significantly decreased in the Ani-AKH 1mg/kg group ($p<0.05$), MK-801 0.1-alone group ($p<0.001$) and combination groups ($p<0.001$) compared with that in the vehicle control group.

Two-way ANOVA revealed no significant effect of Lia-AKH [$F(1,47)=0.96$; $p=0.33$], MK-801 [$F(1,47)=2.87$; $p=0.09$] and interaction [$F(1,47)=0.03$; $p=0.84$] on the ASR data. There was also no significant effect of Lia-AKH [$F(1,47)=0.09$; $p=0.75$] and interaction [$F(1,47)=0.003$; $p=0.95$] while there was significant effect of MK-801 [$F(1,47)=6.38$; $p=0.01$] on the habituation data although post hoc tests showed no significant difference between the groups. In the PPI test, when the Lia-AKH groups were evaluated (Fig 6), two-way ANOVA revealed significant effect of Lia-AKH [$F(1,47)=15.99$; $p=0.0002$], MK-801 [$F(1,47)=51.80$; $p<0.0001$] and interaction [$F(1,47)=4.21$; $p=0.04$]. Bonferroni post hoc test found that %PPI was significantly decreased in the MK-801 0.1-alone ($p<0.01$) and combination groups ($p<0.001$) compared with that in the vehicle control group. There was also a significant effect between the MK-801-alone and combination groups ($p<0.001$).

Two-way ANOVA revealed no significant effect of Pht-HrTH [$F(1,47)=0.01$; $p=0.89$], MK-801 [$F(1,47)=3.74$; $p=0.05$] and interaction [$F(1,47)=0.20$; $p=0.65$] on the ASR data. There was also no significant effect of Pht-HrTH [$F(1,47)=2.29$; $p=0.13$] and interaction [$F(1,47)=0.002$; $p=0.95$] while there was significant effect of MK-801 [$F(1,47)=4.59$;

p=0.03] on the habituation data although post hoc tests showed no significant difference between the groups. In the PPI test, when the Pht-HrTH groups were evaluated (Fig 7), two-way ANOVA revealed no significant effect of Pht-HrTH [$F(1,47)=1.69$; $p=0.19$] and interaction [$F(1,47)=1.39$; $p=0.24$] while there was significant effect of MK-801 [$F(1,47)=15.18$; $p=0.0003$]. Bonferroni post hoc test found that %PPI was significantly decreased in the MK-801 0.1-alone ($p<0.01$) and combination group ($p<0.01$) compared with that in the vehicle control group.

DISCUSSION

The main finding of our study was that subchronic treatment with all hormones could reverse the MK-801-induced cognitive memory impairment effects in the AAPA task. Furthermore, the MK-801-induced increase in locomotion was reversed by Ani-AKH and partially by Lia-AKH but not by Pht-HrTH. In the PPI test, Lia-AKH potentiated the deficit induced by MK-801, while Ani-AKH showed partial potentialization, and Pht-HrTH had no effect. Finally, none of the three hormones alone induced memory deterioration while only Ani-AKH 1mg/kg induced sensorimotor gating deficit.

MK-801 in animal models is known to induce positive-like symptoms (e.g., hyperlocomotion and stereotypy), sensorimotor processing and cognitive deficits [13]. Classical antipsychotics like haloperidol were known not to have improving effects on cognition in MK-801 induced schizophrenia models [13], while new generation antipsychotics had improving effects [13, 23]. It is well known that MK-801 disturbes memory similar to schizophrenia in our previous studies and antipsychotics like olanzapine, clozapine and sertindole had improving effects on MK-801 induced memory impairment [13, 23, 24]. Similar to previous studies, in our study, MK-801 caused memory deterioration and hypermotility in the AAPA test and disturbed sensorimotor gating in the PPI test. In the AAPA test, we administered the drugs subchronically and observed no memory deficit in naive animals. Similarly, AKH caused no memory deterioration in the passive avoidance test after acute administration in our recent study [18]. This supports that the AKH/RPCH family of peptides do not cause psychosis or memory deterioration in naive animals. The effects of the chronic administration of AKH on memory and sensorimotor gating can also be investigated in future studies.

We obtained contrasting results when we evaluated the effects of three hormones on MK-801-induced memory deterioration and MK-801-induced sensorimotor gating deficits. We found that all the hormones reversed MK-801-induced cognition deficits in the AAPA test, although AKH did not reverse MK-801-induced PPI disruption, although Lia-AKH significantly increased the impaired effect of MK-801. In our previous study [9], we showed that the chronic administration of AKH increased brain-derived neurotrophic factor (BDNF) and c-AMP response element binding protein (CREB) levels in the hippocampus of naive mice after two weeks of intraperitoneal administration. It was shown that, in depression and schizophrenia, the BDNF and CREB levels were decreased in the hippocampus of patients while antidepressant and antipsychotic therapy increased the diminished levels of BDNF and CREB levels, which is thought to be responsible for the pro-cognitive and mood enhancement effect of these drugs [25, 26, 27]. In our study, one explanation for the reversing effect of AKH on memory deterioration can be the enhancement of the decreased neurotrophic factors in schizophrenia [26, 28]. Additionally, in the AAPA test, some nonspecific factors, such as anxiety, can affect the results. In our recent study, we showed antidepressant and anxiolytic effects of AKH both after acute and chronic administration [9, 18]. NMDA antagonists such as MK-801 are also known to possess anxiolytic effects [29]. Thus, the anxiolytic effect of AKH and modulating effect with MK-801 on anxiety could have increased the memory performance of animals in the AAPA test. In the AAPA test, the rat can enter the shock zone in two ways, either passively or actively, and in a similar way, it can go out of the zone actively or passively. In our study, MK-801 increased total distance moved in the AAPA test while Ani-AKH and Lia-AKH reversed this effect. This situation can cause some nonspesific effects to the cognitive task. Anxiolytic effect of AKH can affect locomotion and cognitive performance of the animals in the AAPA test although there was significant effect of all three hormones on all parameters of cognition in the AAPA test.

Adipokinetic and hypertrehalosemic peptides control fat, carbohydrate and protein metabolism in insects. These hormones have direct effects on the mobilization of carbohydrates and lipids and/or the utilization of such substrates by flight muscles [3]. It was also shown that these insect peptides of the adipokinetic hormone family cause lipid mobilization in humans [30]. Adipokinetic hormone in insects is known as the analog of glucagon in humans [31]. They use similar pathways for the mobilization of lipids and carbohydrates. During the metabolism of lipids and carbohydrates by AKH, endogenous AKHs bind to a Gq-protein-coupled receptor and activate phospholipase C, and the resulting

inositol trisphosphate releases Ca^{2+} from internal stores. In insects, which use lipids for sustained flight, adenylate cyclase is activated after the AKHs bind to their Gs protein-coupled receptor. The resulting cyclic AMP, together with the messengers intra- and extracellular Ca^{2+}, activate a triacylglycerol lipase, which results in the production of 1,2 diacylglycerols or free fatty acids [32]. These second messenger pathways may also play a role in the central effects of AKH in experimental animal models and should be investigated in further studies.

In a recent study, the physiological role of membrane lipids in the modulation of NMDAR activity was shown [33]. In that study, it was shown that acute and chronic pretreatments resulting in cell cholesterol depletion profoundly diminished NMDAR responses and increased NMDAR desensitization in cultured rat cerebellar granule cells. The lipid mobilization effect of AKH/RPCH family peptides may cause the deactivation of NMDA receptors, and this can explain some synergistic effects of AKH with MK-801, which we observed in our study.

In a previous study, it was shown that adipokinetic hormone release, which can be induced by anticholinesterases, is reduced by depleting the content of monoamines in the nervous system of insects [34]. In this study, anticholinesterases increased hemolymph lipid by increasing adipokinetic hormone. Reserpin reversed the effect of AKH by depleting monoamines. The alpha adrenergic receptor blocker phentolamine, beta adrenergic receptor blocker propranolol, dopaminergic receptor blockers chlorpromazine and flupenthixol, serotonergic receptor blockers methysergide and gramine all reversed the increased effect in hemolymph lipid by adipokinetic hormone [34]. According to this study, it can be hypothesized that an increase in cholinergic activity may increase AKH, while a decrease in dopaminergic and serotoninergic activity may decrease AKH. It is known that increased cholinergic activity in the brain shows improved effects in Alzheimer's disease [35]. In our study, we can speculate that alterations in cholinergic activity by AKH may cause the improved effects on memory in a schizophrenia model while alterations in dopamine/serotonin receptors may be responsible for the increase in MK-801-induced sensorimotor gating deficits.

Classical antipsychotics such as haloperidol are known to improve the positive symptoms of schizophrenia but have no effect on negative symptoms such as cognition deficits [36, 37]. Atypical antipsychotics such as clozapine, risperidone, olanzapine, quetiapine and

aripiprazole have also demonstrated improved effects on negative symptoms such as disturbed memory and emotion [36, 37]. The results of our study showed that AKH did not act as a classical or an atypical antipsychotic drug because of the tendency to improve MK-801-induced hyperlocomotion in the AAPA test; however, no improvement effects were demonstrated on sensorimotor gating deficits in the PPI test. The controversy between our results can also be due to the different structures and functions of the insect hormones and different brain parts playing various roles in cognition, locomotion and sensorimotor gating deficits. Difference between the two tests can be also sourced from the different doses of the drugs and different strains of animals we used in our study. Long-Ewans rats were used for the AAPA test since they were used more frequently in the cognition tests while we used Wistar-albino rats for the PPI test as in previous studies [13, 19, 20].

PPI and AAPA tests were related with different tasks. APPA test is related with cognition in which we investigated effects on memory deterioration in the schizophrenia model while PPI test is related with sensoriomotor gating deficits which includes morely positive symptoms of schizophrenia. So in our study, we couldn't find effect of AKH in the PPI test which is related with positive symptoms of schizophrenia but we found improving effects in the AAPA test which shows the improving effects of AKH on memory in the schizophrenia model. Since many antipsychotic drugs have improving effects on the positive symptoms but not on memory, this finding can be also important for further studies.

Oxytocin is a nine-amino acid peptide that plays an important role in the regulation of normal cognitive function and behavior, which are disturbed in schizophrenia [6]. Nasally administered oxytocin has been reported to reduce fear, possibly by inhibiting the amygdala [38]. Oxytocin also produces antidepressant-like effects in animal models of depression [39], and its deficit may be involved in the pathophysiology of depression in humans [40]. The intranasal administration of oxytocin has been proposed as a possible route of delivery to the central nervous system for oxytocin [41]. AKH has a similar structure to that of oxytocin, and its central effects seem to be comparable to oxytocin in this study and in previous studies [9, 18]. However, oxytocin has more distinct effects than AKH in schizophrenia models in recent studies [6].

In this study, we also found that some behavioral effects could differ after the administration of different types of insect hormones. For example, in the AAPA test, Ani-AKH reversed hyperlocomotion caused by MK-801 while Lia-AKH had a partial effect, while Pht-HrTH increased the total distance moved when combined with MK-801. In our recent studies, we found that when these hormones were administered chronically, they all increased locomotion [9], while Ani-AKH diminished locomotion at the 4-mg/kg dose when administered acutely [18]. In the AAPA test, we administered the drugs subchronically and Ani-AKH decreased MK-801-induced hyperlocomotion, a finding that corresponded with our previous study findings [18]. In the PPI test, we found that Lia-AKH significantly enhanced the impairing effect of MK-801, while Ani-AKH had a partial effect and Pht-HrTH had no effect. This effect was also in line with our previous study in which we found that Pht-HrTH caused the least neurodegeneration and highest neurogenesis, while Lia-AKH caused the highest neurodegeneration and least neurogenesis in the hippocampus dentate gyrus of mice after two weeks of intraperitoneal injection [9].

CONCLUSION

This study documents that the AKH/RPCH family peptides could improve cognitive dysfunction in schizophrenia, while Pht-HrTH seems to have a superior effect to those of Ani-AKH and Lia-AKH because it did not alter the sensorimotor gating deficits induced by MK-801 in the PPI test.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1 illustrates the total distance moved (m) data obtained by intraperitoneally (i.p.) administering vehicle (n=8), Ani-AKH (*Anax Imperator Mauricianus* AKH) (2 mg/kg) (n=8), Lia-AKH (*Libellula Auripennis* AKH) (2 mg/kg) (n=7), Pht-HrTH (*Phormia-Terrae* HrTH) (2 mg/kg) (n=8), MK-801 (0.15 mg/kg) (n=8), Ani 2+MK-801 (n=8), Lia 2+MK-801 (n=8), Pht-HrTH 2+MK-801 (n=7) for 4 days in the active allothetic place avoidance test (AAPA) in Long-Ewans rats. The data are indicated as the means ± standard error of the mean (*p<0.05, **p<0.01, ***p<0.001 compared with the control group; #p<0.05 compared with the MK-801-alone group).

Figure 2 illustrates the number of entries data obtained by intraperitoneally administering (i.p.) vehicle (n=8), Lia-AKH (*Libellula Auripennis* AKH) (2 mg/kg) (n=7), Pht-HrTH (*Phormia-Terrae* HrTH) (2 mg/kg) (n=8), MK-801 (0.15 mg/kg) (n=8), Ani 2+MK-801 (n=8), Lia 2+MK-801 (n=8), Pht-HrTH 2+MK-801 (n=7) for 4 days in the active allothetic place avoidance test (AAPA) in Long-Ewans rats. The data are indicated as the means ± standard error of the mean (**p<0.01 compared with the control group; ##p<0.01, ###p<0.001 compared with the MK-801-alone group).

Figure 3 illustrates the number of shocks data obtained by intraperitoneally (i.p.) administering vehicle (n=8), Ani-AKH (*Anax Imperator Mauricianus* AKH) (2 mg/kg) (n=8), Lia-AKH (*Libellula Auripennis* AKH) (2 mg/kg) (n=7), Pht-HrTH (*Phormia-Terrae* HrTH) (2 mg/kg) (n=8), MK-801 (0.15 mg/kg) (n=8), Ani 2+MK-801 (n=8), Lia 2+MK-801 (n=8), Pht-HrTH 2+MK-801 (n=7) for 4 days in the active allothetic place avoidance test (AAPA) in Long-Ewans rats. The data are indicated as the means ± standard error of the mean (*p<0.05, **p<0.01 compared with the control group; ##p<0.01, ###p<0.001 compared with the MK-801-alone group).

Figure 4 illustrates the maximum time of avoidance (s) data obtained by intraperitoneally (i.p.) administering vehicle (n=8), Ani-AKH (*Anax Imperator Mauricianus* AKH) (2 mg/kg) (n=8), Lia-AKH (*Libellula Auripennis* AKH) (2 mg/kg) (n=7), Pht-HrTH (*Phormia-Terrae* HrTH) (2 mg/kg) (n=8), MK-801 (0.15 mg/kg) (n=8), Ani 2+MK-801 (n=8), Lia 2+MK-801 (n=8), Pht-HrTH 2+MK-801 (n=7) for 4 days in the active allothetic place avoidance test (AAPA) in Long-Ewans rats. The data are indicated as the means ± standard error of the

mean (*p<0.05, **p<0.01 compared with the control group; #p<0.05, ##p<0.01, ###p<0.001 compared with the MK-801-alone group).

Figure 5 illustrates the data on prepulse inhibition (% PPI) obtained by intraperitoneally (i.p.) administering vehicle (n=15), Ani-AKH (*Anax Imperator Mauricianus* AKH) (1 mg/kg) (n=12), MK-801 (0.1 mg/kg) (n=12), and Ani 1+MK-801 (n=12) acutely before the prepulse inhibition test (PPI) in Wistar rats. The data are indicated as the means ± standard error of the mean (*p<0.05, **p<0.01, ***p<0.001 compared with the control group).

Figure 6 illustrates the data on prepulse inhibition (% PPI) obtained by intraperitoneally (i.p.) administering vehicle (n=15), Lia-AKH (*Libellula Auripennis* AKH) (1 mg/kg) (n=12), MK-801 (0.1 mg/kg) (n=12), and Lia 1+MK-801 (n=12) acutely before the prepulse inhibition test (PPI) in Wistar rats. The data are indicated as the means ± the standard error of the mean (**p<0.01, ***p<0.001 compared with the control group; ###p<0.001 compared with the MK-801 alone group).

Figure 7 illustrates the data on prepulse inhibition (% PPI) obtained by intraperitoneally (i.p.) administering vehicle (n=15), Pht-HrTH (*Phormia-Terrae* HrTH) (1 mg/kg) (n=12), MK-801 (0.1 mg/kg) (n=12), and Pht-HrTH 1+MK-801 (n=12) acutely before the prepulse inhibition test (PPI) in Wistar rats. The data are indicated as the means ± standard error of the mean (**p<0.01 compared to the control group).

Figures

Fig 1a

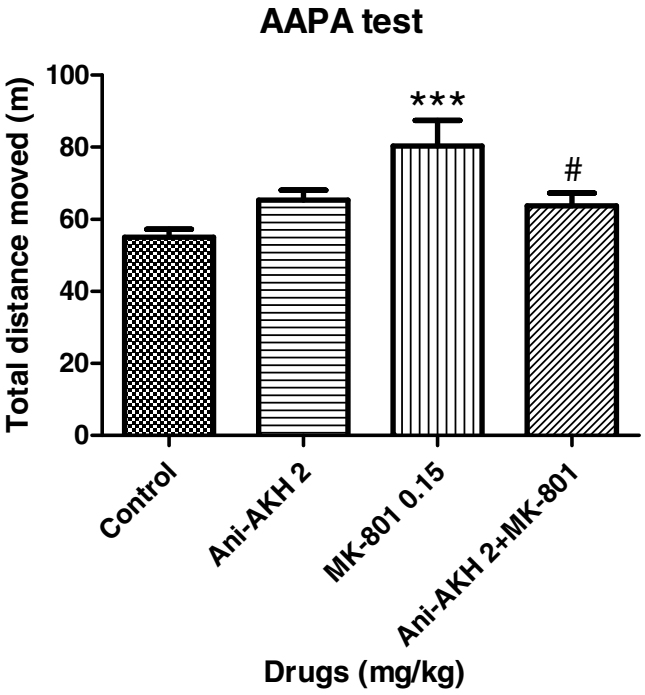
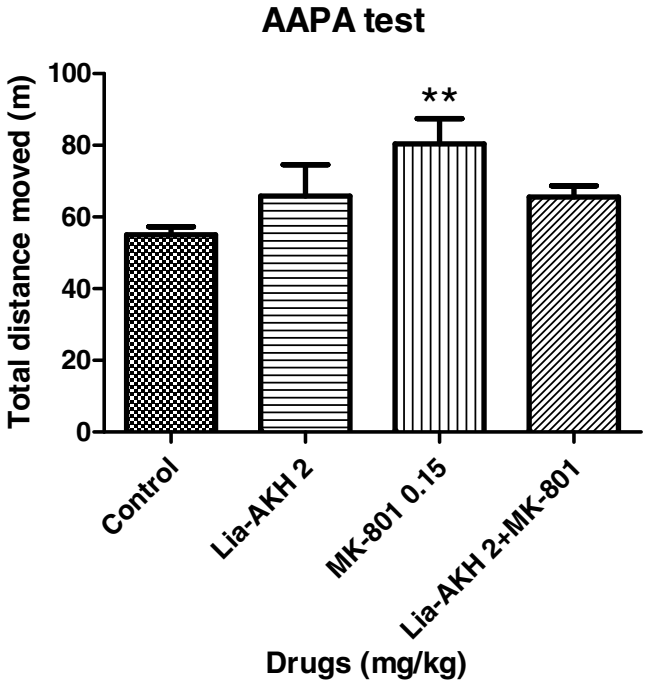


Fig 1b



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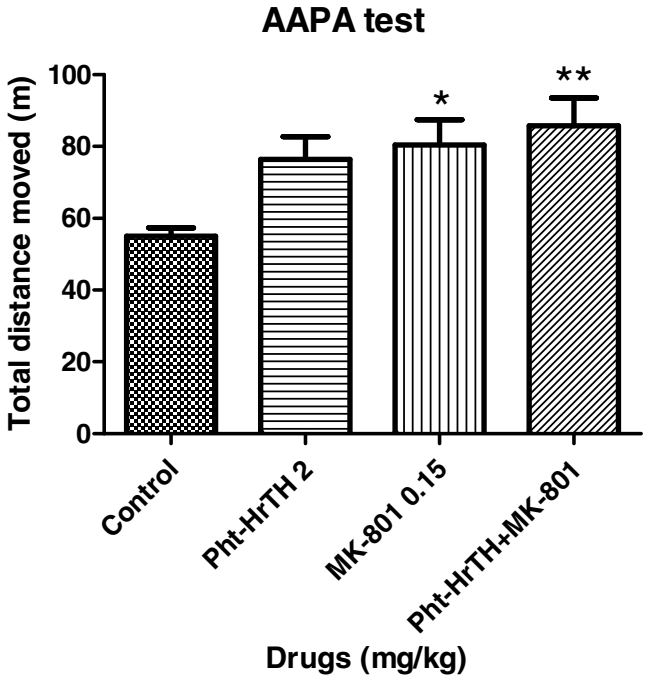

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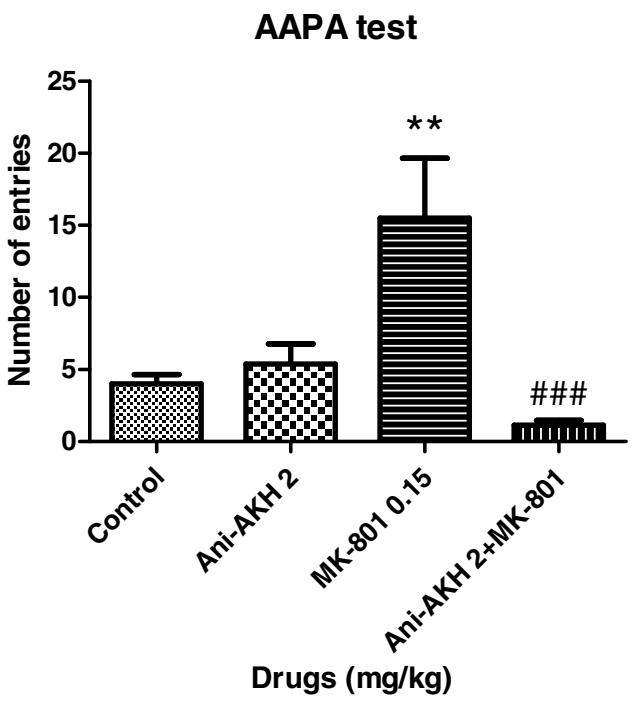
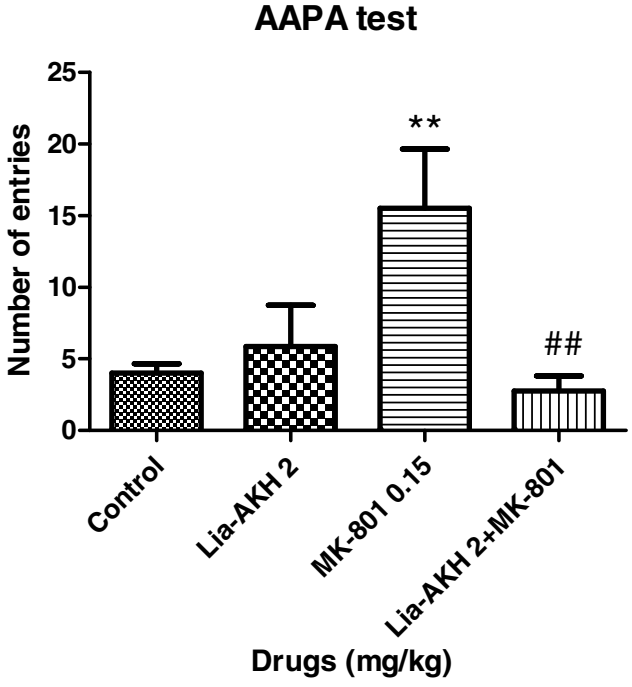


Fig 2b



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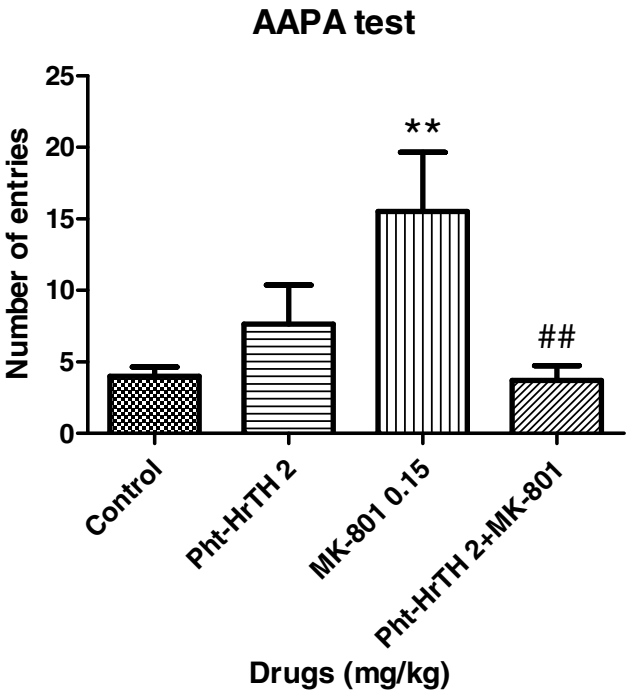

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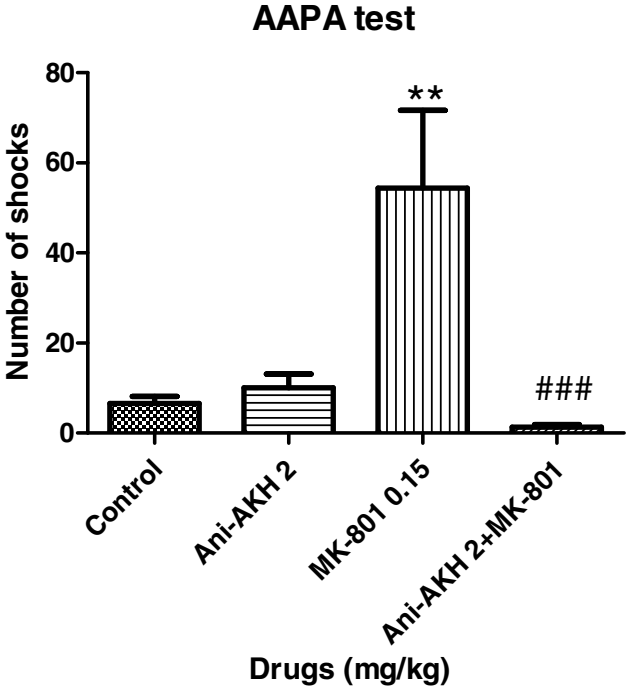

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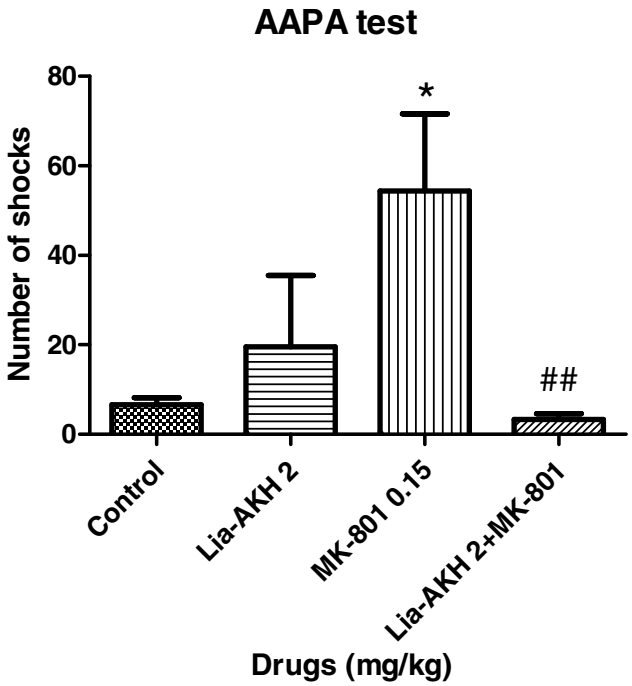

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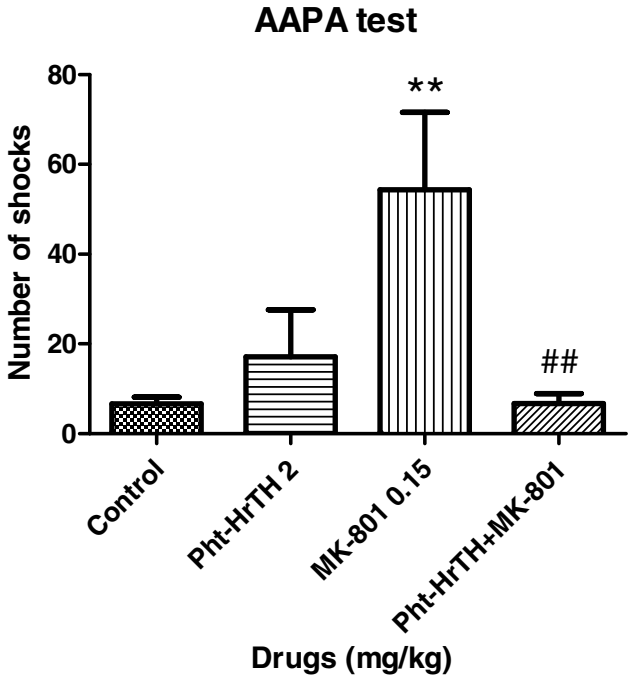

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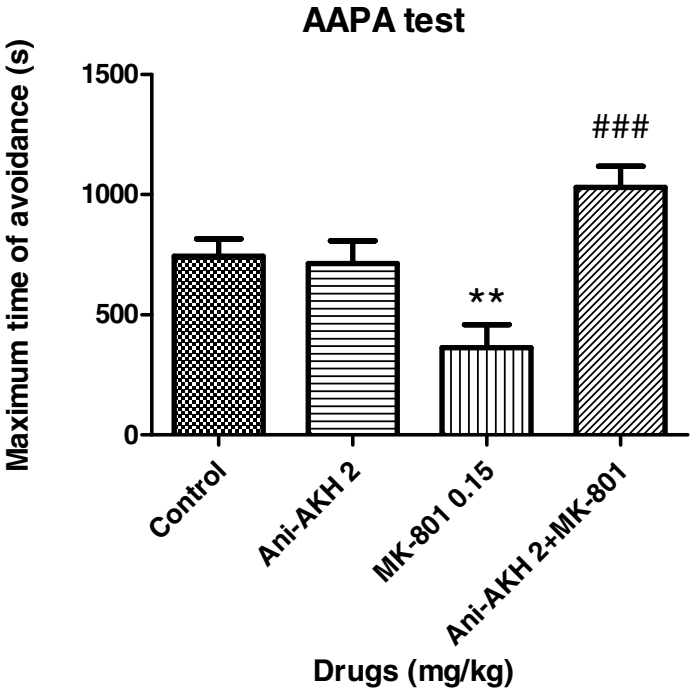

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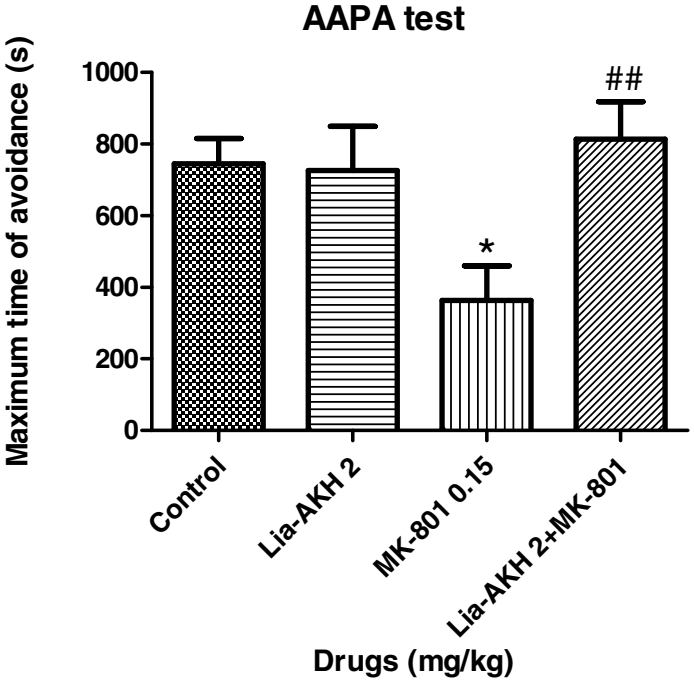

Fig 4c

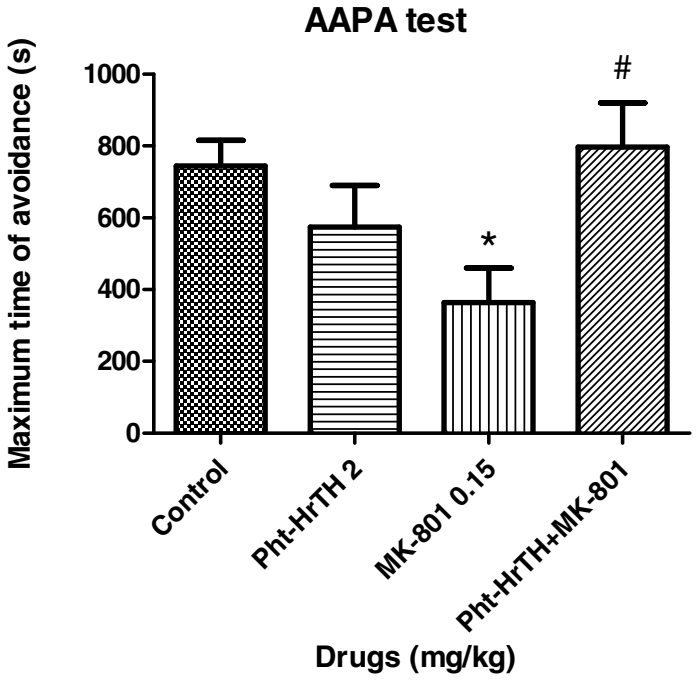

Fig 5

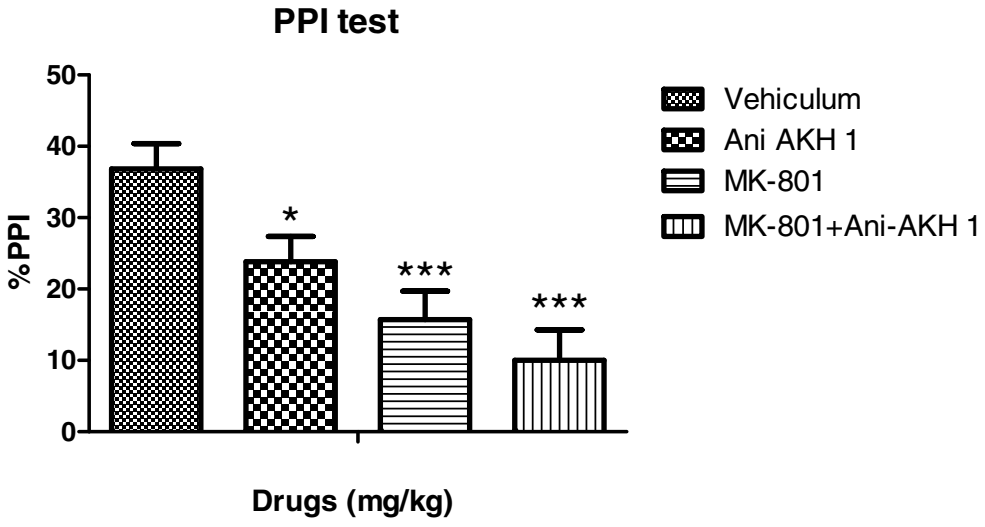

Fig 6

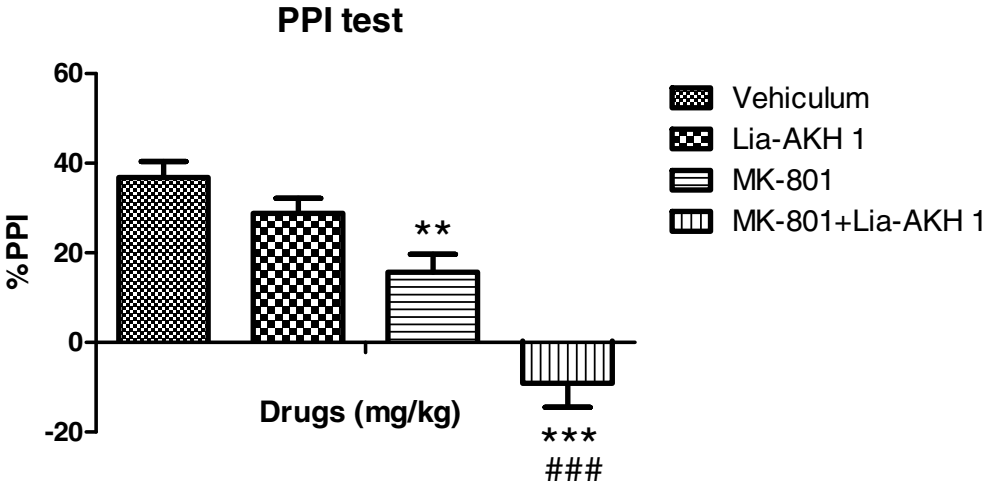

Fig 7