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Neurochemical Effects of the Adipokinetic Hormone/Red Pigment Concentrating Hormone Family of Peptides in MK-801-Induced Schizophrenia Rat Model

Adipokinetik Hormon/Kırmızı Pigment Konsantre Hormon Peptid Ailesinin MK-801'in İndüklediği Şizofreni Sıçan Modelinde Nörokimyasal Etkileri

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ABSTRACT

Objective: Adipokinetic hormone (AKH) plays a role in sugar and lipid metabolism in insects. Previous studies of AKH showed memory improvements in a schizophrenia rat model that displayed memory impairment and reduced depression in a rat olfactory bulbectomy model. In this study, we investigated the effects of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides on brain neurotransmitter levels and brain neurochemistry in a schizophrenia rat model.

Materials and Methods: We administered AKH/RPCH peptides for 4 days sub-chronically, both in naive rats and also in the MK-801-induced schizophrenia rat model. Liquid chromatography-mass spectrometry apparatus was used for targeted and untargeted analysis of rat neurochemistry.

Results: Increased brain glutamate levels characteristic of MK-801 peptide-treated rats were significantly reduced by AKH. Furthermore, AKH also increased brain dopamine levels in both naive and MK-801 rats. Metabolomic studies have shown that AKH affects lipid and glutamate metabolism, while hypertrehalosaemic hormone plays a role in sugar metabolism and inflammation.

Conclusions: According to our results, AKH might affect dopaminergic and glutamatergic systems and reverse the effects of MK-801, possibly affecting NMDA receptors.

Keywords: Adipokinetic hormone, neurochemistry, neurotransmitters, schizophrenia

ÖZ

Amaç: Adipokinetik hormon (AKH) böceklerde şeker ve lipid metabolizmasında rol oynar. Önceki çalışmalarda AKH sıçan şizofeni modelinde belleği ve sıçan olfaktör bulbektomi modelinde depresyonu düzelttiği gösterilmiştir. Bu çalışmada, adipokinetik hormon/kırmızı pigment konsantre edici hormon (AKH/RPCH) ailesi peptidlerinin sıçan şizofreni modelinde beyin nörotransmitter seviyesi ve beyin nörokimyası üzerine etkisi araştırılmıştır.

Materyal ve Metot: Peptidleri hem naif sıçanlarda hem de MK-801 ile indüklenmiş sıçan şizofreni modelinde 4 gün boyunca subkronik olarak uyguladık. Likid kromatografi-kütle spektrometri cihazı nörokimyanın hedeflenmiş ve hedeflenmemiş analizi için kullanıldı.

Bulgular: AKH anlamlı olarak MK-801 uygulanmış sıçanlarda artmış beyin glutamat seviyesini düşürmüştür. AKH ayrıca hem naif sıçanlarda hem MK-801 uygulanmış sıçanlarda beyin dopamin seviyesini artırdı. Metabolomik çalışmalar adipokinetik hormonun lipid ve glutamat metabolizmasını etkilediğini, hipertrehalosemik hormonun ise şeker metabolizması ve enflamasyonda rol oynadığını gösterdi.

Sonuç: Sonuçlarımıza göre AKH dopaminerjik ve glutamaterjik sistemi etkileyebilir ve MK-801'in etkilerini muhtemelen NMDA reseptörleri üzerinden tersine çeviriyor olabilir.

Anahtar Kelimeler: Adipokinetik hormon, nörokimya, nörotransmitter, şizofreni

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INTRODUCTION

Adipokinetic hormone (AKH) and hypertrehalosaemic hormone are the members of the adipokinetic hormone/red pigment concentrating hormone (AKH/RPCH) family of peptides. They are secreted from the corpora cardiaca of insects, which is a special region found in the brain of the insect family.[1,2] This region of insects is the analog region of the hypothalamus-hypophysis system found in human beings. AKH/RPCH peptides consist of a small amino acid chain starting with pyroglutamic acid and ending with an amide. These peptides especially play a role in the lipid and sugar mobilisation of insects. It is known that AKH has more function in lipid metabolism, while hypertrehalosaemic hormone plays a role in sugar metabolism.[1,2] These peptides support the energy needed for the flight and locomotion of insects. In previous studies, these peptides were also suggested to function in the human neuronal system.[3] In the literature, it is also mentioned that AKH receptors also function similarly to gonadotropin-releasing hormone receptors.[4] This peptidergic system was also found in rat brains.[5]

In addition to these, in recent studies, we showed that the AKH/RPCH peptide family might exert improving effects on depression, anxiety, pain and locomotor disorders and also had neuroprotective and improving effects on memory in MK-801-induced schizophrenia and olfactory bulbectomy of depression-models.[6-9] The current invention relates to the investigation of the effects of these peptides on brain neurotransmitter levels and neurochemistry after 4 days of subchronic injection in the MK-801-induced rat schizophrenia model.[10]

Adipokinetic hormone (AKH) plays a role in sugar and lipid metabolism in insects. Previous studies of AKH show memory improvements in a schizophrenia rat model that displays memory impairment and reduced depression in a rat olfactory bulbectomy model. In this study, we investigated the effects of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides on brain neurotransmitter levels and brain neurochemistry in a schizophrenia rat model.

MATERIALS AND METHODS

Ethics Committee Approval: All procedures respected the Guidelines of the European Union (86/609/EU) and the National Committee for the Care and Use of Laboratory Animals, Czech Republic. (Date:17/10/2016, decision no: 59449/2016-MZE-17214).

Animals: Male Long Evans rats obtained from the Academy of Science, Prague, were used in this study. Rats were kept at standard laboratory conditions, including 21°C temperature and 12/12 light/dark cycle, for two weeks before starting the experiments and during the experiments.

Experimental Groups and Drug Administration: Long Evans rats were treated with Anax imperator AKH (Ani-AKH) (1 and 2 mg/kg) (n=8), Libellula auripennis AKH (Lia-AKH) (1 and 2 mg/kg) (n=8), Phormia-Terra hypertrehalosemic hormone (Pht-HrTH) (1 and 2 mg/kg) (n=8), MK-801 (0.15 mg/kg) (n=8), Ani-AKH (2 mg/kg)+MK-801 (0.15 mg/kg) (n=8), Lia-AKH (2 mg/kg)+MK-801 (0.15 mg/kg) (n=8), Pht-HrTH (2 mg/kg)+MK-801 (0.15 mg/kg) (n=8) or vehicle (control group=saline with 15% DMSO) (n=8) for 4 days (subchronically) in a volume of 2 ml/kg body weight. The doses of the drugs were chosen according to previous studies.[6,7]

Drugs: (+)-MK-801 maleate (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a, d)-cyclo-hepten-5,10-imine maleate) was obtained from Tocris Bioscience, United Kingdom. Anax imperator AKH (Ani-AKH), Libellula auripennis AKH (Lia-AKH) and Phormia-Terra hypertrehalosemic hormone (Pht-HrTH) were purchased from TRC (Toronto/Canada). MK-801 was dissolved in saline, while AKH was dissolved in saline mixed with 15% DMSO. Saline with 15% DMSO was used as a control group.

Liquid Chromatography-Mass Spectrometry (LC-MS)

Targeted Analysis

Reagents: Dopamine (DA), 5-hydroxy tryptamine (5-HT), γ-aminobutyric acid (GABA), and glutamate (Glu) were purchased from Sigma–Aldrich (USA). LC-MS grade acetonitrile and methanol were obtained from Honeywell-Research Chemicals (France). High-purity water was provided by a Milli-Q system, Aqual Elga Flex3. LC-MS grade formic acid was purchased from Sigma–Aldrich (USA).

Sample Preparation for Targeted LC-MS/MS Analysis: Whole rat brains were weighed directly after the sacrification and deeply frozen (-84°C) immediately. After thawing was added ice-cold methanol to each sample (for 1g of tissue 4 mL of liquid), and each sample was homogenised, vortex-mixed (1 min) and underwent the centrifugation (18.000 x g for 10 min at 0°C). The supernatant was transferred and evaporated to dryness by SpeedVac (Hanil Modul 4080C). The dry residue was reconstituted in 100 µl of methanol, and the aliquot of 10 µl was injected into the LC-MS system for analysis.

LC-MS/MS Conditions: LC-MS/MS was run on an AB SCIEX QTRAP 6500 spectrometer equipped with an ESI ion source and a Thermo Scientific Ultimate 3000 HPLC system with an autosampler. The analytes were separated on a Phenomenex Kinetex C18 column (2.1 mm x 50 mm, 1.7 µm) used at 30° C. The mobile phase consisting of 0.1% formic acid

in water (Solvent A) and methanol (Solvent B) was used with a gradient elution: 0–1.5 min, 2% B; 7 min, 98% B; 8.5 min, 98% B; 10 min, 2% B, 11.5 min, 2% B; at a flowrate of 0.37 mL/min. MS acquisition of 5-HT, GABA, Glu and DA was performed in electrospray positive ionisation multiple reaction monitoring (MRM) mode.

Untargeted Analysis

Reagents: LC-MS grade acetonitrile and methanol were obtained from Honeywell-Research Chemicals (France). High-purity water was provided by a Milli-Q system, Aqual Elga Flex3. LC-MS grade formic acid was purchased from Sigma–Aldrich (USA).

Sample Preparation for Untargeted LC-MS/MS Analysis: Whole rat brains were weighed directly after the sacrificial and deeply frozen (-84°C) immediately. After thawing was added ice-cold methanol to each sample (for 1g of tissue 4 mL of liquid), and each sample was homogenised, vortex-mixed (1 min) and underwent the centrifugation (18.000 x g for 10 min at 0°C). The supernatant was transferred and evaporated to dryness by speedvac (Hanil Modul 4080C). The dry residue was reconstituted in 100 µl of methanol, and the aliquot of 10 µl was injected into the LC-MS system for analysis.

LC-MS/MS Conditions: LC-MS/MS was run on an AB SCIEX Triple TOF 5600 spectrometer equipped with an ESI ion source and a Thermo Scientific Ultimate 3000 HPLC system with an autosampler. The analytes were separated on a Phenomenex Kinetex C18 column (3 mm x 150 mm, 2.6 µm) used at 30°C. The mobile phase consisting of 0.1% formic acid in water (Solvent A) and methanol (Solvent B) was used with a gradient elution: 0–4.5 min, 20% B; 17.5 min, 99% B; 25.5 min, 99% B; 27 min, 20% B, 30 min, 20% B; at a flowrate of 0.5 mL/min. MS acquisition was performed in electrospray positive ionisation.

Statistical Analysis: The results of the neurotransmitters were evaluated by one-way ANOVA followed by Tukey's post-hoc test when significant differences were detected. The data were expressed as the mean values ± SEM (standard error mean). The differences were considered to be statistically significant when p was less than 0.05. The results of the untargeted analysis were evaluated by the software called Metaboanalyst. This analysis includes identifying metabolites, quantifying their abundance, and mapping them to known metabolic pathways. Additionally, it helps in understanding the interactions between metabolites and their impact on various biological processes, making it a crucial component in systems biology research.

RESULTS

There was a significant difference between the groups when effect of hormones on glutamate levels in the brain of rats after 4 days of subchronic injections is evaluated in Ani-AKH, Lia-AKH and Pht-HrTH groups [F (4.33)=19.85; p<0.0001; F (4.33) =13.78; p<0.0001; F(4.32)=18.83; p<0.0001; respectively]. Glutamate levels were significantly increased in MK-801 0.15 mg/kg group (p<0.001), Ani-AKH 2 mg/kg (p<0.01), Pht 1 mg/kg (p<0.01) and 2 mg/kg (p<0.001) groups compared to the control group. Ani 2 mg/kg (p<0.001), Lia 2 mg/kg (p<0.01) and Pht-HrTH 2 mg/kg (p<0.001) significantly decreased the increased glutamate levels in the MK-801 alone group when combined together (Fig.1).

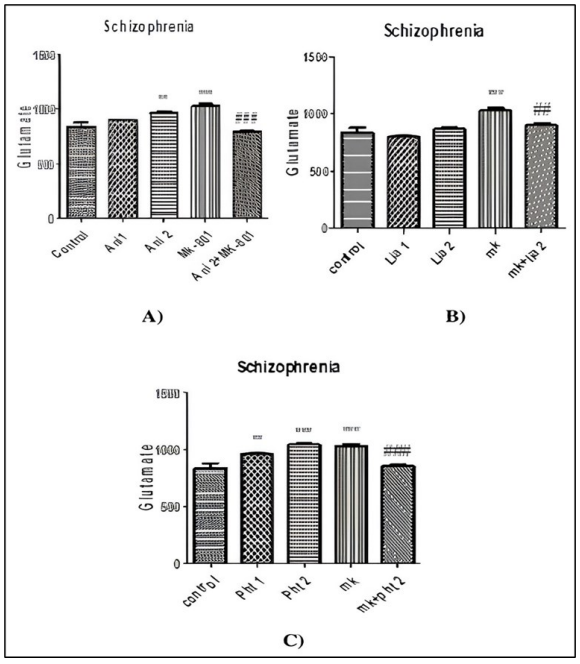

Figure 1. Glutamate levels in the brain of rats after 4 days of intraperitoneal (i.p.) administration.

**: p<0.01; ***: p<0.001; ##: p<0.01; ###: p<0.001; Ani 1: Anax imperator AKH 1 mg/kg; Ani 2: Anax imperator AKH 2 mg/kg; MK-801: MK-801 maleate (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a, d)-cyclo-hepten-5,10-imine maleate; Lia 1: Libellula auripennis AKH 1 mg/kg; Lia 2: Libellula auripennis AKH 2 mg/kg; Pht 1: Phormia-Terra hypertrehalosemic hormone 1 mg/kg; Pht 2: Phormia-Terra hypertrehalosemic hormone 2 mg/kg.

Figure 1 illustrates glutamate levels in the brain of rats after intraperitoneally (i.p.) administration for 4 days in all groups in the schizophrenia model of Long-Ewans rats. The data are indicated as the means ± standard error of the mean. (**p<0.01, ***p<0.001 compared to control group; ##p<0.01, ###p<0.001 compared to MK-801 group) (Fig.1).

There was a significant difference between the groups when effect of drugs on GABA levels in the brain of rats after 4 days of subchronic injections is evaluated in Ani-AKH, Lia-AKH and Pht-HrTH groups [F (4.33)=45.41; p<0.0001; F (4.33)=16.61; p<0.0001; F (4.32)=27.41; p<0.0001; respectively]. GABA levels were significantly increased in Ani-AKH 1 mg/kg (p<0.001), Pht 1 and 2 mg/kg groups (p<0.001) while significantly decreased in Lia-AKH 1 mg/kg group (p<0.001) compared to the control group. Also, Ani 2 mg/kg (p<0.001) and Lia 2 mg/kg (p<0.01) significantly decreased GABA levels compared to the MK-801 alone group (Fig.2).

Figure 2 illustrates GABA levels in the brain of rats after intraperitoneally (i.p.) administration for 4 days in all groups in the schizophrenia model of Long-Ewans rats. The data are indicated as the means ± standard error of the mean. (***p<0.001 compared

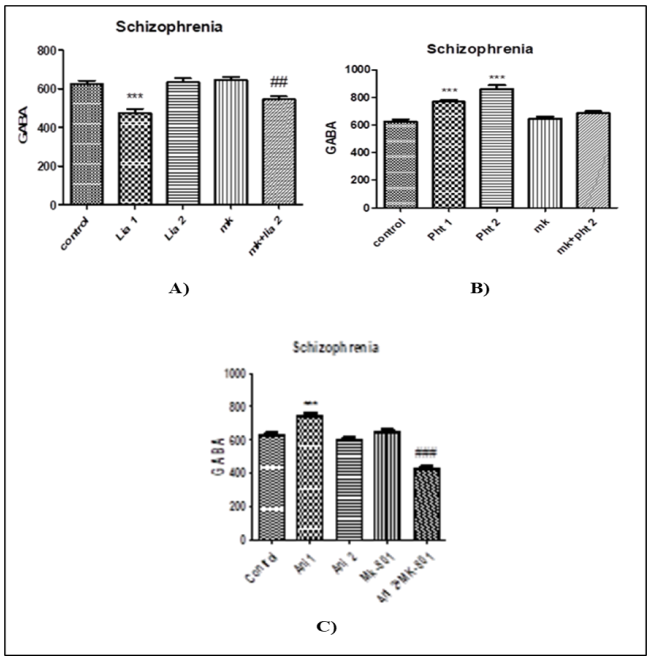

Figure 2. GABA levels in the brain of rats after 4 days of intraperitoneal (i.p.) administration.

***: p<0.001; ##: p<0.01; ###: p<0.001; Ani 1: Anax imperator AKH 1 mg/kg; Ani 2: Anax imperator AKH 2 mg/kg; MK-801: MK-801 maleate .(5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a, d)-cyclo-hepten-5,10-imine maleate; Lia 1: Libellula auripennis AKH 1 mg/kg; Lia 2: Libellula auripennis AKH 2 mg/kg; Pht 1: Phormia-Terra hypertrehalosemic hormone 1 mg/kg; Pht 2: Phormia-Terra hypertrehalosemic hormone 2 mg/kg; GABA: γ-aminobutyric acid.

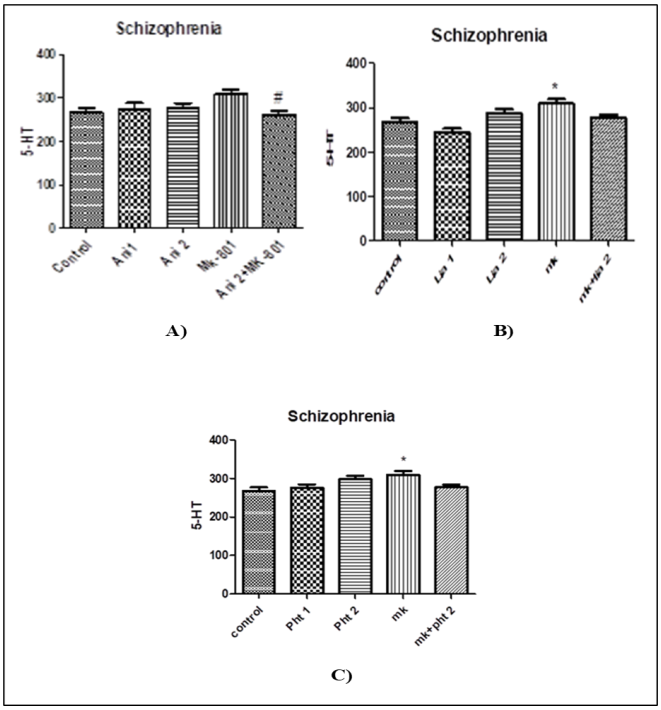

Figure 3. 5-HT levels in the brain of rats after 4 days of intraperitoneal (i.p.) administration.

*: p<0.05; #: p<0.05; Ani 1: Anax imperator AKH 1 mg/kg; Ani 2: Anax imperator AKH 2 mg/kg; MK-801: MK-801 maleate (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a, d)-cyclo-hepten-5,10-imine maleate; Lia 1: Libellula auripennis AKH 1 mg/kg; Lia 2: Libellula auripennis AKH 2 mg/kg; Pht 1: Phormia-Terra hypertrehalosemic hormone 1mg/kg; Pht 2: Phormia-Terra hypertrehalosemic hormone 2 mg/kg; 5-HT: 5-hydroxy tryptamine.

to control group; ##p<0.01, ###p<0.001 compared to MK-801 group) (Fig.2).

There was a significant difference between the groups when the effect of drugs on 5-HT levels in the brain of rats after 4 days of subchronic injections is evaluated in Ani-AKH, Lia-AKH and Pht-HrTH groups [F (4.33)=2.83; p=0.03; F (4.33)=7.99; p=0.0001; F(4.32)=3.64; p=0.01; respectively]. 5-HT levels were increased in the MK-801 group (p<0.05) compared to the control, while this effect was reversed by the Ani-AKH 2 mg/kg (p<0.05) group (Fig.3).

The figure illustrates 5-HT levels in the brain of rats after intraperitoneally (i.p.) administration for 4 days in all groups in the schizophrenia model of Long-Ewans rats. The data are indicated as the means ± standard error of the mean. (*p<0.05 compared to control group; #p<0.05 compared to MK-801 group) (Fig.3).

There was a significant difference between the groups when the effect of drugs on dopamine levels in the brain of rats after 4 days of subchronic injections is evaluated in Ani-AKH, Lia-AKH and Pht-HrTH groups [F (4.33)=117.6; p<0.0001; F (4.33) =168.7; p<0.0001; F (4.32)=245.5; p<0.0001; respectively]. Dopamine levels were significantly decreased in the MK-801 0.15 mg/kg group (p<0.001) while significantly increased in Ani-AKH 1 mg/kg (p<0.001), Lia 2 mg/kg (p<.001), Pht 1 mg/kg (p<0.001) and 2 mg/kg (p<0.001) groups compared to the control group. Ani 2 mg/kg (p<0.001), Lia 2 mg/kg (p<0.001), and Pht-HrTH 2 mg/kg (p<0.001) significantly increased decreased dopamine levels in the MK-801 alone group (Fig.4).

Figure 4 illustrates dopamine levels in the brain of rats after intraperitoneally (i.p.) administration for 4 days in all groups in the schizophrenia model of Long-Ewans rats. The data are indicated as the means ± standard error of the mean. (***p<0.001 compared to control group; ###p<0.001 compared to MK-801 group) (Fig.4).

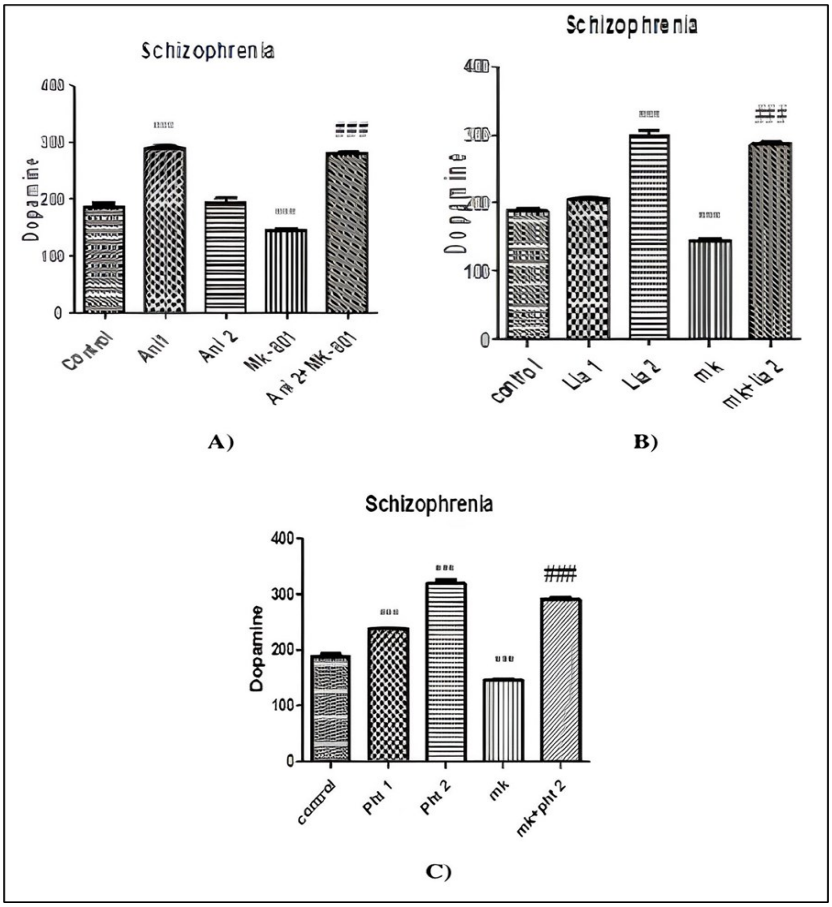

Figure 4. Dopamine levels in the brain of rats after 4 days of intraperitoneal (i.p.) administration.

***: p<0.001; ###:: p<0.001; Ani 1: Anax imperator AKH 1 mg/kg; Ani 2: Anax imperator AKH 2 mg/kg; MK-801: MK-801 maleate (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a, d)-cyclo-hepten-5,10-imine maleate; Lia 1: Libellula auripennis AKH 1 mg/kg; Lia 2: Libellula auripennis AKH 2 mg/kg; Pht 1: Phormia-Terra hypertrehalosemic hormone 1 mg/kg; Pht 2: Phormia-Terra hypertrehalosemic hormone 2 mg/kg.

There was a significant difference between control and MK-801 groups in pathways steroid hormone biosynthesis (p=0.004), fatty acid biosynthesis (p=0.006), sphingolipids metabolism (p=0.009), primary bile acid biosynthesis (p=0.03), D-glutamine and D-glutamate metabolism (p=0.04) (Fig 5a). There was a significant difference between control and Ani-AKH administered groups in pathways steroid biosynthesis (p=0.01), purine metabolism (p=0.02), alanine aspartate and glutamate metabolism (p=0.04), arginine and proline metabolism (p=0.04), tyrosine metabolism (p=0.06) (Fig 5b). There was a significant difference between control and Lia-AKH administered groups in pathways aminoacyl-tRNA biosynthesis (p=0.01), steroid biosynthesis (p=0.01), arachidonic acid metabolism (p=0.04), biosynthesis of unsaturated fatty acids (p=0.07) (Fig 5c). There was a significant difference between control and Pht-HrTH administered groups in pathways alanine, aspartate and glutamate metabolism (p=0.009), purine metabolism (p=0.01), sphingolipid metabolism (p=0.02), porphyrin metabolism (p=0.02), histidine metabolism (p=0.03) (Fig 5d). There was significant difference between MK-801 and MK-801+Ani-AKH 2 mg/kg groups in pathways aminoacyl-tRNA biosynthesis (p=0.009), biosynthesis of unsaturated fatty acids (p=0.01), valine leucine and isoleucine biosynthesis (p=0.01), D-glutamine and D-glutamate metabolism (p=0.01), steroid biosynthesis (p=0.01) (Fig 5e). There was a significant difference between MK-801 and MK-801+Lia-AKH 2 mg/kg groups in pathways aminoacyl-tRNA biosynthesis (p=0.008), steroid biosynthesis (p=0.01), alanine aspartate and glutamate me-

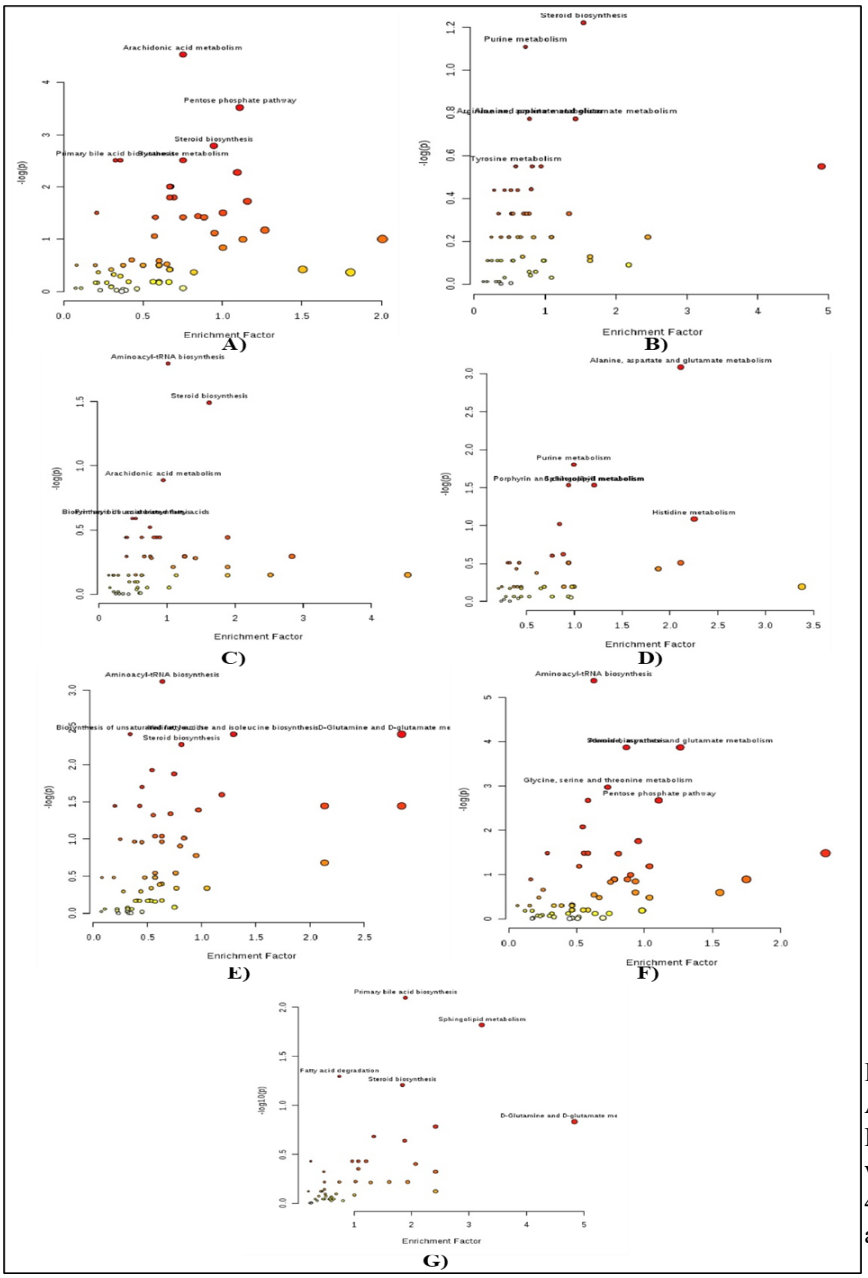

Figure 5. Effects of Ani-AKH, Lia-AKH and Pht-HrTH on metabolomic pathways in the brain of rats after 4 days of intraperitoneal (i.p.) administration.

tabolism (p=0.01), glycine serine and threonine metabolism (p=0.01), pentose phosphate pathway (p=0.01) (Fig 5f). There was a significant difference between MK-801 and MK-801+Pht-HrTH 2 mg/kg groups in pathways arachidonic acid metabolism (p=0.01), pentose phosphate pathway (p=0.01), steroid biosynthesis (p=0.01), primary bile acid biosynthesis (p=0.02), butanoate metabolism (p=0.02) (Fig 5g).

DISCUSSION AND CONCLUSION

AKH/RPCH peptides have many functions, including metabolic, behavioural, developmental or reproductive in insects. In our previous studies, we investigated behavioural effects of AKH in animal depression, anxiety, schizophrenia, olfactory bulbectomy and posttraumatic stress disorder models,[8,9] and now we investigated the effect of these peptides on neurotransmitter levels in schizophrenia model in rat brain after 4 days of subchronic injection. We found that Ani-AKH, Lia-AKH and Pht-HrTH significantly reversed increased brain glutamate levels in MK-801-administered rats. All the hormones increased dopamine levels both in naive and MK-801-administered rats. Ani-AKH and Pht-HrTH also enhanced GABA levels in naive rats, while there was a partial increase effect of Pht-HrTH on 5-HT levels compared to the control group.

The N-methyl-D-aspartate (NMDA) receptor is one of the subgroups of glutamate receptors. It was shown that NMDA receptor antagonists like ketamine, phencyclidine or MK-801 may be used to induce schizophrenia models in rodents.[8] MK-801 disturbs memory in different learning and memory tasks and produces disturbing effects on rodent behaviour.[8] In this study, we used MK-801 to create an animal model which exerts symptoms of schizophrenia.

In the literature, it was found that pyroglutamyl peptides starting with pGlu possess antidepressant, anxiolytic and analgesic effects in animal models.[11] In previous studies, pGlu showed nootropic effects; it reversed memory disturbance in the scopolamine-induced Alzheimer model, increased cholinergic activity and also prevented dementia in old rats. Pyroglutamyl peptides may play a role in the effect mechanism of AKH. It was also found that AKH/RPCH peptides improved memory impairments in MK-801-induced schizophrenia models, showing that they may affect NMDA receptors. In the literature, it was demonstrated that pyroglutamic acid transport to the brain and functions in glutamate storage, and also acts to oppose the action of glutamate and may have a partial agonistic effect on the glutamatergic system.[12] Effect of pyroglutamyl peptides and AKH on NMDA receptors should be investigated to enlighten the effect mechanism of AKH on the glutamatergic system. Also, in this study, the increasing effect of AKH on dopamine and GABA levels in rat brains may cause the behavioural effects of AKH, which we observed in previous studies.[9]

According to our untargeted analysis, primary bile acid biosynthesis, sphingolipid metabolism, fatty acid degradation, steroid biosynthesis, D-glutamine, and D-glutamate metabolism cause metabolic pathway changes in the schizophrenia model compared to the control group. The untargeted analysis found that Ani-AKH mainly affects steroid biosynthesis and purine metabolism; Lia-AKH mostly affects aminoacyl-tRNA biosynthesis and steroid biosynthesis, while Pht-HrTH may mostly affect alanine, aspartate and glutamate metabolism when compared to the control group. Ani-AKH and Lia-AKH mainly affected aminoacyl-tRNA biosynthesis, steroid biosynthesis and glutamate metabolism. In contrast, Pht -HrTH mainly affected arachidonic acid metabolism, pentose phosphate pathway and steroid biosynthesis in the schizophrenia model.

According to these results, our metabolomic study points to changes in lipid metabolism, phospholipids, glycerophospholipids, bile acids and glutamate pathways in plasma in the rat schizophrenia model. Ani-AKH and Lia-AKH seem to have more effect on lipid and glutamate metabolism, while Pht-HrTH plays a role in sugar metabolism, inflammation and changes in energy metabolism. These results support the physiological function of these hormones because it is known that AKH plays more of a role in lipid metabolism. In contrast, the hypertrehalosaemic hormone plays more of a role in sugar metabolism.[9]

In conclusion, other neurobiological features of schizophrenia are alteration in glutamatergic and dopaminergic systems, and all three hormones reversed MK-801-induced changes in these neurotransmitter systems. AKH may affect the dopaminergic and glutamatergic system and reverse the effects of MK-801, possibly affecting NMDA receptors. However, further studies investigating the role of AKH on NMDA receptors should be conducted to elucidate the underlying mechanisms of its effects.

Ethics Committee Approval: All procedures respected the Guidelines of the European Union (86/609/EU) and the National Committee for the Care and Use of Laboratory Animals, Czech Republic. (Date:17/10/2016, decision no: 59449/2016-MZE-17214).

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – ŞNBB, OM; Supervision – ŞNBB, OM, PT; Materials – ŞNBB, OM, PT, PÇ, DRB, DK, KV; Data Collection and/or

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