UNIVERSITY OF PÉCS

Doctoral School of Biology

The effects of non-adenosine nucleosides and uric acid on absence epileptic activity in WAG/Rij rats

PhD Thesis

Renáta Krisztina Lakatos

Supervisor:

Zsolt Kovács, PhD

Associate professor

Pécs, 2024.

TABLE OF CONTENTS

1. ABBREVIATIONS

A_1R, $A_{2A}R$, $A_{2B}R$, A_3R, A_4R: adenosine receptor subtypes

COX: cyclooxygenase

EEG: electroencephalography, electroencephalogram

$GABA_AR$: gamma-aminobutyric acid type A receptor

i.p.: intraperitoneal

MK-801: (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate

SCH 58261: 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo(1,5-c)pyrimidine

SWD: spike-wave discharge

WAG/Rij: Wistar Albino Glaxo/Rijswijk

2. INTRODUCTION

The drugs currently available for the treatment of epilepsy are not sufficiently effective in all cases. As a result, the seizures of approximately one-third of the patients can not be alleviated, or the epileptic activity can attenuate with only insufficient effectiveness, also in the case of patients with absence epilepsy (Löscher et al.; 2011; Crunelli et al., 2020; Katyayan and Diaz-Medina, 2021; Rinaldi et al., 2021). Furthermore, some antiepileptic drugs can also cause serious side effects (Avoli et al., 2005; Howard et al., 2011; Perucca and Gilliam, 2012). Although the absence epileptic seizures may decrease or even disappear with age in the majority of patients, more severe forms of seizures may also develop in others (Callenbah et al., 2009). The above-mentioned facts show that the development of additional antiepileptic treatment options and drugs, as well as more thorough understanding of the pathomechanism of epilepsy disease would be essential.

Not only adenosine, but also non-adenosine nucleoside inosine, guanosine, and uridine has a modulatory role, for example, in regulating the processes of sleep and epilepsy (Kimura et al., 2001a; Porkka-Heiskanen et al., 2002; Vinadé et al., 2003; Boison, 2008; Beamer et al., 2021; Nascimento et al., 2021). Different nucleoside derivatives, nucleoside metabolic inhibitors and nucleoside uptake inhibitors are being used in the researches in connection with drug development for the treatment of various diseases related to the central nervous system (Merighi et al., 2003; Nascimento et al., 2021; Guo and Li, 2022). The effect of agonists and antagonists of different adenosine receptor subtypes (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R) on epileptic activity is thoroughly investigated. Non-adenosine nucleosides and their analogues can enhance endogenous antiepileptic mechanisms, which is also a promising anticonvulsant therapeutic method in case of drug resistant epilepsies: for example, they can influence the shift in the balance of stimulation and inhibition (hyperexcitation or hypoinhibition) in the central nervous system (Tomé et al., 2010; Kovács et al., 2014), they can modify not only neuronal, but also astrocyte dysfunctions (Tomé et al., 2010; Héja, 2014), furthermore, they probably do not or to a lesser extent cause an intensification of the mechanisms that reduce the effectiveness of the treatment (e.g. increased expression and function of multidrug resistance-associated proteins) (Löscher et al., 2011). In earlier studies, inosine (Skolnick et al., 1979; Lewin and Bleck, 1983, 1985; Ganzella et al., 2011), guanosine (Schmidt et al., 2000, 2007; Vinadé et al., 2003), and uridine (Connolly and Duley, 1999; Zhao et al., 2006, 2008; Al-Otaibi et al., 2021) reduced epileptic activity effectively in different epilepsy models. In addition, all of these non-adenosine nucleosides have been shown to be well-tolerated agents with low toxicity (Kimura et al.,

2001a, 2001b; Kovács and Dobolyi, 2013; Wang et al., 2018; Al-Otaibi et al., 2021). However, there are only limited and insufficient research data related to the effects and the potential mechanisms of action of non-adenosine nucleosides on absence epileptic activity. Purine nucleoside metabolite uric acid also has a role in different physiological and pathophysiological processes. Modulation of uric acid level is a strategy that has been successfully used in the treatment of several diseases (Togha et al., 2007; Kutzing and Firestein, 2008), but only limited amount of information is available about its effect on epileptic seizure activity. For example, elevated uric acid level is able to induce seizures (Thyrion et al., 2016) while reduced uric acid level shows anticonvulsant/antiepileptic effects (Togha et al., 2007). In addition, the effect of uric acid on absence epileptic activity is unknown.

Earlier investigations revealed that application of non-adenosine nucleosides and modulation of uric acid level in different seizure models are effective and safe methods that is why we assumed that they may also have beneficial effects on absence epileptic seizure activity.

3. AIMS OF THE THESIS

The objectives in the three experimental groups were the followings:

We investigated whether:

- the intraperitoneally (i.p.) administered inosine, guanosine, and uridine have modulatory effects on absence epileptic activity in Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats (**1st experimental group**).
- higher dose of guanosine attenuates more effectively the absence epileptic seizure activity in WAG/Rij rats than lower doses we used in the first experimental group, and whether this effect is modified by A_{2A}Rs (**2nd experimental group**).
- uric acid and allopurinol can modulate absence epileptic activity in WAG/Rij rats (**3rd experimental group**).

4. MATERIALS AND METHODS

During our experiments divided into three groups, we implanted EEG electrodes in WAG/Rij male rats (age: 9-10 months; n=230; body weight: 270-360 grams), which rat strain

is one of the well-known and valid animal models of absence epilepsy (Coenen and Van Luijtelaar, 2003). During the treatments and surgeries, we acted in accordance with the relevant international and national regulations and our project permits (project numbers: 00332/0004/2011, VA/ÉBNTF02/85-8/2016). Rats were implanted with stainless steel screw electrodes for electroencephalographic (EEG) recording, which electrodes were placed into the bone above different cortical areas, e.g. above primary motor cortex and somatosensory cortex. Electroencephalogram were recorded from freely moving animals, by an EEG device (NIHON-COHDEN, Tokyo, Japan) or a differential preamplifier (SUPERTECH Bioamp 4, Hungary) attached to an A/D converter (CED 1401 mkII data capture and analysis device, Cambridge Electronic Design Ltd., Cambridge, UK; the sampling rate: 500 Hz; the bandwidth of the EEG recording: 0.3 Hz to 150 Hz). Electroencephalogram were split into 60 min sections and these were evaluated separately. Spike-wave discharges - which are a series of asymmetric spikes and waves, with a sharp starting and ending "spike", their average amplitude is at least twice the basic EEG activity, and their frequency is 7-11 Hz - were separated from the EEG manually with Spike2 software, and checked by Fast Fourier Transform (FFT). For statistical analysis, two-way repeated measure analysis of variance (ANOVA) tests, subsequently, Bonferroni's post hoc tests were performed. We examined the effects of different doses of i.p. administered agents (alone or in combination) with EEG mainly on the number and duration of SWDs between 30 and 270 minutes of post-injection.

In the first experimental group, we investigated the effects of administration of the following drugs alone on absence epileptic activity (SWDs) in WAG/Rij rats: inosine (i.p. 500 and 1000 mg/kg), guanosine (i.p. 20 and 50 mg/kg), uridine (i.p. 500 and 1000 mg/kg), gamma-aminobutyric acid type A receptor ($GABA_AR$) agonist muscimol (i.p. 1 and 3 mg/kg), $GABA_AR$ antagonist bicuculline (i.p. 2 and 4 mg/kg), non-selective adenosine receptor antagonist theophylline (i.p. 5 and 10 mg/kg) and non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801; i.p. 0.0625 and 0.1250 mg/kg). Furthermore, we examined the effect of combined use of inosine, guanosine, and uridine with muscimol, bicuculline, theophylline, and MK-801 on absence epileptic seizure activity.

In the second experimental group, we investigated the effect of guanosine (i.p. 50 mg/kg and 100 mg/kg) alone on SWDs. In the following, we used theophylline (i.p. 5 mg/kg) alone and in combination with different doses of guanosine (i.p. 50 mg/kg and 100 mg/kg). We also examined the effects of the selective $A_{2A}R$ antagonist 7-(2-phenylethyl)-5-amino-2-(2-

furyl)-pyrazolo[4,3-e]-1,2,4-triazolo(1,5-c)pyrimidine (SCH 58261; i.p. 1 mg/kg), and the cyclooxygenase-1 and 2 (COX-1 and COX-2) inhibitor indomethacin (i.p. 10 mg/kg) alone and in combination with guanosine (i.p. 50 mg/kg and 100 mg/kg) on SWDs.

In the third experimental group, we investigated the effects of uric acid (i.p. 100 mg/kg and 200 mg/kg), xanthine oxidase inhibitor allopurinol (i.p. 50 mg/kg and 100 mg/kg), indomethacin (i.p. 10 mg/ kg) and inosine (i.p. 500 mg/kg) alone on SWDs. Furthermore, we examined the effects of combined application of allopurinol (i.p. 50 mg/kg) with uric acid (i.p. 100 mg/kg), allopurinol (i.p. 50 mg/kg) with inosine (i.p. 500 mg/kg), indomethacin (i.p. 10 mg/kg) with uric acid (i.p. 100 mg/kg), as well as inosine (i.p. 500 mg/kg) with uric acid (i.p. 100 mg/kg) on seizure activity.

5. RESULTS

In the first experimental group we showed that i.p. application of uridine (500 and 1000 mg/kg) and guanosine (20 and 50 mg/kg) significantly and dose-dependently reduced, while inosine (500 and 1000 mg/kg) increased the number and total duration of SWDs. Muscimol administered alone (i.p. 1 mg/kg and 3 mg/kg) significantly increased, while bicuculline (2 mg/kg and 4 mg/kg), and theophylline (5 mg/kg and 10 mg/kg) significantly decreased SWD number. Administration of two doses of MK-801 (i.p. 0.0625 mg/kg and 0.1250 mg/kg) alone reduced SWD number dose-dependently and significantly. Combined use of nucleosides (inosine, guanosine, uridine) with other agents (muscimol, bicuculline, theophylline, MK-801) resulted in a significant increase (muscimol + inosine, bicuculline + inosine, theophylline + inosine, muscimol + uridine) or decrease (guanosine + MK-801, theophylline + guanosine, theophylline + uridine, bicuculline + uridine) in SWD number compared to the control SWD numbers. However, combined application of muscimol with inosine increased the number of SWDs compared to the effect of inosine applied alone. Similarly, we experienced an increase in the number of SWDs after the combined use of muscimol and uridine compared to the effect of uridine alone.

In the second experimental group we showed that higher dose of guanosine (i.p. 100 mg/kg) increased SWD number, in contrast to the effects of the lower doses of guanosine. Theophylline (i.p. 5 mg/kg), indomethacin (i.p. 10 mg/kg), and SCH 58261 (i.p. 1 mg/kg) administered alone, significantly reduced SWD number compared to control values. The

combined use of different drugs significantly reduced SWD number (i.p. 5 mg/kg theophylline + 50 mg/kg guanosine: compared to control values; i.p. 5 mg/kg theophylline + 100 mg/kg guanosine: compared to the effect of guanosine administered alone; i.p. 1 mg/kg SCH 58261 + 100 mg/kg guanosine, and i.p. 10 mg/kg indomethacin + 100 mg/kg guanosine: compared to control values and the effect of guanosine administered alone).

In the third experimental group we verified that i.p. administered uric acid (100 mg/kg and 200 mg/kg), as well as allopurinol (50 mg/kg and 100 mg/kg) alone significantly and dose-dependently increased SWD number. Combined use of allopurinol (i.p. 50 mg/kg) and uric acid (i.p. 100 mg/kg) significantly increased SWD number compared to control values and the effect of allopurinol alone. Combined administration of allopurinol (i.p. 50 mg/kg) and inosine (i.p. 500 mg/kg) significantly increased SWD number compared to control values and the effect of allopurinol and inosine alone too. Combined use of indomethacin (i.p. 10 mg/kg) and uric acid (i.p. 100 mg/kg) significantly reduced SWD number compared to the effect of uric acid alone. However, administration of inosine with uric acid caused an increase in the number of SWDs compared to the control values and to the effect of inosine and uric acid alone.

6. DISCUSSION AND CONCLUSIONS

Based on the literature data and the results of i.p. administration of drugs (alone and in combination) we can assume that the effects on SWD number and duration (i) may occur mainly through the stimulation of A_{2A}Rs and/or $GABA_A$Rs in the case of inosine (Skolnick et al., 1979; Haskó et al., 2004), furthermore, (ii) in the case of guanosine – after the administration of lower doses of guanosine – these effects may occur possibly via its own guanosine receptors (Johansson and Fredholm, 1995; Traversa et al., 2003; Di Iorio et al., 2004; Volpini et al., 2011) and/or A_1Rs (Ciruela et al., 2006; Kovács et al., 2017; Brunner et al., 2021a), whereas in the case of higher doses of guanosine, the modulation of SWD number and duration may develop due to the stimulation of A_{2A}Rs (Cunha, 2005; Ciruela et al., 2006; Cunha et al., 2008; Ferre et al., 2008), (iii) and regarding uridine, it can exert its effects through its own uridine receptors (Guarneri et al., 1985; Kimura et al., 2001a, 2001b; Kovács et al., 2013) as well as A_1Rs (Brunner et al., 2021b). However, uric acid may exert its SWD number increasing effect via interleukin-1 receptor (IL-1R)/COX-2/prostaglandin E2 (PGE2) system (Kovács et al., 2006; Van Luijtelaar et al., 2012; Russo et al., 2013, 2014; Citraro et al., 2015), as well as through A_{2A}Rs (Dixon and Thurlow, 1924; Barber and Siegel, 1982; Tan et al., 1993).

Our results also suggest that among the investigated non-adenosine nucleosides, uridine could be a promising (additional) option in the treatment of absence epilepsy. Regarding the effect of guanosine on SWDs, further studies are needed to determine whether this non-adenosine nucleoside can at least theoretically be used to treat absence epilepsy. Our experiments also show that the shift of purine nucleoside metabolism, which results in elevated guanosine and/or inosine, adenosine, as well as uric acid level, may increase absence epileptic activity. Furthermore, the therapeutic use of allopurinol in patients with absence epilepsy can also theoretically aggravate absence epileptic activity. However, we received our results from experiments, which were carried out on an animal model of absence epilepsy – WAG/Rij rats –, therefore the application (translatability) of the conclusions derived from these results are currently doubtful in connection with human absence epilepsy disease.

7. OUTLOOK

Regarding the above-mentioned results and conclusions, it would be important (i) to investigate and strengthen our results in connection with the modulation of the levels of non-adenosine nucleosides and their effects on SWDs on other animal models, (ii) to confirm the assumed changes in the nucleoside levels in the brain occurred as a result of our treatments, (iii) to clarify the exact mechanisms of action of nucleosides and their metabolites, as well as (iv) to investigate in further experiments whether they can be used as antiepileptic drugs. Thus, for example, it would be important to reveal the mechanisms of action by local application of non-adenosine nucleosides, adenosine receptor and $GABA_AR$ inhibitors, and by investigation of changes in the induced signal transduction mechanisms.

8. REFERENCES

- Al-Otaibi, A.; AlAyed, A.; Al Madhi, A.; Saeed, L.; Ng G.B.; Freeze H.H.; Almannai, M. Uridine monophosphate (UMP)-responsive developmental and epileptic encephalopathy: A case report of two siblings and a review of literature. Mol. Genet. Metab. Rep., 2021, 30:100835. doi: 10.1016/j.ymgmr.2021.100835
- Avoli, M.; Louvel, J.; Pumain, R.; Köhling, R. Cellular and molecular mechanisms of epilepsy in the human brain. Prog. Neurobiol., 2005, 77, 166-200. doi: 10.1016/j.pneurobio.2005.09.006.
- Barber, M.J.; Siegel, L.M. Oxidation-reduction potentials of molybdenum, flavin, and iron-sulfur centers in milk xanthine oxidase: variation with pH. Biochemistry, 1982, 21, 1638-1647. doi: 10.1021/bi00536a026.
- Beamer, E.; Kuchukulla, M.; Boison, D.; Engel, T. ATP and adenosine - Two players in the control of seizures and epilepsy development. Prog. Neurobiol., 2021, 204:102105. doi: 10.1016/j.pneurobio.2021.102105.
- Boison, D. Adenosine as a neuromodulator in neurological diseases. Curr. Opin. Pharmacol., 2008, 8, 2-7. doi: 10.1016/j.coph.2007.09.002.
- Brunner, B.; Ari, C.; D'Agostino, D.P.; Kovács, Z. Adenosine Receptors Modulate the Exogenous Ketogenic Supplement-Evoked Alleviating Effect on Lipopolysaccharide-Generated Increase in Absence Epileptic Activity in WAG/Rij Rats. Nutrients, 2021a, 13:4082. doi: 10.3390/nu13114082.
- Brunner, B.; Rauch. E.; Ari, C.; D'Agostino, D.P.; Kovács, Z. Enhancement of Ketone Supplements-Evoked Effect on Absence Epileptic Activity by Co-Administration of Uridine in Wistar Albino Glaxo Rijswijk Rats. Nutrients, 2021b, 13:234. doi: 10.3390/nu13010234.
- Callenbach, P.M.; Bouma, P.A.; Geerts, A.T.; Arts, W.F.; Stroink, H.; Peeters, E.A.; van Donselaar, C.A.; Peters, A.C.; Brouwer, O.F. Long-term outcome of childhood absence epilepsy: Dutch Study of Epilepsy in Childhood. Epilepsy Res., 2009, 83, 249-256. doi: 10.1016/j.eplepsyres.2008.11.011.
- Ciruela, F.; Casadó, V.; Rodrigues, R.J.; Luján, R.; Burgueño, J.; Canals, M.; Borycz, J.; Rebola, N.; Goldberg, S.R.; Mallol, J.; Cortés, A.; Canela, E.I.; López-Giménez, J.F.; Milligan, G.; Lluis, C.; Cunha, R.A.; Ferré, S.; Franco, R. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J. Neurosci., 2006, 26, 2080-2087. doi: 10.1523/JNEUROSCI.3574-05.2006.
- Citraro, R.; Leo, A.; Marra, R.; De Sarro, G.; Russo, E. Antiepileptogenic effects of the selective COX-2 inhibitor etoricoxib, on the development of spontaneous absence seizures in WAG/Rij rats. Brain Res. Bull., 2015, 113, 1-7. doi: 10.1016/j.brainresbull.2015.02.004.
- Coenen, A.M.; Van Luijtelaar, E.L. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav. Genet., 2003, 33, 635-655. doi: 10.1023/a:1026179013847.
- Connolly, G.P.; Duley, J.A. Uridine and its nucleotides: biological actions, therapeutic potentials. Trends Pharmacol. Sci., 1999, 20, 218-225. doi: 10.1023/a:1026179013847.
- Crunelli, V.; Lőrincz, M.L.; McCafferty, C.; Lambert R.C.; Leresche, N.; Di Giovanni, G.; David, F. Clinical and experimental insight into pathophysiology, comorbidity and therapy of absence seizures. Brain, 2020, 143, 2341-2368. doi: 10.1093/brain/awaa072.
- Cunha, R.A. Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. Purinergic Signal., 2005, 1, 111-134. doi: 10.1007/s11302-005-0649-1.

- Cunha, R.A.; Ferré, S.; Vaugeois, J.M.; Chen, J.F. Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders. Curr. Pharm. Des., 2008, 14, 1512-1524. doi: 10.2174/138161208784480090
- Di Iorio, P.; Ballerini, P.; Traversa, U.; Nicoletti, F.; D'Alimonte, I.; Kleywegt, S.; Werstiuk, E.S.; Rathbone, M.P.; Caciagli, F.; Ciccarelli, R. The antiapoptotic effect of guanosine is mediated by the activation of the PI 3-kinase/AKT/PKB pathway in cultured rat astrocytes. Glia, 2004, 46, 356-368. doi: 10.1002/glia.20002.
- Dixon, M.; Thurlow, S. Studies on xanthine oxidase: the dynamics of the oxidase system. Biochem. J., 1924, 18, 976-988. doi: 10.1042/bj0180976.
- Ferre, S.; Ciruela, F.; Borycz, J.; Solinas, M.; Quarta, D.; Antoniou, K.; Quiroz, C.; Justinova, Z.; Lluis, C.; Franco, R.; Goldberg, S.R. Adenosine A1-A2A receptor heteromers: new targets for caffeine in the brain. Front. Biosci., 2008, 13:2391-9. doi: 10.2741/2852.
- Ganzella, M.; Faraco, R.B.; Almeida, R.F.; Fernandes, V.F.; Souza, D.O. Intracerebroventricular administration of inosine is anticonvulsant against quinolinic acid-induced seizures in mice: an effect independent of benzodiazepine and adenosine receptors. Pharmacol. Biochem. Behav., 2011, 100, 271-274. doi: 10.1016/j.pbb.2011.09.001.
- Guarneri, P.; Guarneri, R.; La Bella, V.; Piccoli, F. Interaction between uridine and GABA-mediated inhibitory transmission: studies in vivo and in vitro. Epilepsia, 1985, 26, 666-671. doi: 10.1111/j.1528-1157.1985.tb05709.x.
- Guo, M.; Li, T. Adenosine dysfunction in epilepsy and associated comorbidities. Curr. Drug. Targets, 2022, 23, 344-357. doi: 10.2174/1389450122666210928145258.
- Haskó, G.; Sitkovsky, M.V.; Szabó, Cs. Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol. Sci., 2004, 25, 152-157. doi: 10.1016/j.tips.2004.01.006.
- Héja, L. Astrocytic target mechanisms in epilepsy. Curr. Med. Chem., 2014, 21, 755-763. doi: 10.2174/09298673206613119160445.
- Howard, P.; Twycross, R.; Shuster, J.; Mihalyo, M.; Rémi, J.; Wilcock, A. Anti-epileptic drugs. J. Pain Symptom Manage., 2011, 42, 788-804. doi: 10.1016/j.jpainsymman.2011.10.007.
- Johansson, B.; Fredholm, B.B. Further characterization of the binding of the adenosine receptor agonist [3H]CGS 21680 to rat brain using autoradiography. Neuropharmacology, 1995, 34, 393-403. doi: 10.1016/0028-3908(95)00009-u.
- Katyayan, A.; Diaz-Medina, G. Epilepsy: epileptic syndromes and treatment. Neurol. Clin., 2021, 39, 779-795. doi: 10.1016/j.ncl.2021.04.002.
- Kimura, T.; Ho, I.K.; Yamamoto, I. Uridine receptor: discovery and its involvement in sleep mechanism. Sleep, 2001a, 24, 251-260. doi: 10.1093/sleep/24.3.251.
- Kimura, T.; Miki, M.; Ikeda, M.; Yonemoto, S.; Watanabe, K.; Kondo, S.; Ho, I.K.; Yamamoto, I. Possible existence of a novel receptor for uridine analogues in the central nervous system using two isomers, N3-(S)-(+)- and N3-(R)-(-)-alpha-hydroxy-betaphenethyluridines. Biol. Pharm. Bull., 2001b, 24, 729-731. doi: 10.1248/bpb.24.729.
- Kovács, Z.; Kékesi, K.A.; Szilágyi, N.; Ábrahám, I.; Székács, D.; Király, N.; Papp, E.; Császár, I.; Szegő, É.; Barabás, K.; Péterfy, H.; Erdei, A.; Bártfai, T.; Juhász, G. Facilitation of spike-wave discharge activity by lipopolysaccharides in Wistar Albino Glaxo/Rijswijk rats. Neuroscience, 2006, 140, 731-742. doi: 10.1016/j.neuroscience.2006.02.023.
- Kovács, Z.; Slézia, A.; Bali, Z.K.; Kovács, P.; Dobolyi, A.; Szikra, T.; Hernádi, I.; Juhász, G. Uridine modulates neuronal activity and inhibits spike-wave discharges of absence epileptic Long Evans and Wistar Albino Glaxo/Rijswijk rats. Brain Res. Bull., 2013, 97, 16-23. doi: 10.1016/j.brainresbull.2013.05.009.
- Kovács, Z.; Dobolyi, A. Anatomical distribution of nucleoside system in the human brain and implications for therapy. In: Adenosine: a key link between metabolism and brain

activity; Masino, S.A.; Boison, D., Eds.; Springer Science, Business Media: New York, 2013; 621-656.
- Kovács, Z.; Kékesi, A.K.; Juhász, G.; Dobolyi, A. The antiepileptic potential of nucleosides. Curr. Med. Chem., 2014, 21, 788-821. doi: 10.2174/13816128196661311919154505.
- Kovács, Z.; D'Agostino, D.P.; Dobolyi, A.; Ari, C. Adenosine A1 Receptor Antagonism Abolished the Anti-seizure Effects of Exogenous Ketone Supplementation in Wistar Albino Glaxo Rijswijk Rats. Front. Mol. Neurosci., 2017, 10:235. doi: 10.3389/fnmol.2017.00235.
- Kutzing, M.K.; Firestein, B.L. Altered uric acid levels and disease states. J. Pharmacol. Exp. Ther., 2008, 324, 1-7. doi: 10.1124/jpet.107.129031.
- Lewin, E.; Bleck, V. Electroshock raises pentylenetetrazol threshold: possible role of inosine. Life Sci., 1983, 32, 433-436. doi: 10.1016/0024-3205(83)90135-2.
- Löscher, W.; Luna-Tortós, C.; Römermann, K.; Fedrowitz, M. Do ATP-binding cassette transporters cause pharmacoresistance in epilepsy? Problems and approaches in determining which antiepileptic drugs are affected. Curr. Pharm. Des., 2011, 17, 2808-2828. doi: 10.2174/138161211797440212.
- Merighi, S.; Mirandola, P.; Varani, K.; Gessi, S.; Leung, E.; Baraldi, P.G.; Tabrizi, M.A.; Borea, P.A. A glance at adenosine receptors: novel target for antitumor therapy. Pharmacol. Ther., 2003, 100, 31-48. doi: 10.1016/s0163-7258(03)00084-6.
- Nascimento, F.P.; Macedo-Júnior, S.J.; Lapa-Costa, F.R.; Cezar-Dos-Santos, F.; Santos, A.R.S. Inosine as a tool to understand and treat central nervous system disorders: A neglected actor? Front. Neurosci., 2021, 24:15:703783. doi: 10.3389/fnins.2021.703783.
- Perucca, P.; Gilliam, F.G. Adverse effects of antiepileptic drugs. Lancet Neurol., 2012, 11, 792-802. doi: 10.1016/S1474-4422(12)70153-9.
- Porkka-Heiskanen, T.; Alanko, L.; Kalinchuk, A.; Stenberg, D. Adenosine and sleep. Sleep Med. Rev., 2002, 6, 321-332. doi: 10.1053/smrv.2001.0201. doi: 10.1053/smrv.2001.0201.
- Rinaldi, V.E.; Di Cara, G.; Mencaroni, E.; Verrotti, A. Therapeutic options for childhood absence epilepsy. Pediatr. Rep., 2021, 13, 658-667. doi: 10.3390/pediatric13040078.
- Russo, E.; Citraro, R.; Donato, G.; Camastra, C.; Iuliano, R.; Cuzzocrea, S.; Constanti, A.; De Sarro, G. mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy. Neuropharmacology, 2013, 69, 25-36. doi: 10.1016/j.neuropharm.2012.09.019
- Russo, E.; Andreozzi, F.; Iuliano, R.; Dattilo, V.; Procopio, T.; Fiume, G.; Mimmi, S.; Perrotti, N.; Citraro, R.; Sesti, G.; Constanti, A.; De Sarro, G. Early molecular and behavioral response to lipopolysaccharide in the WAG/Rij rat model of absence epilepsy and depressive-like behavior, involves interplay between AMPK, AKT/mTOR pathways and neuroinflammatory cytokine release. Brain Behav. Immun., 2014, 42, 157-168. doi: 10.1016/j.bbi.2014.06.016.
- Schmidt, A.P.; Lara, D.R.; De Faria Maraschin, J.; Da Silveira Perla, A.; Onofre Souza, D. Guanosine and GMP prevent seizures induced by quinolinic acid in mice. Brain Res., 2000, 864, 40-43. doi: 10.1016/s0006-8993(00)02106-5.
- Schmidt, A.P.; Lara, D.R.; Souza, D.O. Proposal of a guanine based purinergic system in the mammalian central nervous system. Pharmacol. Ther., 2007, 116, 401-416. doi: 10.1016/j.pharmthera.2007.07.004.
- Skolnick, P.; Syapin, P.J.; Paugh, B.A.; Moncada, V.; Marangos, P.J.; Paul, S.M. Inosine, an endogenous ligand of the brain benzodiazepine receptor, antagonizes pentylenetetrazole-evoked seizures. Proc. Natl. Acad. Sci. U.S.A., 1979, 76, 1515-1518. doi: 10.1073/pnas.76.3.1515.

- Tan, S.; Radi, R.; Gaudier, F.; Evans, R.A.; Rivera, A.; Kirk, K.A.; Parks, D.A. Physiologic levels of uric acid inhibit xanthine oxidase in human plasma. Pediatr. Res., 1993, 34, 303-307. doi: 10.1203/00006450-199309000-00013.
- Thyrion, L.; Raedt, R.; Portelli, J.; Van Loo, P.; Wadman, W.J.; Glorieux, G.; Lambrecht, B.N.; Janssens, S.; Vonck, K.; Boon, P. Uric acid is released in the brain during seizure activity and increases severity of seizures in a mouse model for acute limbic seizures. Exp. Neurol., 2016, 277, 244-251. doi: 10.1016/j.expneurol.2016.01.001.
- Togha, M.; Akhondzadeh, S.; Motamedi, M.; Ahmadi, B.; Razeghi, S. Allopurinol as adjunctive therapy in intractable epilepsy: a double-blind and placebo-controlled trial. Arch. Med. Res., 2007, 38, 313-316. doi: 10.1016/j.arcmed.2006.10.010.
- Tomé, A.R.; Silva, H.; Cunha, R.A. Role of the purinergic neuromodulation system in epilepsy. Open Neurosci. J., 2010, 4, 64-83. doi: 10.2174/1874082001004010064
- Traversa, U.; Bombi, G.; Camaioni, E.; Macchiarulo, A.; Costantino, G.; Palmieri, C.; Caciagli, F.; Pellicciari, R. Rat brain guanosine binding site. Biological studies and pseudo-receptor construction. Bioorg. Med. Chem., 2003, 11, 5417-5425. doi: 10.1016/j.bmc.2003.09.043.
- Van Luijtelaar, G.; Lyashenko, S.; Vastyanov, R.; Verbeek, G.; Oleinik, A.; Van Rijn, C.; Volokhova, G.; Shandra, A.; Coenen, A.; Godlevsky, L. Cytokines and absence seizures in a genetic rat model. Neurophysiology, 2012, 43, 478-486. doi: 10.1007/s11062-012-9252-6
- Vinadé, E.R.; Schmidt, A.P.; Frizzo, M.E.; Izquierdo, I.; Elisabetsky, E.; Souza, D.O. Chronically administered guanosine is anticonvulsant, amnesic and anxiolytic in mice. Brain Res., 2003, 977, 97-102. doi: 10.1016/s0006-8993(03)02769-0.
- Volpini, R.; Marucci, G.; Buccioni, M.; Dal Ben, D.; Lambertucci, C.; Lammi, C.; Mishra, R.C.; Thomas, A.; Cristalli, G. Evidence for the existence of a specific g protein-coupled receptor activated by guanosine. Chem. Med. Chem., 2011, 6, 1074-1080. doi: 10.1002/cmdc.201100100.
- Wang, T.; Zhou, X.; Bai, Y.; Zhang, L.; Li, L.; Wu, C. Antiepileptic effect of uridine may be caused by regulating dopamine release and receptor expression in corpus striatum, Brain Res., 2018, 1688, 47-53. doi: 10.1016/j.brainres.2018.03.011.
- Zhao, Q.; Marolewski, A.; Rusche, J.R.; Holmes, G.L. Effects of uridine in models of epileptogenesis and seizures. Epilepsy Res., 2006, 70, 73-82. doi: 10.1016/j.eplepsyres.2006.03.003
- Zhao, Q.; Shatskikh, T.; Marolewski, A.; Rusche, J.R.; Holmes, G.L. Effects of uridine on kindling. Epilepsy Behav., 2008, 13, 47-51. doi: 10.1016/j.yebeh.2008.02.002.

9. LIST OF PUBLICATIONS

Scientometric data:

- MTMT ID: 10071510
- Number of scientific journal articles published in peer-reviewed journals: 6
- Total impact factor: 18.614
- Number of citations: 74 (independent: 47)

Publications related to the thesis topic

1. Kovács, Z.; Kékesi, K.A.; Dobolyi, Á.; **Lakatos, R.**; Juhász, G. Absence epileptic activity changing effects of non-adenosine nucleoside inosine, guanosine and uridine in Wistar Albino Glaxo Rijswijk rats. Neuroscience, 2015, 300, 593–608. doi: 10.1016/j.neuroscience.2015.05.054. (**Q2, IF 3.231**; independent citations: 17)

2. **Lakatos, R.K**., Dobolyi, Á., Todorov, M.I., Kékesi, K.A., Juhász, G., Aleksza, M., Kovács, Z. Guanosine may increase absence epileptic activity by means of A2A adenosine receptors in Wistar Albino Glaxo Rijswijk rats. Brain Res. Bull., 2016, 124, 172-181. doi: 10.1016/j.brainresbull.2016.05.001 (**Q2, IF 3.033**; independent citations: 6)

3. **Lakatos, R.K**.; Dobolyi, Á.; Kovács, Z. Uric acid and allopurinol aggravate absence epileptic activity in Wistar Albino Glaxo Rijswijk rats. Brain Res., 2018, 1686, 1-9. doi: 10.1016/j.brainres.2018.02.012. (**Q1; IF 2.929**; independent citations: 4)

Further publications related to the thesis topic

1. Kovács, Z.; Kékesi, K.A.; Juhász, G.; Barna, J.; Héja, L.; **Lakatos, R**.; Dobolyi, Á. Non-adenosine Nucleoside Inosine, Guanosine and Uridine as Promising Antiepileptic Drugs: a Summary of Current Literature. Mini-Rev. Med. Chem., 2014, 14(13), 1033-1042. doi: 10.2174/1389557514666141107120226. (**Q2, IF: 2.841**; independent citations: 17)

2. Kovács, Z.; Kardos, J.; Kékesi, K.A.; Juhász, G.; **Lakatos, R.**; Héja, L. Effects of Nucleosides on Glia - Neuron Interactions Open up New Vistas in the Development of More Effective Antiepileptic Drugs. Curr, Med, Chem., 2015, 22(12), 1500-1514. doi: 10.2174/0929867322666150212153210. (**Q2, IF: 3.455**; independent citations: 1)

3. Kovács, Z.; **Lakatos, R.K.**; Barna, J.; Dobolyi, Á. Absence epileptic activity in Wistar Albino Glaxo Rijswijk rat mothers. Brain Res., 2017, 1657, 368-376. doi: 10.1016/j.brainres.2017.01.005 (**Q1, IF 3.125**; independent citations: 2)

Conferences related to the thesis topic

Poster presentations

1. **Renáta Krisztina Lakatos**, Árpád Dobolyi, Katalin A. Kékesi, Magdolna Aleksza, Zsolt Kovács: Guanosine may increase absence epileptic activity in Wistar Albino Glaxo Rijswijk rats. https://pcongress.hu/pdf/lakatos_68_110.pdf In Program Booklet of FENS Regional Meeting. Pécs, Hungary. 20-23. September 2017.
http://fensfrm.hu/uploads/docs/1/fens2017booklet.pdf 2016

2. **Renáta Lakatos**, Árpád Dobolyi, Katalin A. Kékesi, Gábor Juhász, Zsolt Kovács: Inosine, guanosine and uridine modulate the lipopolysaccharide-evoked changes in spike-wave discharge activity in Wistar Albino Glaxo/Rijswijk rats. IBRO Workshop. Budapest, Hungary. Hungarian Academy of Sciences. 21-22. January 2016.
http://www.ibro2016.hu/images/downloads/IBRO_poster_sessions.pdf 2015

3. Zsolt Kovács, Katalin A. Kékesi, Árpád Dobolyi, **Renáta Lakatos**, Gábor Juhász: Inosine, guanosine and uridine change the absence epileptic activity in Wistar Albino Glaxo/Rijswijk rats. XV. Biannual Conference of the Hungarian Neuroscience Society. Budapest, Hungary. Hungarian Academy of Sciences. 22-23. January 2015.
http://www.mitt2015.hu/files/4414/2132/1205/MITT2015_Abstract_Boo k_v_20150115.pdf

Lecture presentations

1. **Renáta Krisztina Lakatos**, Zsolt Kovács: The effect of guanosine on absence epileptic activity in WAG/Rij rats. 12th Regional Conference of Natural Sciences. Szombathely, Hungary. NYME-SEK TTMK, 25. January 2017.

2. **Renáta Krisztina Lakatos**, Zsolt Kovács: The effects of non-adenosine nucleosides on the lipopolysaccharide-induced absence epileptic activity in WAG/Rij rats. 11th Regional Conference of Natural Sciences. Szombathely, Hungary. NYME SEK TTMK. 27. January 2016.

10. ACKNOWLEDGEMENTS

I would like to express my gratitude towards my supervisor, Zsolt Kovács, PhD (associate professor, Eötvös Loránd University, Savaria University Centre, BDTTC, Department of Biology). I sincerely appreciate his professional guidance.

I would like to thank to the Doctoral School of Biology of University of Pécs, and the Department of Biology of Eötvös Loránd University Savaria University Center, as well as our colleagues for their professional support and providing the infrastructural background.

I am grateful to family for their support they provided me during my doctoral studies.