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ABSENCE EPILEPTIC ACTIVITY CHANGING EFFECTS OF NON-ADENOSINE NUCLEOSIDE INOSINE, GUANOSINE AND URIDINE IN WISTAR ALBINO GLAXO RIJSWIJK RATS

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Abstract—Adenosine (Ado) and non-adenosine (non-Ado) nucleosides such as inosine (Ino), guanosine (Guo) and uridine (Urd) may have regionally different roles in the regulation of physiological and pathophysiological processes in the central nervous system (CNS) such as epilepsy. It was demonstrated previously that Ino and Guo decreased quinolinic acid (QA)-induced seizures and Urd reduced penicillin-, bicuculline- and pentylenetetrazole (PTZ)-induced seizures. It has also been demonstrated that Ino and Urd may exert their effects through GABAergic system by altering the function of $GABA_A$ type of gamma-aminobutyric acid receptors ($GABA_A$ receptors) whereas Guo decreases glutamate-induced excitability through glutamatergic system, which systems (GABAergic and glutamatergic) are involved in pathomechanisms of absence epilepsy. Thus, we hypothesized that Ino and Guo, similarly to the previously described effect of Urd, might also decrease absence epileptic activity. We investigated in the present study whether intraperitoneal (i.p.) application of Ino (500 and 1000 mg/kg), Guo (20 and 50 mg/kg), Urd (500 and 1000 mg/kg), $GABA_A$ receptor agonist muscimol (1 and 3 mg/kg), $GABA_A$ receptor antagonist bicuculline (2 and 4 mg/kg), non-selective Ado receptor antagonist theophylline (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, 0.0625 and 0.1250 mg/kg) alone and in combination have modulatory effects on absence epileptic activity in Wistar Albino Glaxo Rijswijk (WAG/Rij) rats. We found that Guo decreased the number of spike-wave discharges (SWDs) whereas Ino increased it dose-dependently. We strengthened that Urd can decrease absence epileptic activity. Our results suggest that Guo, Urd and their analogs could be potentially effective drugs for treatment of human absence epilepsy.

Key words: absence epilepsy, inosine, guanosine, uridine, WAG/Rij rats.

INTRODUCTION

Regional differences in nucleoside levels as well as metabolic enzymes, transporters and receptors of nucleosides were demonstrated in the central nervous system (CNS) suggesting region-dependent functions of nucleosides in the brain (Kovács et al., 2010, 2011; Kovács and Dobolyi, 2013). Indeed, adenosine (Ado) and non-adenosine (non-Ado) nucleosides (e.g., inosine/Ino, guanosine/Guo and uridine/Urd) have a role in the regulation of physiological and pathophysiological processes in the brain, such as the regulation of sleep and memory, Parkinson's disease, Alzheimer's disease and epilepsy (Burnstock et al., 2011; Kovács and Dobolyi, 2013; Kovács et al., 2014). Therefore, several nucleoside uptake inhibitors, nucleoside metabolic inhibitors and nucleoside derivatives are being used in drug development for the treatment of different CNS diseases (Boison, 2011; Kovács and Dobolyi, 2013; Kovács et al., 2014).

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Abbreviations: A_{2A} receptors, A_{2A} type of Ado receptors; Ado, adenosine; ANOVA, analysis of variance; EEG recording, electroencephalographic recording; $GABA_A$ receptors, $GABA_A$ type of gamma-aminobutyric acid receptors; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; Guo, guanosine; i.p., intraperitoneal; Ino, inosine; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine maleate; NMDA, N-methyl-D-aspartate; Non-Ado, non-adenosine; PTZ, pentylenetetrazole; QA, quinolinic acid; REM, rapid eye movement; S.E.M., standard error of the mean; SWD, spike-wave discharge; SWS, slow wave sleep; Urd, uridine; UTP, uridine triphosphate; WAG/Rij, Wistar Albino Glaxo/Rijswijk.

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Non-Ado nucleoside Ino, Guo and Urd may enter the brain via the blood–brain barrier (Lewin and Bleck, 1985; Slézia et al., 2004; Soares et al., 2004; Cansev, 2006; Ipata, 2011) and are also derived from intracellular and/or extracellular metabolism of nucleotides by nucleotidases in the brain (Ipata, 2011; Kovács et al., 2013a; Ipata and Balestri, 2014). Depending on the local concentration gradient, Ino, Guo and Urd may be transported bidirectionally through plasmamembrane coupled nucleoside transporters of brain cells (Ipata, 2011; Giuliani et al., 2012). It was suggested that Guo may be released from synaptosomes (Fredholm and Vernet, 1979) and Guo as well as Urd may bind to their putative specific receptors (Kimura et al., 2001a; Traversa et al., 2003; Volpini et al., 2011). Non-Ado nucleosides may modulate (i) the effects of glutamatergic system (by means of Guo), (ii) the release of Ado (Guo), as well as (iii) functioning of GABAergic system (Ino and Urd) and adenosinergic system (Ino, Guo and Urd) (Skolnick et al., 1979; Ciccarelli et al., 2000; Kimura et al., 2001a; Haskó et al., 2004; Schmidt et al., 2007). Thus, Ino, Guo and Urd may act as signaling/neuromodulator molecules in the CNS.

Antiepileptic effects of Ado and Ado receptor agonists as well as antagonists are extensively investigated but our knowledge relating to effect of non-Ado nucleosides on epileptic activity is far from complete (Boison, 2011; Masino et al., 2012; Kovács et al., 2014, 2015). For instance, it was revealed that (i) Ino showed an anticonvulsant effect on quinolinic acid (QA)-induced seizures (Ganzella et al., 2011), (ii) Guo may decrease QA-induced seizures dose-dependently (Schmidt et al., 2000) and (iii) Urd decreased epileptic activity in the rat hippocampal kindling model (Zhao et al., 2008) and in a model of human absence epilepsy Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats (Kovács et al., 2013b) suggesting the antiepileptic potential of non-Ado nucleosides. It is well known that GABAergic system (e.g., $GABA_A$ type of gamma-aminobutyric acid receptors: $GABA_A$ receptors), glutamatergic system (e.g., via glutamate-evoked excess hyperexcitability) and adenosinergic system (e.g., A_{2A} type of Ado receptors, A_{2A} receptors) are involved in the pathomechanisms of absence epilepsy (Snead, 1995; Coenen and Van Luijtelaar, 2003; Pisu et al., 2008; D'Alimonte et al., 2009). Thus, we hypothesized that (i) Ino and Urd may have effects on absence epilepsy through $GABA_A$ and Ado receptors (Skolnick et al., 1979; Kimura et al., 2001a; Haskó et al., 2004; Kovács et al., 2015), (ii) Guo may change absence epileptic activity via the glutamatergic system (Frizzo et al., 2003; De Oliveira et al., 2004; Schmidt et al., 2007; Kovács et al., 2015), (iii) Ino and Guo may modulate absence epilepsy through Ado receptors indirectly by their degradation to Ado or by stimulation of Ado release (Guo) (Zimmermann, 1996; Ciccarelli et al., 2000; Ipata, 2011; Kovács et al., 2015) and, theoretically, (iv) Guo and Urd may exert their effects on absence epileptic activity via own putative receptors (Kimura et al., 2001a, 2001b; Traversa et al., 2003; Volpini et al., 2011; Kovács et al., 2015). These effects of Ino, Guo and Urd on absence epileptic activity have not been investigated yet except absence epileptic activity decreasing influence of Urd injected alone in Long Evans and WAG/Rij rats (Kovács et al., 2013b).

We carried out our experiments on WAG/Rij rats. WAG/Rij rat strain is one of the most investigated rodent absence models of human absence epilepsy (Coenen and Van Luijtelaar, 2003). Electroencephalographic (EEG) recordings of all WAG/Rij rats (older than 3 months) contain bilaterally synchronous and spontaneously occurring spike-wave discharges (SWDs). In relation to SWD genesis, it was demonstrated that firstly the hyperexcitable neurons of cortical focus in the perioral/lateral region of the somatosensory cortex initiate SWDs and lead the thalamus (cortical focus theory) but after the first 500 ms of the SWDs, the cortical focus and the thalamus drive each other by cortico-thalamo-cortical oscillatory networks in WAG/Rij rats (Coenen and Van Luijtelaar, 2003; Depaulis and Van Luijtelaar, 2005).

We addressed in the present study whether Ino, Guo and Urd and their combined application with $GABA_A$ receptor agonist muscimol, $GABA_A$ receptor antagonist bicuculline, non-selective Ado receptor antagonist theophylline 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) have modulatory effects on absence epileptic activity in WAG/Rij rats. We demonstrated that not only Urd but also Ino and Guo result in changes in the absence epileptic activity in WAG/Rij rats: Guo and Urd decreased whereas Ino increased absence epileptic activity dose-dependently.

EXPERIMENTAL PROCEDURES

Animals

All animal treatments and surgery procedures were carried out according to the local ethical rules, which are in conformity with the guidelines of the European Communities Council Directive 24 November 1986 (86/609/EEC) to use and treat animals in experimental laboratories. All efforts were made to minimize pain and suffering of the animals and to reduce the number of animals used.

WAG/Rij male rats (10 months old; $n = 110$) weighting 340–360 g were used (breeding colony of WAG/Rij rats at University of West Hungary, Savaria Campus, Szombathely, Hungary). Animals were housed in groups 3–4 and they were separated after surgery under standard laboratory conditions (12:12 h light–dark cycle, light was on from 08.00 AM to 08.00 PM; free access to water and food; air-conditioned room at 22 ± 2 °C).

Electroencephalography

Implantation of animals for EEG recording. Rats were implanted under Halothane-air mixture (1%) anesthesia with 0.8 mm od. stainless steel screw electrodes for EEG recording (Kovács et al., 2006). Briefly, screws were