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Research report

Uridine modulates neuronal activity and inhibits spike-wave discharges of absence epileptic Long Evans and Wistar Albino Glaxo/Rijswijk rats

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ABSTRACT

Pharmacological and functional data suggest the existence of uridine (Urd) receptors in the central nervous system (CNS). In the present study, simultaneous extracellular single unit recording and microiontophoretic injection of the pyrimidine nucleoside Urd was used to provide evidence for the presence of Urd-sensitive neurons in the thalamus and the cerebral cortex of Long Evans rats. Twenty-two neurons in the thalamus (24% of recorded neurons) and 17 neurons in the cortex (55%) responded to the direct iontophoresis of Urd. The majority of Urd-sensitive neurons in the thalamus and cortex (82% and 59%, respectively) increased their firing rate in response to Urd. In contrary, adenosine (Ado) and uridine 5′-triphosphate (UTP) decreased the firing rate of all responding neurons in the thalamus, and the majority of responding neurons in the cortex (83% and 87%, respectively). Functional relevance of Urd-sensitive neurons was investigated in spontaneously epileptic freely moving Long Evans and Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. Intraperitoneal (i.p.) injection of 500 mg/kg Urd decreased epileptic activity (210–270 min after injection) in both rat strains. Intraperitoneal administration of 1000 mg/kg Urd decreased the number of spike-wave discharges (SWDs) between 150–270 min and 90–270 min in Long Evans and WAG/Rij rats, respectively. The effect of Urd was long-lasting in both rat strains as the higher dose significantly decreased the number of SWDs even 24 h after Urd injection. The present results suggest that Urd-sensitive neurons in the thalamus and the cerebral cortex may play a role in the antiepileptic action of Urd possibly via modulation of thalamocortical neuronal circuits.

Abbreviations: 3-AP, 3-aminopyridine; Ado, adenosine; ANOVA, analysis of variance; ATP, adenosine 5′-triphosphate; CNS, central nervous system; d.w., distilled water; EEG, electroencephalogram; FFT, Fast Fourier Transform; GABA, gamma-aminobutyric acid; i.p., intraperitoneal; NMDA, N-methyl-D-aspartate; S.E.M., standard error of the mean; SWD, spike-wave discharge; UDP, uridine 5′-diphosphate; Urd, uridine; UTP, uridine 5′-triphosphate; WAG/Rij, Wistar Albino Glaxo/Rijswijk.

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1. Introduction

Nucleosides participate in the physiological and pathophysiological mechanisms of the CNS such as memory, sleep, depression, schizophrenia, epilepsy, Alzheimer's disease and Parkinson's disease. Therefore, increasing attention has been paid to nucleoside mechanisms in the CNS (Burnstock, 2007; Kovacs and Dobolyi, 2013).

Uridine is an endogenous pyrimidine nucleoside, which serves as a precursor of UTP synthesis required for RNA synthesis. Following the discovery of the neuromodulatory function of adenosine 5′-triphosphate (ATP), UTP, and the purine nucleoside Ado reviewed recently (Burnstock, 2007), the specific neuroactive role of Urd has also been hypothesized (Connolly and Duley, 1999). Based on the sleep-promoting effect of Urd (Borbely and Tobler, 1989), the existence of a Urd receptor was proposed (Kimura et al., 2001). Indeed, Urd was shown to activate fast transmembrane Ca^{2+} ion fluxes in rat brain homogenates and a specific Urd binding site has also been characterized (Kardos et al., 1999; Kovács et al., 2003). We demonstrated an uneven distribution of Urd in the CNS (Kovacs et al., 2010). Furthermore, elevated levels of extracellular Urd were found in response to depolarizing agents in the brain of anesthetized rats (Dobolyi et al., 1999, 2000) and also during 3-aminopyridine (3-AP)-induced epileptic seizures (Slézia et al., 2004).

In the present study, we aimed to identify Urd-sensitive neurons in the cerebral cortex and the thalamus. The effect of locally iontophoretized Urd was examined on maintained extracellular firing activity of neurons intrinsic to the cerebral cortex and the thalamus of Long Evans rats. The effects of microiontophoretically applied Ado and UTP on neuronal spiking activity were also examined: Ado is an established neuromodulator nucleoside (Burnstock, 2007; Kovacs and Dobolyi, 2013) and uridine could have direct effects, too. In addition, Urd may be metabolized to UTP; thus Urd may exert its action also via the G-protein coupled P2Y2, P2Y4 and P2Y6 receptors of UTP (Köles et al., 2005; Richardson et al., 2003). In turn, released UTP is considered to be a potential source of Urd in the extracellular space (Zimmermann, 1996). Based on these data Ado, Urd and UTP were investigated in this study.

Ketamine is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, which changes the excitatory/inhibitory balance in brain areas involved in genesis of absence epilepsy. Under ketamine treatment absence seizure generators may be inhibited and, as a consequence, SWDs disappear. Indeed, ketamine may decrease/abolish absence epileptic activity dose-dependently similarly to other NMDA receptor antagonists (Midzyanovskaya et al., 2004; Peeters et al., 1990). Thus, in the first experiment, when single-neuron electrophysiological recordings were performed under ketamine anaesthesia (similarly as described previously by Kovács and Hernádi (2006), spontaneous SWDs were suppressed. Therefore, in a second experiment, to test the functionality of Urd-sensitive neurons, the effect of Urd was examined on the spontaneous epileptic activity of freely moving Long Evans rats. Besides, to investigate whether i.p. administered Urd evokes strain specific effects on SWDs in Long Evans rats, we tested the antiepileptic effects of Urd also in WAG/Rij rats. Awake, freely moving animals were injected with two different doses of Urd (500 mg/kg, and 1000 mg/kg i.p.) and the effects on spontaneous absence epileptic activity were analyzed on different time scales.

2. Material and methods

2.1. Animals

Eight months old adult WAG/Rij male rats (housed at the Department of Zoology, University of West Hungary, Savaria Campus, Szombathely, Hungary) and 6–8 months old male hooded Long Evans rats (Charles River Laboratories, Gödöllő, Hungary, housed at the Institute of Biology, University of Pécs, Hungary) were used in the experiments. Animals were initially kept in groups of 3–4 under standard laboratory conditions (12:12 h light–dark cycle, light was on from 08.00 AM to 08.00 PM), with free access to water and food pellets. Rats were maintained in air-conditioned rooms at $22 \pm 2\,^{\circ}C$ and were housed individually after surgery and during the experiments.

Animal treatment and surgery procedures were carried out according to the local ethical rules in accordance with the Hungarian Act of Animal Care and Experimentation (1998. XXVIII. Section 243/1998) in conformity with the regulations for animal experimentation in the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain and suffering and to reduce the number of animals used.

2.2. Microiontophoresis and extracellular unit recording

2.2.1. Surgical treatments

Electrophysiological recordings were performed under ketamine anaesthesia following single i.p. injection of 100 mg/kg ketamine (CP Ketamine, RG, Hungary). Long Evans rats ($n = 25$) were placed in a stereotaxic apparatus and a hole was drilled in the skull. Stereotaxic coordinates for the track of multibarrel micropipettes selected on the basis of the rat brain stereotaxic atlas by Paxinos and Watson (2005) were AP: −3.6 mm; L: 2.5 mm; V (from the dura): 1–7 mm.

2.2.2. Single-unit recording and microiontophoresis

Seven-barreled micropipettes were used for electrophysiological recording and microiontophoresis, with tips of 8–10 micrometre in total diameter (Carbostar-7, Kation Scientific Ltd., MN, USA). The impedance of the central, recording channel was 0.4–0.8 MΩ (at 50 Hz), whereas the impedance for each drug channel was 20–200 MΩ. One drug channel (filled with 0.5 M NaCl) was used for the application of a continuous balancing current, while each of the remaining pipettes were filled with one of the following five bioactive substances: kainate (Sigma, 60 mM, dissolved in distilled water (d.w.)); gamma-aminobutyric acid (GABA; Sigma, 500 mM, dissolved in d.w.); Ado (Sigma, 250 mM, dissolved in 50% dimethyl sulfoxide 50% d.w.); UTP (Sigma, 125 mM, dissolved in d.w.); and Urd (Sigma, 100 mM, dissolved in d.w.), respectively. Ejection currents were controlled by individual constant current circuits (Neurophore BH-2, Medical Systems Corp., NY, USA). Extracellular action potentials were passed to a high performance biological amplifier (Supertech Ltd., Pécs, Hungary), then to an analogue-digital conversion interface (Power 1401, CED, Cambridge, UK) and were stored and archived on PC.

2.2.3. Statistical analysis

Waveform data were analyzed with the Spike2 software (CED, Cambridge, UK). In case of ambiguous neuronal identification, waveform dependent spike-sorting routines were run off-line. Firing rates of individual neurons were tested before (as control) and during iontophoretic drug administration (treatment). Each neuron was tested 3–5 times and firing rate data was averaged for each neuron. Data were expressed as mean percentage of control firing rate ± standard error of the mean (S.E.M.). Statistics between control and treatment firing activity were performed using one-way analysis of variance (ANOVA) and Student's paired and unpaired *t*-tests, where applicable; $p < 0.05$ was set as threshold for significance.

2.3. Recording and analysis of absence epileptic activity

2.3.1. Electrode implantation

Long Evans ($n = 10$) and WAG/Rij ($n = 10$) rats were anaesthetized by halothane–air mixture (0.8–1%). Stainless steel screw (outer diameter of the thread: 0.8 mm) electrodes were placed into the bone above the primary motor cortex (AP: 0.8 mm; L: 1.8 mm) and the somatosensory cortex (AP: 0.2 mm; L: 6.2 mm) based on the stereotaxic atlas of the rat brain by Paxinos and Watson (2005). A stainless steel reference electrode (a plate of 3 mm × 4 mm) was implanted under the skin and over the masseter muscle while a screw electrode was placed above the cerebellum as ground electrode. All electrodes were soldered to a ten-pin socket and were fixed to the skull bone with acrylic dental cement. Rats were allowed to recover from surgery for 2 weeks.

2.3.2. EEG recording

A differential biological amplifier (Bioamp4, Supertech Ltd., Pécs, Hungary) was connected to a CED 1401 mII data capture and analysis device and the Spike2 software (CED, Cambridge, UK) was used for electroencephalogram (EEG) data recording. To detect SWDs (generated in reverberating thalamocortical neuronal circuitry and manifested in EEG) primary motor cortex-plate and somatosensory cortex-plate leads were recorded. The bandwidth of the EEG signal filtering was 0.53–150 Hz. The analogue signal was digitized at 1 kHz sampling rate and raw EEG data were stored on a PC for further analysis.

2.3.3. Experimental design and data analysis

We measured the number of SWD occurrences and duration between 30 and 270 min of post-injection time. The first 30 min post injection time was omitted from analysis as stress induced by i.p. injection is known to change the frequency of SWDs for up to 30 min (Kovacs et al., 2006). SWDs were extracted from the raw data files