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Research report

Lipopolysaccharide induced increase in seizure activity in two animal models of absence epilepsy WAG/Rij and GAERS rats and Long Evans rats

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ABSTRACT

We showed previously that the number and time of spike–wave discharges (SWDs) were increased after intraperitoneal (i.p.) injection of lipopolysaccharide (LPS), an effect, which was completely abolished by cyclooxygenase-2 (COX-2) inhibitor indomethacin (IND) pretreatment in Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. These and other results suggest that injection of LPS to genetically absence epileptic animals, such as WAG/Rij rats, may allow us to investigate relationships between absence epilepsy and LPS evoked neuroinflammation processes. However, LPS may evoke different effects on absence epileptic activity in various animal strains. Thus, to extend our previous results, we injected two doses of LPS (50 μg/kg and 350 μg/kg i.p.) alone and in combination with IND (10 mg/kg IND i.p. +50 μg/kg LPS) into rats of two model animal strains (WAG/Rij rats; GAERS rats: Genetic Absence Epileptic Rats from Strasbourg) and into Long Evans rats. The effects of treatments on SWD number and SWD duration were examined. Both doses of LPS increased the SWD number and the total time of SWDs dose-dependently during the whole 4-h recording period, which was abolished by IND pretreatment in all three investigated strains. These results extend our previous results suggesting that our methods using LPS injection into freely moving absence epileptic rats is applicable not only in well-established animal models of absence epilepsy such as WAG/Rij rats and GAERS rats but also in Long Evans rats to investigate links between inflammation and absence epilepsy.

1. Introduction

It has been shown that enhanced pro-inflammatory cytokine levels can increase the susceptibility to epileptic seizure (Galic et al., 2008; Vezzani et al., 2008). Indeed, pro-inflammatory cytokines (e.g., interleukin-1 β (IL-1β) and tumor necrosis factor-α (TNF-α)) synthesized by glial cells in the central nervous system (CNS) elevate neuronal excitability (Rodgers et al., 2009; Vezzani et al., 2008; Vezzani and Granata, 2005). Changes in IL-1β and TNF-α levels may have a role in SWD generation/precipitation in two animal models of human absence epilepsy, GAERS rats and WAG/Rij rats (Akin et al., 2011; Van Luijtelaar et al., 2012). Lipopolysaccharide evokes rapid excitation in the cortex (Wang and White, 1999), enhances seizure susceptibility (Sayyah et al., 2003), increases absence epileptic activity and body temperature (Kovács et al., 2006, 2011) and changes protein expression in the WAG/Rij rat

Abbreviations: AMPA receptor, alpha-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid receptor; CNS, central nervous system; COX-2, cyclooxygenase-2; EEG, electroencephalogram; GAERS rats, Genetic Absence Epileptic Rats from Strasbourg; HVRS, high-voltage rhythmic spike; IL-1β, interleukin-1 β; IL-1R, interleukin-1 receptor; IND, indomethacin; i.p., intraperitoneal; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; PTC day, post-treatment control day; SWD, spike–wave discharge; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α; WAG/Rij rats, Wistar Albino Glaxo/Rijswijk rats.

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brain (Györffy et al., 2014). These effects of LPS may be mediated by increased levels of pro-inflammatory cytokines and interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) signaling in the CNS (Turrin et al., 2001; Vezzani et al., 2011).

Lipopolysaccharide, a cell wall component of gram-negative bacteria, belongs to the pathogen-associated molecular patterns (PAMPs) (Vezzani et al., 2011), which induce rapid synthesis of other cytokines and COX-2 via Toll-like receptor 4 (TLR4) evoked induction of transcriptional factors (e.g., nuclear factor κB, NFκB) in the CNS (Vezzani and Granata, 2005). Systemic injection of LPS to rats is a well-studied and described way for precipitation of inflammatory reactions in the CNS (Vezzani and Granata, 2005), which is also suitable for investigation of links between immune system and absence epileptic activity (Kovács et al., 2006). Lipopolysaccharide injected into genetically absence epileptic animals such as WAG/Rij rats, the animal strain, which is one of the most appropriate animal model strains for the study of spontaneous childhood absence epilepsy (Depaulis and Van Luijtelaar, 2005), could provide an opportunity for the investigation of the relationships between non-convulsive types of epilepsies such as absence epilepsy and LPS/cytokine evoked neuroinflammatory processes (Kovács et al., 2006), which is still incompletely understood. We previously demonstrated that i.p. application of LPS dose-dependently increased the absence epileptic activity in freely moving WAG/Rij rats (Kovács et al., 2006). Other absence epileptic animals may also be promising models to investigate the links between inflammation and epilepsy. However, the effects of LPS on absence epileptic activity in freely moving animals have not been investigated in genetically epileptic GAERS rats and Long Evans rats. It was recently demonstrated that Long Evans rats also produce SWDs similarly to WAG/Rij rats and GAERS rats (Depaulis and Van Luijtelaar, 2005; Huang et al., 2012; Polack and Charpier, 2006; Shaw, 2004, 2007; Shaw et al., 2009; Van Luijtelaar et al., 2011) allowing the investigation of SWDs in this strain as well. Therefore, to validate and extend our previous results, in which we demonstrated the LPS induced increase in absence epileptic seizures in WAG/Rij rats (Kovács et al., 2006) we injected two doses of LPS (50 μg/kg and 350 μg/kg i.p.) alone, and 50 μg/kg LPS in combination with 10 mg/kg IND into WAG/Rij rats, GAERS rats and Long Evans rats to investigate the effects of treatments on absence epileptic activity by detecting and evaluating SWDs.

2. Materials and methods

2.1. Animals

Eight months old male WAG/Rij rats, GAERS rats and Long Evans rats (housed at the Department of Zoology, University of West Hungary, Savaria Campus, Szombathely, Hungary) were used in the experiments. Animals were 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 food pellets and water. Rats were maintained in air-conditioned rooms at 22 ± 2 °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 reduce the number of animals used and to minimize pain and suffering.

2.2. Recording of absence epileptic activity and body temperature

2.2.1. Implantation of animals for EEG recording and body temperature measuring

Wistar Albino Glaxo/Rijswijk rats ($n = 20$), GAERS rats ($n = 20$) and Long Evans rats ($n = 20$) were anaesthetized by halothane-air mixture (0.8–1%) and implanted with screw electrodes as described earlier (Kovács et al., 2006). Briefly, stainless steel screw electrodes were placed into the bone above the frontal (AP: 2.0 mm; L: 2.1 mm) and parietal (AP: −6.5 mm; L: 2.1 mm) cortices (Paxinos and Watson, 2005) for electroencephalogram (EEG) recording, whereas a screw (ground) electrode was placed above the cerebellum. A stainless steel plate (3 mm × 4 mm, one side insulated) was used as a reference electrode which was implanted under the skin and over the masseter muscle. All electrodes were soldered to a ten-pin socket.

To detect the well-known effect of LPS and IND on body temperature, aluminum holders containing thermo-resistors (Pt 100) were placed above the frontal bone (Kovács et al., 2006) of three – three animals of all animal groups (Table 1). We measured the body temperature with a thermometer (SUPERTECH, Hungary) in each 10 min. Electrodes and aluminum holders were fixed to the skull bone with acrylic dental cement. Rats were allowed to recover from surgery for 2 weeks.

2.2.2. EEG recording and SWD scoring

Electroencephalogram was recorded by a differential biological amplifier (Bioamp4, Supertech Ltd., Pécs, Hungary) connected to a CED 1401 mII data capture and analysis device. Spike2 software (CED, Cambridge, UK) was used for recording the EEG. To detect SWDs, frontal cortex – plate and parietal cortex – plate leads were recorded. The bandwidth of the EEG signal filtering was 0.53–150 Hz. The analog signal was A/D converted at 1 kHz sampling rate and raw EEG data were stored on a PC for further analysis (Kovács et al., 2006).

The main properties of a typical SWD of WAG/Rij rats, GAERS rats and Long Evans rats (WAG/Rij/GAERS/Long Evans) are as follows: power spectra 7–11/7–9/6–12 Hz (Fig. 1b, d and f), amplitude 0.2–1.0/0.3–1.0/0.1–2.0 mV, duration 1–30/5–60/1–40 s (Akman et al., 2010; Coenen and Van Luijtelaar, 2003; Depaulis and Van Luijtelaar, 2005; Polack and Charpier, 2006; Shaw, 2004). The number of SWDs may vary between 15–40/20–80/6–90 (per hour) in WAG/Rij rats, GAERS rats and Long Evans rats depending on the time of the day and age of animals (Akman et al., 2010; Coenen and Van Luijtelaar, 2003; Depaulis and Van Luijtelaar, 2005; Polack and Charpier, 2006; Shaw, 2004). To avoid the modulatory effect of age and sleep-wake cycle on SWD number, electrophysiological recordings were carry out on eight months old animals (±1 week) between 4.00 PM and 8.00 PM. In this relatively short period of circadian activity, the LPS evoked changes in SWD number are not the consequence of reduced waking state (Kovács et al., 2006). After the different treatments, the EEG recording periods were split into 60 min sections and evaluated separately (Kovács et al., 2006). The SWDs (Fig. 1a, c and e) were selected, and SWD numbers were measured in all animal groups (groups 1–12; Table 1). The durations of SWDs were also measured in animals treated i.p. LPS injection alone (groups 1–6; Table 1). Both i.p. injection and handling evoked stress could influence SWD number during first 30 min (Depaulis and Van Luijtelaar, 2005; Kovács et al., 2006, 2012). Consequently, the first half hour of data after the i.p. injections were not included into the analysis. In relation to individually different SWD number and duration (Kovács et al., 2006; Polack and Charpier, 2006; Shaw, 2004), the changes in SWD numbers and duration were expressed in percentage of average control measures (three-day