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Research report

Intracerebroventricularly administered lipopolysaccharide enhances spike–wave discharges in freely moving WAG/Rij rats

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ABSTRACT

Peripheral lipopolysaccharide (LPS) injection enhances spike–wave discharges (SWDs) in the genetic rat model of absence epilepsy (Wistar Albino Glaxo/Rijswijk rats: WAG/Rij rats) parallel with the peripheral proinflammatory cytokine responses. The effect of centrally administered LPS on the absence-like epileptic activity is not known, however despite the important differences in inflammatory mechanisms. To examine the effect of centrally administered LPS on the pathological synchronization we intracerebroventricularly (i.c.v.) injected LPS into WAG/Rij rats and measured the number and duration of SWDs. I.c.v. injected LPS increased the number and duration of SWDs for 3 h, thereafter, a decrease in epileptic activity was observed. To further investigate the nature of this effect, a non-steroid anti-inflammatory drug (indomethacin; IND) or a competitive N-methyl-D-aspartate (NMDA) receptor antagonist (2-amino-5-phosphonopentanoic acid; AP5) was injected intraperitoneally (i.p.), preceding the i.c.v. LPS treatment. IND abolished the i.c.v. LPS induced changes in SWDs, while AP5 extended it for 5 h. As control treatments, both IND and AP5 application by themselves decreased the number of SWDs for 2 and 3 h, respectively. Our results show that centrally injected LPS, likewise the peripheral injection, can increase the number and duration of SWDs in the WAG/Rij rat, and the effect invoke inflammatory cytokines as well as excitatory neurotransmitters.

1. Introduction

Recent studies suggest that there may be a link between certain types of epilepsy syndromes and the immune system [2,7,42,71,72,74]. In several different epilepsy syndromes patients have increased post-ictal serum cytokine levels [64] and increased proinflammatory cytokine levels can enhance the epileptic seizure susceptibility [12,19,60,71]. Proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are synthesized by the glial cells in the brain [50,71,76] and it looks that there is a functional interaction between the cytokines and classical neurotransmitters in the brain [14,71]. In fact, the cortical innate immune response, through IL-1β and TNF-α, increases local neuronal excitability that can lead to epileptic seizures [57,74]. Despite the extensive studies on the interaction of proinflammatory cytokines and epilepsy, the possible role of neuroinflammatory processes in the pathophysiology of absence seizures is poorly investigated.

LPS, the biologically active cell wall component of gram-negative bacteria, binds to Toll-like receptor 4 (TLR4) [49] of the innate immune system and induce proinflammatory cytokine release from the systemic immune cells. We previously revealed that in the genetically epileptic WAG/Rij rat strain [9] the absence-like epileptic seizure activity was facilitated by i.p. LPS administration, in parallel with increased cytokine levels [35]. However, LPS might have a different effect when it applied into the brain directly [37,45,56,63]. Therefore, to examine this question we directly injected LPS into the brain (i.c.v.) and investigated its effect on the absence-like epileptic activity of WAG/Rij rats.

Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, 2-amino-5-phosphonopentanoic acid; COX-2, cyclooxygenase-2; i.c.v., intracerebroventricular; IL-1β, interleukin-1β; IL-1R, interleukin-1 receptor; IL-1ra, endogenous antagonist of interleukin-1 receptor; IL-6, interleukin-6; IL-10R, interleukin-10 receptor; IND, indomethacin; i.p., intraperitoneal; LPS, lipopolysaccharide; NMDA receptor, N-methyl-D-aspartate receptor; NSAIDs, non-steroid anti-inflammatory drugs; PGE_2, prostaglandin E_2; PTC day, post-treatment control day; REM sleep, rapid eye movement sleep; SWD, spike–wave discharge; SWS, slow-wave sleep; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α; WAG/Rij, Wistar Albino Glaxo/Rijswijk.

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Our results revealed, that i.c.v. injected LPS enhanced the SWD frequency in WAG/Rij rats for 3 h. To further investigate the observed effect, the i.c.v. LPS injection was preceded by i.p. administration of IND or AP5. While IND abolished the effect of i.c.v. LPS treatment, AP5 extended it for 5 h.

2. Materials and methods

2.1. Animals

The care and treatment of all animals conformed to Council Directive 86/609/EEC, the Hungarian Act of Animal Care and Experimentation (1998, XXVIII), and local regulations for the care and use of animals in research. All efforts were taken to minimize the animals' pain and suffering and to reduce the number of animals used.

Adult WAG/Rij male rats (8 months old; $n = 46$) weighing 280–340 g were used. Animals were housed under standard laboratory conditions [35]. All experiments were done between 1.30 PM and 8.00 PM.

2.2. Implantation of animals for EEG recording, i.c.v. LPS injection and measurement of body temperature

Rats were implanted under Halothane–air mixture (1%) anaesthesia with 0.8 mm screw electrodes for EEG recording as described earlier [34–36]. Briefly, screw electrodes were placed into the bone above the frontal (A 2.0, L 2.1) and parietal (A −6.5, L 2.1) cortices [51] whereas the ground electrode was implanted above the cerebellar cortex. Reference electrode (stainless steel plate of 3 × 4 mm, one side insulated) was implanted under the skin and over the masseter muscle (the insulated side facing to the masseter muscle). One stainless steel guide cannula (27G) was inserted into the lateral ventricle (A −0.8, L 1.4, V 3.5) in each animals to i.c.v. injection of LPS (Sigma; E. coli, serotype O111:B4) or artificial cerebrospinal fluid (ACSF) by infusion pump (WPI, Germany). We verified the location of the cannula tip in the lateral ventricle by the free outflow of cerebrospinal fluid through the guide cannula and by histology after the experiments. Patency of guide cannula was maintained with a sterile stainless steel dummy stylet which was removed before i.c.v. injection.

To detect the effect of LPS on body temperature changes, all animals belonged to the third animal group ($n = 6$; see Table 1) were implanted with thermo-resistors as described previously [35]. Briefly, thermo-resistors (Pt 100) were implanted onto the surface of the skull of the animals into an aluminum holder that was placed above the frontal bone. We measured the body temperature with a thermometer (SUPERTECH, Hungary) in each 10 min. To measure the effect of IND and AP5 on the body temperature and their effects on LPS induced body temperature changes, we implanted thermo-resistors into two-two animals in group 1, group 2 and groups 4–6 ($n = 2$–2), as it was described above. Rats were allowed to recover after surgery for at least 2 weeks.

2.3. EEG recording, SWD scoring and analysis of sleep–waking ratios

EEG were recorded by an electroencephalograph (NIHON-COHDEN, Japan) attached to a CED 1401 μII a data capture and analysis device using SPIKE 2 software (Cambridge, UK). The bandwidth of the EEG recording was 0.53–150 Hz and it was A/D converted at 500 Hz sampling rate.

After the different treatments (see Section 2.4 and Table 1) the SWDs (Fig. 1) were selected and changes in SWD numbers were measured in all animal groups as it was described previously [35]. Briefly, the main properties of a typical SWD: a train of asymmetric spikes and slow waves starting and ending with sharp spikes, power spectra 7–11 Hz and the average amplitude at least twice as high as the EEG delta activity. The durations of SWDs were also measured in case of the i.c.v. LPS alone treatment (third group). The first 30 min of data after the injections were not included into the analysis because injection evoked stress could influence SWD number during this time [35].

Both the number and duration of SWDs varied individually in the control recordings (SWD number: 8–37/h, SWD duration: 2.8–36.1 s/SWD), therefore, the changes in SWD numbers and duration were expressed in percentage of average control measures (three-day control period) and evaluated by Student's *t*-tests.

Sleep–waking ratios of all animals were evaluated in each recording hour. Recordings were analyzed offline by visual evaluation of the raw EEG. Briefly, we distinguished wakefulness, slow wave sleep (SWS) and rapid eye movement sleep (REM) [23]. Rats were in wakefulness when the EEG contained dominantly beta (30–40 Hz) and theta (6–8 Hz) activity as well as motor artifacts (moving and chewing). We considered the rats as being in SWS when the EEG was synchronized in the delta range (0.5–4 Hz). REM was characterized with continuous theta activity (6–8 Hz) without motor artifacts.

2.4. The i.c.v. LPS application and i.p. pretreatment with ACSF, IND or AP5

Starting two weeks after surgery, the WAG/Rij rats were handled daily to be adapted to the experimental procedures (for example rats were gently restrained by a towel and were connected to the electroencephalograph and 30 min later were gently restrained by a towel again). Animals were assigned into six groups (six animals were in group 3 whereas eight animals in group 1, group 2 and groups 4–6; 6 + 2 with thermo-resistors) and treated as follows (Table 1). The first, third, fifth and sixth group of WAG/Rij rats were injected with 2 ml ACSF i.p. for three days (three-day control period) to establish average control SWD levels. Animals of the second and fourth group were injected with 2 ml 5% (v/v) ethanol solution for three days (three-day control period) because the solvent of IND (Sigma, Germany), to be injected on the fourth day, was ethanol (see below).

The first group of animals, as an i.c.v. treatment control group, on the fourth day, received 2 ml ACSF i.p. injection and 30 min later an ACSF i.c.v. injection (5 μl/rat) into the lateral ventricle through the guide cannula. During i.c.v. injections the animals were gently restrained by a towel, the dummy stylet was removed from the guide cannula and a stainless steel needle – that was connected by polyethylene tube to the infusion pump – was inserted. The injection flow rate was 0.25 μl/min. On the fifth day, post-treatment control experiments were done with i.p. treatments alone (PTC day, see Table 1).

In case of the second group, as an IND control group, we injected 10 mg/kg IND i.p. (in 5%, v/v ethanol in saline) and 30 min later the i.c.v. ACSF (5 μl/rat) (Table 1).

The third group, 30 min after the 2 ml ACSF i.p. injection on the fourth day, received the i.c.v. LPS injection (3 μg/rat in 5 μl ACSF) into the lateral ventricle.

The fourth group of animals, on the fourth day, received 10 mg/kg IND (i.p.) dissolved in 5% (v/v) ethanol in saline and 30 min later they received the i.c.v. LPS injection, the same way as the third group. The fifth group of animals, on the fourth day, received AP5 (Tocris; 40 mg/kg in 2 ml ACSF; i.p.) and 30 min later only ACSF i.c.v. (5 μl/rat). The sixth group, on the fourth day, received AP5 (40 mg/kg in 2 ml ACSF; i.p.) that was followed by the i.c.v. LPS injection (3 μg/rat in 5 μl ACSF), the same way as the third and fourth group. In all animal groups, on the fifth day, a post-treatment control experiment was done (PTC day, see Table 1) to disclose putative long lasting effects.

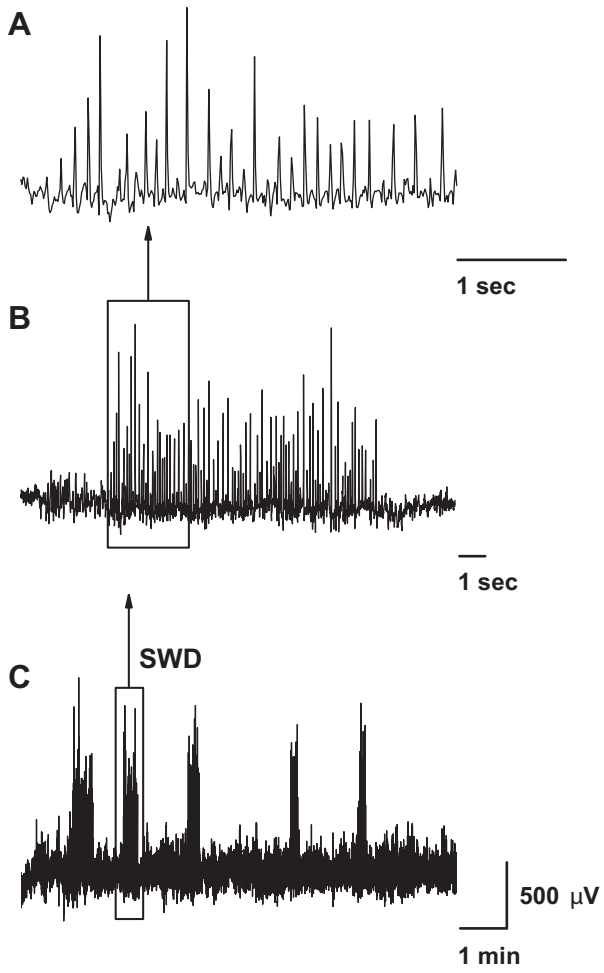

Fig. 1. Representative example of an SWD (A and B) and a typical 10 min compressed EEG (C) recorded from the frontal cortex of a WAG/Rij rat (control recording).