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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.

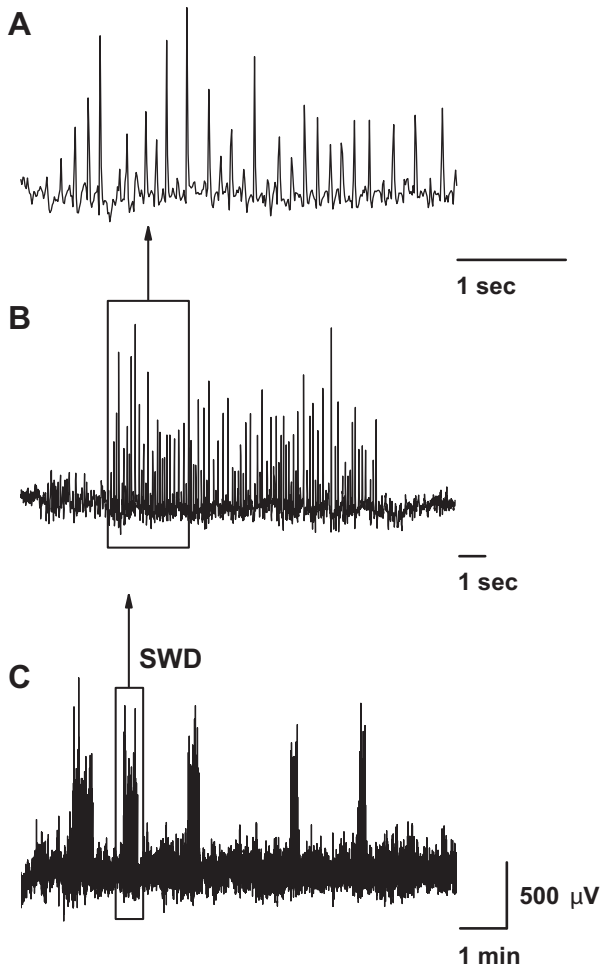

Fig. 1. Representative example of an SWD (A and B) and a typical 10 min compressed EEG (C) recorded from the frontal cortex of a WAG/Rij rat (control recording).

Table 1
The treatment of the six animal groups.

Animal groups	Treatment protocol (according to the days of the treatment)		
	1–3 days	4th day	5th day (PTC day)
Group 1 ($n = 8$)	2 ml ACSF i.p.	1st treatment: 2 ml ACSF i.p. 2nd treatment: 30 min later, ACSF i.c.v.	2 ml ACSF i.p.
Group 2 ($n = 8$)	2 ml 5% (v/v) ethanol solution i.p.	1st treatment: 10 mg/kg IND i.p. 2nd treatment: 30 min later, ACSF i.c.v.	2 ml 5% (v/v) ethanol solution i.p.
Group 3 ($n = 6$)	2 ml ACSF i.p.	1st treatment: 2 ml ACSF i.p. 2nd treatment: 30 min later, LPS i.c.v. (3 μg/rat)	2 ml ACSF i.p.
Group 4 ($n = 8$)	2 ml 5% (v/v) ethanol solution i.p.	1st treatment: 10 mg/kg IND i.p. 2nd treatment: 30 min later, LPS i.c.v. (3 μg/rat)	2 ml 5% (v/v) ethanol solution i.p.
Group 5 ($n = 8$)	2 ml ACSF i.p.	1st treatment: 40 mg/kg AP5 i.p. 2nd treatment: 30 min later, ACSF i.c.v.	2 ml ACSF i.p.
Group 6 ($n = 8$)	2 ml ACSF i.p.	1st treatment: 40 mg/kg AP5 i.p. 2nd treatment: 30 min later, LPS i.c.v. (3 μg/rat)	2 ml ACSF i.p.

3. Results

3.1. Effect of ACSF, IND, AP5 and LPS on body temperature

We did not find body temperature changes after i.c.v. application of ACSF (group 1; data not shown). A rapid elevation of body temperature was observed after i.c.v. LPS injection (group 3; Fig. 2A). It increased continuously for about 150 min and reached 1.2 °C above basal level. It stayed elevated for about an additional 100 min then decreased, but only about 0.3 °C by the end of the measurement (390 min). AP5 alone produced a slight decrease in body temperature between 40 and 90 min after injection (about −0.1 °C) (group 5; Fig. 2A). IND alone (group 2) had no effect on body temperature. Pretreatment with IND (group 4) and AP5 (group 6) abolished the LPS induced increase in body temperature (Fig. 2A).

3.2. Effects of i.c.v. LPS on sleep–waking ratios and SWD number and duration in WAG/Rij rats

We did not find significant changes in the sleep–waking ratios during the six recorded hours after the i.c.v. LPS injection (3 μg/rat; group 3) compared to the control animals (group 1; data not shown). On the other hand, i.c.v. LPS injection (group 3) significantly increased the number of SWDs between 30 and 210 min after injection (Fig. 2B). Thereafter, between 270 and 390 min a significant decrease in SWD number was observed (Fig. 2B). The i.c.v. injection of ACSF into the lateral ventricle did not change the SWD number compared to the three-day control period (group 1; Fig. 2B). On the PTC day, the SWD number returned to the baseline level (data not shown).

The total time of SWD episodes increased in parallel with the change in SWD number between 30 and 210 min after i.c.v. LPS injection in agreement with the result that average duration of SWDs did not changed (group 3; Fig. 2B). On the other hand, both total time and average duration of SWDs decreased significantly between 270 and 390 min post-injection (Fig. 2B).

3.3. Effect of IND alone and on i.c.v. LPS

We tested the effect of IND (10 mg/kg, i.p.) alone (with i.c.v. ACSF; group 2) and its effect on the i.c.v. LPS induced SWD changes (group 4; Fig. 2C). IND alone significantly decreased the number of SWDs between 30 and 150 min after injection (Fig. 2C). The injection of IND 30 min before the i.c.v. LPS injection (3 μg/rat) completely abolished the biphasic effect of i.c.v. LPS on SWD number (Fig. 2C).

3.4. Effect of AP5 alone and on i.c.v. LPS

AP5 (40 mg/kg, i.p.) alone (with i.c.v. ACSF; group 5) decreased the SWD number significantly in the first 3 h of the recordings. The effect of AP5 on the i.c.v. LPS application (group 6; Fig. 2C) was similar to that of the LPS alone treatment, in the first 3 h of the measurement (Fig. 2C). However, in the fourth and fifth hours, the SWD number was significantly higher after the combined AP5 and LPS application than in case of the LPS alone treatment (Fig. 2C). It is interesting to note, that in the last measured hour, the SWD number was significantly lower in both cases compared to the average control SWD level (Fig. 2C).

4. Discussion

The major finding in our study is that i.c.v. injected LPS increased the number of SWDs for 3 h in genetically epileptic WAG/Rij rats. This effect was followed by a decrease in SWD activity. When the i.c.v. LPS injection was preceded by i.p. administration of IND or AP5, the cyclooxygenase-2 (COX-2) inhibitor IND abolished the effect of i.c.v. LPS, while the NMDA receptor antagonist AP5 extended it for 5 h.

The genetically absence epileptic WAG/Rij rat is one of the most investigated models of human absence epilepsy. These rats spontaneously generate absence-like seizures, due to their hypersynchronic thalamocortical and corticothalamic connections, that manifested in SWDs in EEG [8,9]. T-type Ca^{2+} channels, glutamatergic and GABAergic changes may all be involved in the generation of spontaneous SWDs [62,69].

The increase in body temperature, in our experiment after i.c.v. LPS, was similar to those described by others [4,68]. Centrally administered LPS may induce fever via the IL-1β, COX-2/prostaglandin E_2 (PGE_2) system influencing the hypothalamic thermoregulatory center [50]. IL-1β induced fever can be suppressed by the COX-2 inhibitor IND [4,25], however LPS as well as TNF-α can also induce a μ-opioid-receptor-mediated fever [18]. It is noteworthy that μ-opioid-receptors have a role in facilitation of the absence epileptic activity [38] and the opioid receptor antagonist naloxone can reverse the proconvulsant effect of LPS [59].

The body temperature changes after AP5 and IND alone and in combination with LPS were similar to those described previously [15,28,29]. AP5 and IND alone did not cause significant changes in body temperature, while in combination with LPS, they abolished the fever inducing effect of i.c.v. LPS (Fig. 2A), similarly to previous studies [15,29,33]. Since NMDA receptor blocker MK-801 and LY235959 may attenuate the LPS induced fever via inhibition of NMDA receptor-dependent hydroxyl radical/COX-2/PGE2 pathway [27,33], we suggest that AP5 may also use this pathway.

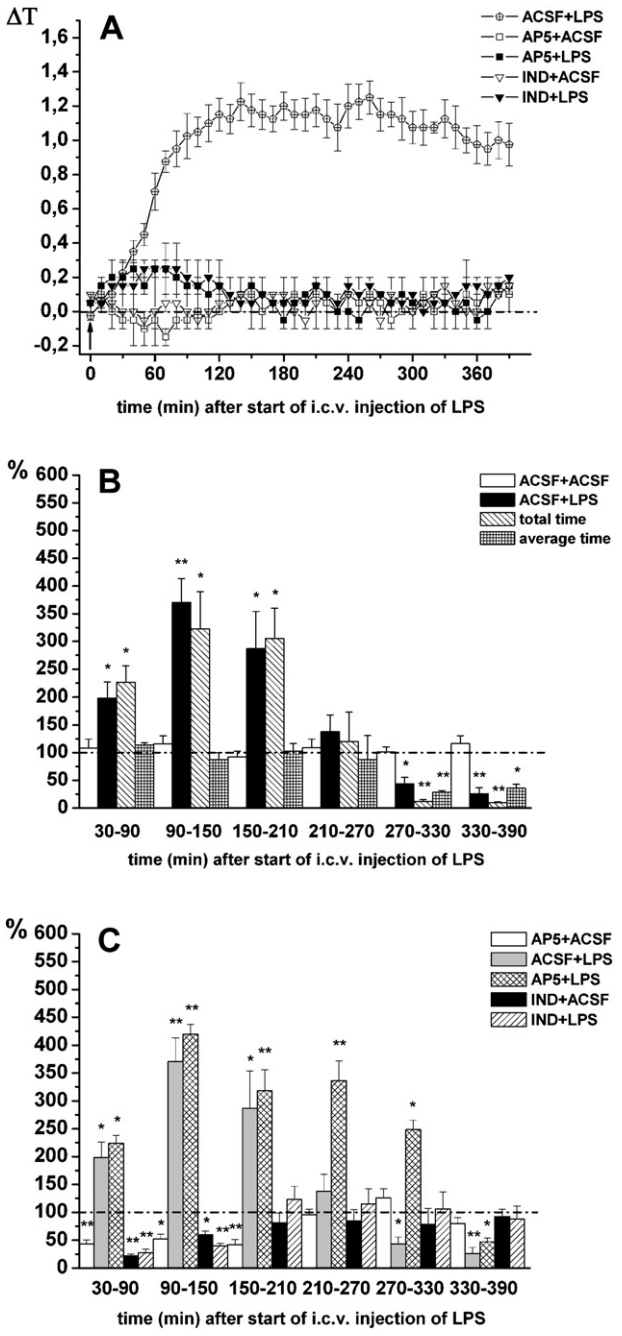

Fig. 2. (A) Body temperature changes after IND (10 mg/kg), LPS (3 μg/rat) and AP5 (40 mg/kg) application alone (group 2, group 3 and group 5) and in combination (group 4 and group 6) (↑ marks the i.c.v. injection). (B) Changes in SWD number after i.p. ACSF + i.c.v. ACSF (open columns, group 1) and i.p. ACSF + i.c.v. LPS injection (black columns; group 3). This part of Fig. 2 also shows the effect of i.c.v. injected LPS (3 μg/rat i.c.v.; group 3) on total SWD time (total time; stripped columns) and average SWD duration (average time; double stripped columns). (C) Effect of AP5 alone (40 mg/kg; group 5: AP5 + ACSF; open columns) and together with LPS (3 μg/rat i.c.v.; group 6: AP5 + LPS; double stripped columns), IND (10 mg/kg i.p.) alone (group 2: IND + ACSF; black columns) and IND (10 mg/kg i.p.) with LPS (3 μg/rat i.c.v.; group 4: IND + LPS; stripped columns) on SWD number in WAG/Rij rats were also showed; for comparison effect of i.c.v. injected LPS alone is plotted again (gray columns, group 3). The changes in SWD numbers and duration were expressed in percentage of average control measures (three-day control period; % on *y*-axis; B and C); * labels $p < 0.05$ and ** labels $p < 0.005$ level of significance.

In our previous paper [35] we demonstrated that LPS increased the absence epileptic activity in the WAG/Rij rat, independently from the i.p. LPS induced body temperature changes: lower doses of LPS caused fever whereas higher dose of LPS induced hypothermia, however, both low and high doses increased the SWD number. Similarly, AP5 and IND by themselves both decreased the SWD number without significant changes in body temperature ([35] and Fig. 2).

In this study, i.c.v. LPS increased the body temperature during the whole 6-h recording period while the SWD number was high only between 30 and 210 min and decreased in the last two measured hours (Fig. 2). Furthermore, AP5 in combination with i.c.v. LPS aggravated the SWD facilitating effect of LPS but abolished its body temperature increasing effect. On the bases of these results, we think that there is no direct connection between the effects of LPS on body temperature and SWD number.

Regarding the way of the LPS application, one can assume that there could be a difference in the dynamics of the responses evoked by i.c.v. or i.p. injected LPS [37,45,56,63], although the direct comparison is difficult, as we do not know which i.p. LPS dose from our previous study fits to the current i.c.v. LPS dose. Our previous [35] and current findings about the i.p. and i.c.v. injected LPS with regard to the SWD enhancing effect show that probably the 350 μg/kg i.p. LPS is the most similar to the current i.c.v. LPS injection, although 270–330 min and 330–390 min were not documented in our previous study. Nonetheless, the i.c.v. injected LPS biphasically influenced the epileptic activity of WAG/Rij rats (Fig. 2B) and the first phase (increase of SWD number) was similar to the 350 μg/kg i.p. LPS [35]. On the PTC day after the i.c.v. LPS application, the SWD number was not decreased, in contrast to those of the i.p. LPS application [35], suggesting that somewhat different mechanisms were activated by the i.c.v. and i.p. LPS [56,63].

The i.c.v. injected LPS can also be regarded as an experimental model of acute brain inflammation which induces direct cerebral responses via the ventricular system and meninges [81,82]. With this model we directly activated the innate immune system in the brain [48] as the molecular complex of glycosylphosphatidylinositol-anchored glycoprotein CD14, accessory protein MD-2 and TLR4 can evoke a complete immune response [49]. LPS activates glial cells via TLR4 [40] and they can release such proinflammatory cytokines as IL-1β, interleukin-6 (IL-6) and TNF-α. Accordingly in previous studies after i.c.v. injected LPS, increased TLR4, IL-1β and TNF-α immunostaining was found in the rat brain meninges, ependymal cells of the ventricle and/or neighboring brain tissue [82] and bioactive IL-1 was detected in the brainstem and diencephalon 2 h after LPS injection [54]. It is known that i.c.v. LPS evokes an increase in mRNA expression of IL-1β, IL-6 and TNF-α within 1–2 h [13,22]. IL-1β and TNF-α may facilitate excitatory, glutamatergic neurotransmission via enhancing NMDA receptor-mediated Ca^{2+} influx into neurons [53,71,77] and attenuate the chloride current [50,78]. TNF-α may increase α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)-dependent excitation and decrease $GABA_A$-mediated inhibition [66,71]. All of these could increase local neuronal excitability and might lead to epileptic seizures [26,57,71,74]. Therefore, via interleukin-1 receptor (IL-1R) expressed on thalamic neurons [83], IL-1β, released by i.c.v. injected LPS, could increase the excitation in the thalamo-cortical circuitry which could promote epileptic seizures in the WAG/Rij rats [65]. IL-1R expression was also demonstrated in the cortex [1,41,80] and LPS increases glutamate release and excitation in the cortex [79].

Interleukin-10 (IL-10) is one of the anti-inflammatory cytokines which can decrease the level of inflammatory cytokines in the brain [39,50]. Level of interleukin-10 receptor (IL-10R) mRNA was measured to be increased within 3.5 h after i.c.v. LPS injection [39]. It was also described that the endogenous antagonist of interleukin-1 receptor (IL-1ra) mRNA level was enhanced after i.c.v. LPS injection [20,32]. IL-1ra, by blocking the IL-1β actions, has been revealed

to be antiepileptic [75]. Thus, in the biphasic effect of i.c.v. LPS, the decrease of SWD number in the later stage might result from the increased IL-1ra and/or IL-10R levels. Another explanation for the decrease of SWD number in the second phase could be that the number of TLR4 could be down-regulated hours after the LPS injection [37].

It is known, that WAG/Rij rats show intracortical hyperexcitability during SWDs because of the imbalanced intracortical inhibitory and excitatory mechanisms [43,46]. IL-1β decreases the seizure threshold and enhances neuronal hyperexcitability via NMDA receptors [71,73]. IL-1β may also increase the glutamate release [45] and attenuate the glutamate uptake of astrocytes [84]. Consequently, LPS evoked IL-1β release may increase absence epileptic activity in WAG/Rij rats via glutamate evoked hyperexcitability which is in agreement with the cortical theories of SWD genesis [46,69].

NMDA receptor antagonists decrease both the number and mean duration of SWDs in WAG/Rij rats [11,16,17,47,52] suggesting the involvement of NMDA receptors in SWD genesis. In agreement with this, we found significantly decreased SWD number between 30 and 210 min after AP5 i.p. injection (Fig. 2C). This result can be explained by the inhibition of initial excitatory postsynaptic potentials (EPSPs) required to trigger SWD genesis [11,65]. As IL-1β and TNF-α could facilitate the glutamatergic neurotransmission via the NMDA receptor [53,71,77] one might have thought that NMDA receptor antagonism by AP5 would ameliorate the SWD enhancing effect of i.c.v. LPS. Surprisingly, AP5 had not blocked the effect of i.c.v. LPS. Moreover, it aggravated its effect in the later stage. This suggests that via the interaction of inflammatory cytokines and glutamatergic and/or GABAergic neurotransmission, a simple antagonism of NMDA receptors by AP5 could extend the absence-like seizures genesis [10,21,35,65].

IND injected alone significantly decreased the SWD number and completely abolished the effect of i.c.v. LPS (Fig. 2C) and peripheral LPS [35] on SWDs, and decreased the neocortical spike-and-wave spindling numbers of DBA/2J mice [5]. IND is a COX-2 inhibitor and COX-2 mRNA and its protein are induced by i.c.v. LPS in blood vessels near the cerebral ventricles and subarachnoidal space [4]. It is known that prostaglandins can be involved in LPS-induced seizure threshold decrease [59] and PGE_2 may act as a proconvulsant mediator which promotes glutamate release [67]. However, IND, beside its COX-2 inhibitor effect, also decreased the T-type Ca^{2+} channel evoked Ca^{2+} current *in vitro* and attenuate absence epileptic activity in WAG/Rij rats [24,55,62]. It is widely accepted that T-type Ca^{2+} channel has a role in the genesis of absence seizure [58]. Mibefradil (a blocker of T-type Ca^{2+} channel evoked Ca^{2+} current) decreased absence epileptic activity in WAG/Rij rats dose dependently [3] and several anti-absence drugs such as ethosuximide exert their effect mainly through the T-type Ca^{2+} channel [24,44,61,62,70].

Inhibition of COX by non-steroid anti-inflammatory drugs (NSAIDs) without direct Ca^{2+} channel effects is not plausible as increased level of arachidonic acid also inhibits the T-type Ca^{2+} channels [55]. Additionally, NSAIDs have several other effects on several neurotransmitters and hormones [10,55], and cross-talk between intracellular pathways can all modulate the T-type Ca^{2+} channel [6,30,31].

Therefore we cannot easily separate the two main effect of IND, the T-type Ca^{2+} channel inhibition and COX/PGE2 inhibition, in our case. We think that IND alone may decrease the absence-like epileptic activity mainly via T-type Ca^{2+} channel inhibition whereas its effect on the LPS injections may also involve its COX inhibitory effect.

In conclusion, we have demonstrated that centrally applied LPS increased the number and duration of SWDs in the WAG/Rij rats similarly to the peripheral injection. This effect was abolished by IND and extended by AP5 suggesting an interaction between centrally secreted inflammatory cytokines and glutamatergic neurotransmission.

Conflict of interest

The authors declare that they have no competing financial interests.

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