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Volumetric response of the adult brain to seizures depends on the developmental stage when systemic inflammation was induced

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ABSTRACT

Inflammation has detrimental influences on the developing brain including triggering the epileptogenesis. On the other hand, seizure episodes may induce inflammatory processes and further increase of brain excitability. The present study focuses on the problem whether transitory systemic inflammation during developmental period may have critical importance to functional and/or structural features of the adult brain. An inflammatory status was induced with lipopolysaccharide (LPS) in 6- or 30-day-old rats. Two-month-old rats which experienced the inflammation and untreated controls received injections of pilocarpine, and the intensity of their seizure behavior was rated during a 6-hour period. Three days thereafter, the animals were perfused; their brains were postfixed and subjected to magnetic resonance imaging (MRI) scans. Then, volumes of the brain and of its main regions were assessed. LPS injections alone performed at different developmental stages led to different changes in the volume of adult brain and also to different susceptibility to seizures induced in adulthood. Moreover, the LPS pretreatments modified different volumetric responses of the brain and of its regions to seizures. The responses showed strong inverse correlations with the intensity of seizures but exclusively in rats treated with LPS on postnatal day 30. It could be concluded that generalized inflammation elicited at developmental stages may have strong age-dependent effects on the adult brain regarding not only its susceptibility to action of a seizuregenic agent but also its volumetric reactivity to seizures.

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1. Introduction

Recently, it has been repeatedly underlined that inflammatory response can fulfill a double role in the central nervous system (CNS) [1]. On the one hand, inflammation can play a protective role inducing endogenous adaptive reactions, and on the other, it can be a direct or indirect cause of nervous tissue dysfunction. The effect of inflammation on nerve cell survival and function depends on the type and amount of cytokines produced, duration of nervous tissue exposure to inflammation, and balance between neurotrophic factors and inflammatory mediators produced by immunocompetent cells [2].

Undoubtedly, inflammation developing in the nervous tissue after various brain injuries contributes to triggering of epileptogenic processes [1]. It is also known that even a single seizure episode caused by excitotoxicity, followed by infiltration of leucocytes from peripheral blood, induces an inflammatory process in the nervous tissue [3]. It is accompanied by the activation of glia cells which produce greater amounts of proinflammatory cytokines [4] and by progressive permeabilization of the blood–brain barrier [5], thus, increasing excitability of nerve cells which raises probability of the next seizure attacks [6].

Unfortunately, it is still not much known about the effect of inflammation developing in the periphery on epileptogenic processes. Results of some experimental studies on different models have suggested that past peripheral inflammation decreased the excitability threshold in seizure attacks elicited later [7–10].

On the other hand, however, there are increasingly more frequent reports suggesting that inflammation induced by lipopolysaccharide (LPS) treatment in a specific time window prior to induction of ischemic stroke causes a transient resistance of the nervous tissue to damage called damage-resistant state [11–14]. This state can be induced by exposing the whole organism or specific tissues to a potentially harmful factor (preconditioning stimulus) but of low intensity, in this way, eliciting tolerance to stronger stimuli [15]. Studies of the last 20 years have indicated that the damage-resistant state can be induced also in different models of epilepsy [16]. In relation to tolerance to seizure attacks, it was shown that neuronal death after status epilepticus (SE) could be reduced in animals in which low intensity seizures were elicited earlier (preconditioning seizures) [17–19]. It is also possible to induce cross-tolerance which involves application of a nonseizure preconditioning stimulus, for instance, a short ischemic episode prior to Kainic acid (KA)-induced seizures [20] or inflammation caused by intraperitoneal (i.p.) injection of LPS 72 h before pilocarpine-induced SE [21].

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Studies on cross-tolerance to epileptic attacks induced by transient generalized inflammation as the preconditioning stimulus demonstrated that LPS administration to rats 72 h before seizure attacks reduced SE-induced neurodegenerative changes in the hippocampal formation [21] but had no effect on intensity of pilocarpine-induced seizures. What is more, our last experiment [22] showed for the first time that a transient short-lasting inflammation after i.p. LPS administration to 30-day-old rats significantly reduced intensity of seizures induced by pilocarpine injection in those rats a month later. Moreover, it appeared that changes in morphological parameters of microglia following seizure could be minimized in animals injected with LPS on 30th or 6th day of their postnatal life [22].

The aim of the present experiment was to verify whether a generalized inflammation elicited at different stages of postnatal development will affect intense developmental processes progressing in the nervous system at that time. Specifically, at the end of the first postnatal week, several processes are still advancing in the developing brain which can be distorted by a transient inflammation: programmed cell death, gliogenesis, neurogenesis (especially hippocampal granular cells), cell migration, formation and elongation of nerve cell processes, and formation of neuronal connections [23,24]. The present study was focused on detection of long-term consequences of the past inflammation. It was demonstrated earlier that it could produce permanent proinflammatory changes in the nervous tissue or could alter susceptibility of animals to seizure attacks induced in adulthood; in addition, it was able to change the extent of neurodegenerative changes progressing in the brain after a seizure episode. Considering reports indicating a possible effect of experimental procedures used in this work on the volume of the brain and its structures [25,26], we estimated the volumes using magnetic resonance imaging (MRI). It is the best tool for noninvasive detection of even very subtle volumetric changes [27], which can accompany both inflammation progress and epileptogenesis [28].

2. Methods

All experimental procedures were approved by the Animal Care and Use Committee of the Jagiellonian University in accordance with the European Communities Council Directive (2010/63/EU).

2.1. Induction of generalized inflammation

Procedures used in the first stage of this experiment were described in our previous publication [22]. A solution of LPS (serotype 026:B6; Sigma L3755) in sterile saline (dose 2 mg/kg b.w.) was used here as a tool to evoke generalized inflammation. This solution was injected intraperitoneally on postnatal day 6 (P06) or 30 (P30). The mortality rate of the first group was approximately 20% while all rats from the second group survived. Blood samples were obtained from the animals before the LPS injection, and 2, 4, 6, and 24 h after the injection. Increases in serum levels of TNFα and IL-6 and their duration were determined using commercial ELISA kits to define an inflammatory response. Results of the analysis have already been shown in our previous study [22]. According to Freiman et al. [29], Salvesen et al. [30], and our pilot tests, a single i.p. injection of saline (0.9% sodium chloride aqueous solution) does not significantly alter blood levels of proinflammatory cytokines. Thus, we have resigned from the use of additional control animals treated with saline alone to minimize their numbers following recommendations of the bioethical committee.

2.2. Seizure induction

Two-month-old rats which experienced inflammation and untreated, control rats received single i.p. injections of pilocarpine (250 mg/kg, Sigma P6503). Scopolamine methyl bromide (1 mg/kg, Sigma S8502) was injected i.p. 30 min prior to pilocarpine to reduce its peripheral effects. Pilocarpine was injected between 9 and 10 a.m. to avoid circadian effects of seizure vulnerability. All the animals survived the procedure.

2.3. Behavioral observations

In the present study, we focused on the acute period of SE [31] lasting no longer than 6 h. During the 6-hour period following the pilocarpine injection, the animals were continuously observed without knowledge of their previous experimental treatment. The pilocarpine-induced changes in the animal behavior were rated on a six-point scale with respect to characteristic symptoms and their intensity. The scale was used in our previous studies [32,33]:

(a) Light symptoms:
0.5—immobility, piloerection, salivation, narrowing of eyes, face and vibrissae twitching, and ear rubbing with forepaws;
1.0—head nodding and chewing movements.

(b) Intermediate symptoms:
1.5—clonic movements of forelimbs, mild whole body convulsions, exophthalmia, and aggressive behavior;
2.0—rearing and running with stronger tonic–clonic motions including hindlimbs, tail hypertension, and lockjaw.

(c) Heavy symptoms:
2.5—rearing and falling and eye congestion;
3.0—loss of postural tone with general body rigidity.

The maximal intensity of seizures was rated in each of the successive 10-minute periods within the whole 6 h of the observation time. The recorded scores were summarized separately for each animal (6 h SUM SE).

2.4. Tissue fixation

Animal numbers in the control and experimental groups are shown in Table 1.

Immediately before and 3 days after seizure induction, the animals were weighted, sacrificed by a lethal dose of pentobarbital, and perfused transcardially with 0.9% NaCl followed by 10% formalin in 0.1 M phosphate buffer (pH 7.4). Then brains were removed from skulls, weighted, and postfixed in 10% formalin.

2.5. Ex vivo MRI and volume measurements

The excised and fixed brains were placed in plastic containers filled with formalin of size meeting the requirements of the MRI device and not distorting the brain shape. Structural MRI scans were performed with a 9.4 T Bruker Biospec 94/20 research MRI system (Institute of Nuclear Physics, Department of Magnetic Resonance Imaging, Polish Academy of Sciences), equipped with 1 T/m gradient coils and 35 mm ID birdcage RF coil. Turbo RARE 3D pulse sequence with the following

Table 1
Numbers of animals in each of examined groups.

Animal group	Number of animals
N	15
L06	9
L30	11
NSE	13
L06 SE	16
L30 SE	13

Abbreviations: N — normal rats, P06, and P30 rats injected with LPS alone on postnatal days 6 or 30, respectively. A symbol SE (status epilepticus) added to each of the symbols indicates animal groups which additionally experienced seizures induced with pilocarpine at the age of 2 months.

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