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Inflammation induced at different developmental stages affects differently the range of microglial reactivity and the course of seizures evoked in the adult rat

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ABSTRACT

Background: In the brain, inflammation occurs following a variety of types of brain damage, including epileptic seizures. Proinflammatory cytokines, like IL-1β or TNFα, can increase neuronal excitability and initiate spontaneous seizures or epileptogenesis. Recent studies indicate that the effects can be attenuated or even abolished in animals subjected to inflammation-inducing treatments at earlier developmental stages, termed "preconditioning". Immunocompetent microglial cells display particular sensitivity to subtle brain pathologies showing a morphological continuum from resting to reactive forms. Following inflammation, multiple ramified processes of resting microglia become gradually shorter, and the cells transform into macrophages. Parameters of the morphological variations were used here as indicators of the nervous tissue reactivity to seizures in adult rats experiencing inflammation at earlier stages of postnatal development.
Methods: Systemic inflammation was induced with lipopolysaccharide (LPS) in 6-day-old or 30-day-old rats. In two-month-old survivors of the inflammatory status, seizures were evoked with pilocarpine injection. The seizure intensity was scored during a six-hour continuous observation period following the injection. Brain sections were immunostained for Iba1 to visualize microglia. Thereafter, morphology of microglial cells located in the hippocampal formation was analyzed using parameters such as solidity, circularity, ramification index, and area.
Results: In naïve rats, seizure-induced transformations of microglial cells were reflected by strong changes in the parameters of their morphology. However, in the adult rats pretreated with LPS on their 6th or 30th postnatal days, the seizure-induced changes were significantly reduced, and microglial morphology remained significantly closer to normal. Significant amelioration of the acute phase of seizures was observed only when inflammation was induced in 30-day-old, but not in 6-day-old, rats.
Conclusions: The results confirm previous reports that moderate inflammation protects the nervous tissue from subsequent damage by reducing influences of proinflammatory factors on reactive glial cells. The young-age inflammation may have age-dependent effects on susceptibility to seizures induced in adulthood.

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1. Introduction

Inflammation in the brain is associated with an increased presence of cytokines and inflammatory mediators, which are normally undetectable or present in very low concentrations [1]. During the inflammatory status, endothelial and blood-derived cells, microglia, and astrocytes are involved in enhanced cytokine production resulting, in turn, in activation of metalloproteinases and catabolism of arachidonic acid [2–4]. Proinflammatory factors such as IL-1β or TNFα can directly increase neuronal excitability and facilitate spontaneous seizure activity [5]. These findings highlight a possible critical importance of inflammation for the development of epilepsy. Studies in transgenic mice with increased expression of inflammatory cytokines like IL-1β, TNFα, or IL-6 showed increases in seizure intensity and neurodegenerative changes. On the other hand, in patients suffering from seizures and in corresponding animal models, increased levels of proinflammatory factors were also observed [1,5]. Thus, inflammation may initiate epileptogenesis and epilepsy, which in turn may evoke inflammation [6,7]. Recent experimental studies point to the fact that, depending on the time period after their administration or the developmental stage, these factors may have beneficial, adverse, or completely neutral effects [8].

One of the best characterized and reliable methods to quickly elicit strong inflammatory response in the CNS is the administration of lipopolysaccharide (LPS), a component of the outer wall of Gram-

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negative bacteria, which mimics systemic infection [1]. A generalized inflammation induced with LPS prior to experimental cerebral hypoxia significantly reduces its negative effects [8] and is evidence in support of the concept of *preconditioning*. Its neuroprotective and dose-dependent effect was observed within the period from 24 h to 14 days following LPS administration (therapeutic window) [9,10]. Depending on the experimental model, the effective dose was from 0.02 to 1.0 mg/kg b.w. [11,12]. The LPS protective action has a mechanism which is not yet understood and seems to occur via proinflammatory cytokines such as TNFα or IL-1β [8,13]. It appears reasonable to hypothesize that LPS could also exert a protective influence on hippocampal neurons affected by seizures in animal models of epilepsy. Nevertheless, in rats treated with LPS 72 h before seizure induction, no amelioration in the course of seizures was observed. However, a decrease of neurodegenerative processes occurred in CA1, CA3, and DG regions of the hippocampal formation [14]. Stenzel-Poore et al. [15] reported that LPS administration following a stroke suppressed the microglia and monocyte activation.

The purpose of this study was to verify whether a previously experienced generalized inflammation would affect susceptibility to seizures. We were, however, not focused on an effective LPS dosage or the therapeutic window but on the long-term effects of LPS-induced inflammation. In this case, it could be assumed that inflammation itself could no longer be a factor directly influencing the seizure reactivity because, according to predefined indicators, it disappeared a long time earlier. This directly influencing factor might rather be permanent postinflammatory changes in the nervous tissue. To monitor these changes, we selected the reactive morphological transformation of microglia as particularly sensitive to subtle pathologies of the nervous system [16].

Another aim of this study was to determine whether the generalized inflammation at various developmental stages would affect the susceptibility to seizures induced during adulthood differently and whether the functional change would be accompanied by respective variations of microglial morphology.

2. Material and methods

All experimental procedures were compliant with the European Communities Council Directive (2010/63/EU) and were approved by the Animal Care and Use Committee of the Jagiellonian University (decision no.122/2011).

Lipopolysaccharide (serotype 026:B6; Sigma L3755) solution was injected intraperitoneally (2 mg/kg b.w.) into male Wistar rats on postnatal days 6 (P06s) or 30 (P30s). Blood samples were obtained from the animals before the LPS injection and 2, 4, 6, and 24 h after the injection. Thereafter, serum levels of TNFα and IL-6 were determined using commercial ELISA kits.

Two-month-old rats which survived the inflammation procedure were injected with pilocarpine (250 mg/kg b.w.) to evoke status epilepticus. During a six-hour period following the injection (acute period of status epilepticus), each animal was continuously observed. The intensity of motor manifestations of seizure activity was scored on a 6-point scale (modified Racine's scale) [17,18].

Three days after the seizure induction, the animals were 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. To visualize microglia, free-floating 30-μm-thick brain sections were immunostained using primary antibody against Iba1 (Wako PDN2194, 1:2000) and a Vectastain ABC kit.

Images of the immunostained sections were taken randomly from the Ammon's horn CA3 sector (CA3) and the dentate gyrus (DG) at 40× magnification (Fig. 1) and at different focusing planes with a digital camera mounted on a microscope (Nikon Microphot SA). Using ImageJ free software, the obtained subsets of images were thresholded and processed by a two-step cleaning algorithm including size-based particle exclusion and manual pruning of overlapping cell profiles (Fig. 2). For each cell profile resulting from the procedure, morphological parameters were calculated such as solidity, circularity, ramification index, and area [19].

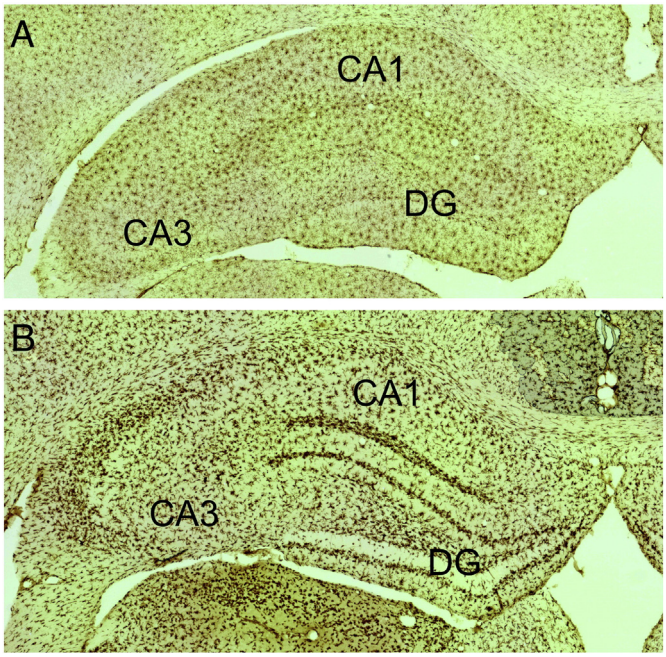

Fig. 1. Microglial cells visualized in the hippocampal formation of 2-month-old normal rats (A) and following pilocarpine-induced seizures (B).

Statistical analysis was performed with the STATISTICA software (Statsoft, Inc.). For normal or nonnormal distribution of data, nonparametric test of Mann–Whitney or one-way ANOVA with the Tukey's post hoc test were performed.

3. Results

3.1. Changes in proinflammatory cytokine concentration

The dynamics of inflammatory response to LPS injections was reflected by changes in the concentration of proinflammatory cytokines — TNFα and IL-6 in blood plasma (Fig. 3). The levels of TNFα peaked 2 h after LPS administration in both age groups, exceeding the control level up to 72-fold in P06s and 33-fold in P30s. Two hours later, the levels remained considerably elevated and came back to normal 6 h after LPS injection.

In P06s, IL-6 plasma concentration peaked 4 h after an LPS injection (22-fold higher than normal) and returned to the normal level during the next 2 h. In P30s, increases in the IL-6 level were approximately 10-fold lower than that in P06s but reached their maximum earlier, i.e., 2 h after LPS injection, remained unchanged for the next 2 h, and then became close to the norm.

3.2. The course of pilocarpine-induced seizures

In the control and two LPS-treated age groups, 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 hour and presented as cumulative graphs (Fig. 4). No statistically significant difference was observed between P06s and the control group. However, in P30s, the sum of maximal seizure scores after each successive hour of the observation period was significantly lower than that in controls and was also significantly lower than that in P06s.

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