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Short communication

Cortical spreading depression decreases Fos expression in rat periaqueductal gray matter

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HIGHLIGHTS

- Cortical spreading depression (CSD) likely underlies migraine aura.
- Periaqueductal gray matter (PAG) controls pain and autonomic functions.
- CSD provocation decreased neuronal Fos in PAG and Edinger–Westphal nucleus of rats.
- The numbers of CSDs correlated negatively with Fos-immunoreactive counts.

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ABSTRACT

The migraine headache involves activation and central sensitization of the trigeminovascular pain pathway. The migraine aura is likely due to cortical spreading depression (CSD), a propagating wave of brief neuronal depolarization followed by prolonged inhibition. The precise link between CSD and headache remains controversial. Our objectives were to study the effect of CSD on neuronal activation in the periaqueductal grey matter (PAG), an area known to control pain and autonomic functions, and to be involved in migraine pathogenesis. Fos-immunoreactive nuclei were counted in rostral PAG and Edinger–Westphal nuclei (PAG–EWn bregma −6.5 mm), and caudal PAG (bregma −8 mm) of 17 adult male Sprague–Dawley rats after KCl-induced CSD under chloral hydrate anesthesia. Being part of a pharmacological study, six animals had received, for the preceding 4 weeks daily, intraperitoneal injections of lamotrigine (15 mg/kg), six others had been treated with saline, while five sham-operated animals served as controls. We found that the number of Fos-immunoreactive nuclei in the PAG decreased after CSD provocation. There was no difference between lamotrigine- and saline-treated animals. The number of CSDs correlated negatively with Fos-immunoreactive counts. CSD-linked inhibition of neuronal activity in the PAG might play a role in central sensitization during migraine attacks and contribute to a better understanding of the link between the aura and the headache.

1. Introduction

In about 20% of subjects, the migraine headache is preceded by neurological aura symptoms [42]. Cortical spreading depression (CSD) is thought to be the underlying mechanism of the migraine aura [19]. The migraine headache involves activation and central sensitization of the trigeminovascular pain pathway. The precise link between CSD and headache remains controversial [18], as does the possibility that CSD-like events may also occur during attacks of migraine without aura [49].

In rat CSD can activate second order trigeminovascular projections of the spinal trigeminal nucleus [23] and provoke behavioral changes comparable to those seen during the migraine attack [2]. Migraine attacks are associated with activation in an upper brain

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stem area that includes periaqueductal grey matter (PAG) [1]. On magnetic resonance imaging (MRI), iron deposition [47], and tissue density [41] were found increased in the PAG of episodic and chronic migraine patients. Lesions in the PAG can provoke migraine-like headaches [17] as can electrodes implanted for neurostimulation therapy of chronic pain [40]. In functional MRI studies the connectivity of the PAG in migraine patients was increased with somatosensory and nociceptive circuits which correlated with disease duration [33]. Specific anti-migraine drugs, like triptans, when injected in the PAG of experimental animals, are able to inhibit trigeminal nociceptor responses induced by dural stimulation [4].

The PAG is a pivotal component of the descending pain inhibitory system but also of emotional behavior and autonomic control [5]. It receives afferents from the cortex [46], where CSD occurs [19], and projects to hypothalamus [46], and raphe nuclei [5] thought to be involved in migraine pathogenesis. Neuronal dysfunction in the PAG may, thus, be involved in various features that accompany the migraine attack [30].

We hypothesized that CSD would be able to modify neuronal activity in the PAG. Fos expression, a validated marker of neuronal activation, was used to estimate activity of PAG neurons after artificial provocation of CSDs over the occipital cortex of anesthetized rats. Since we previously shown that lamotrigine (LTG) is a potent inhibitor of KCl-induced CSD in rat cerebral cortex [6], we included LTG-treated animals in that study to verify if pharmacological suppression of CSDs abolishes the effect of CSDs on PAG.

2. Methods

2.1. Experimental animals

Seventeen adult male Sprague–Dawley rats were randomly chosen among those used in a previously published study on the effect of preventive anti-migraine drugs on CSD [6]. The animals weighed between 300 g and 480 g on the day of recordings. All animals were separated randomly onto three experimental groups during the therapeutic study [6]. Five rats were sham-operated and received no treatment. Two groups of six animals received, for 4 weeks daily, intraperitoneal (IP) injections of saline or lamotrigine (15 mg/kg, a gift by GSK, UK). The number of animals used was kept to a minimum and chosen according to the numbers used in other *c*-Fos studies in PAG [22]. The Ethics Committee of University of Liege approved the study and the guidelines for animal care were followed.

2.2. Anesthesia and surgery

Anesthesia was induced by 400 mg/kg chloral hydrate (8% in saline, 5 ml/kg) and maintained by smaller doses (80 mg/kg) added every hour. Level of anesthesia was monitored by tail pinch and electrocorticogram (ECoG) where applicable. Rectal temperature was maintained between 36.5 and 37.0 °C using a thermostatically controlled heating blanket (ATC 1000®, WPI Inc., USA). The rats were placed in a stereotaxic frame (David Kopf Instruments, USA). Three 1–2 mm wide burr holes were drilled 2 mm off the midline: 7 mm posterior to bregma (P-7; occipital cortex; stimulation site), 4 mm posterior to bregma (P-4; occipito–parietal junction; posterior recording site), and 1 mm anterior to bregma (A + 1; frontal cortex; anterior recording site) [39] to observe the propagation of CSD which was the aim of main study.

2.3. CSD provocation and recordings

The detailed stimulation and recording procedures were described previously [6]. Briefly, after surgery that lasted 30–40 min, in all animals except in the sham-operated group, we induced CSD with a cotton ball soaked with 1 M KCl placed over the occipital cortex (AP-7, R + 2). The cotton ball was kept moist by adding every 20 min a 1.5 μl drop of KCl and left in place for 2 h. For sham-operated animals the cotton ball was soaked with artificial CSF. CSDs and the electrocorticogram (DC-100 Hz) were recorded ipsilaterally from two cortical areas: frontal (bregma AP + 1, R + 2) and occipito-parietal (AP -4, R + 2) at a depth of 1 ± 0.2 mm. Animals were sacrificed immediately after the recordings, i.e., on average 2 h 30 min after induction of the first CSD by an IP nembutal injection (natrium pentobarbital 60 mg/ml:3 ml/kg BW according to the ethical guidelines of the University of Liege).

2.4. Histology

Rats were perfusion-fixed with 500 ml 4% paraformaldehyde in phosphate-buffered saline. After cryoprotection (30% sucrose for two days), 30 μm transverse serial cryostat sections of the brain were cut and serially collected in 12 wells containing cold PBS. Each well received sections at a 360 μm distance throughout the rostro-caudal extent of the brain. Fos expression was assessed using standard immunohistochemistry protocol [8]. Free-floating sections after pretreatment were kept overnight at room temperature in anti-*c*-Fos (Santa Cruz Biotechnology, sc-52-G) primary antiserum at a dilution of 1:1500. The immunocytochemical reaction product was visualized using the Vectastain (Vector Laboratories, Inc., PK-6101) avidin–biotin kit (ABC) with imidazole-intensified 3,3′-diaminobenzidine. Running during each staining procedure a series of sections without the primary antiserum served to control specificity of immunoreactivity.

Fos-immunoreactive (Fos-Ir) nuclei were counted automatically on a digital image analyzer in 2000 × 2000 μm areas of interest manually adjusted. The software package analySIS (Olympus soft imaging solutions GmbH) was used for the automatic counting. Counts were performed on three sections separated by 360 μm at two PAG levels: (1) PAG, bregma −8 mm or (2) PAG + Edinger–Westphal nucleus (EWn), bregma −6,5 mm [39].

As the PAG comprises different functional segments, we separately quantified Fos expression in ventro-lateral, dorso-lateral, and dorso-medial segments of the rostral PAG area and EWn (bregma −6.5 mm). For every image the aqueduct was located; we corrected rotation of sections according to the shape of the aqueduct. The periaqueductal area was then subdivided into six radial segments, as shown in Fig. 1 (top panel). The number of Fos-Ir particles was estimated separately for each of functional segments.

2.5. Statistical analyses

Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analysis. Group values were expressed as means ± 95% confidence intervals. The unit of analysis was a count of Fos-Ir in a single slice for histology and an animal for occurrence of electrophysiologically recorded CSDs during 2 h. Shapiro–Wilk test for normality used for each group separately did not detect violation of normal distribution of the dependent variables; we relied, therefore, on parametric statistics.

We used repeated measures two-way ANOVA to test for the group differences in Fos-Ir expression. Where ANOVA revealed a significant between-group difference, we used post-hoc Scheffe's test for pair-wise comparison of the group means.

Spearman's rank order correlations (*R*) where computed in each CSD group to explore the links between the number of CSDs and of Fos-Ir nuclei in individual animals.