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Rapid Communication

Repetitive trigeminal nociceptive stimulation in rats increases their susceptibility to cortical spreading depression

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ABSTRACT

We examined the ability of trigeminal nerve activation to induce cortical spreading depression in rats. Capsaicin was injected into the bilateral plantar or whisker pad for either 4 or 6 days in rats. The number and duration of cortical spreading depressions induced by potassium were significantly increased in animals injected with capsaicin in the bilateral whisker pad compared with animals injected in the bilateral plantar or in controls, while administration of a $GABA_A$ receptor agonist decreased these effects. Repetitive nociceptive stimulation of the trigeminal nerve lowers the threshold for the induction of cortical spreading depression by altering GABAergic neuronal activity.

The phenomenon of cortical spreading depression (CSD) was initially reported by Leão as a reversible response of the rabbit cerebral cortex that manifested itself as the depolarization of neurons and glial cells followed by a sustained suppression of spontaneous neuronal activity (Leão, 1944). CSD is known to be induced at dissimilar points in the cortex in different animals by electrical, mechanical, and chemical stimulation with potassium. It is also known that CSD spreads from its initiation site through the cortical tissue at a rate of 2–5 mm/min with suppression of the electroencephalogram and a deflection of DC potential (Ayata and Lauritzen, 2015). CSD research in animal models has provided important information regarding its pathophysiological role in many neurological disorders, including migraines (Bolay et al., 2002; Dreier, 2011).

A migraine is a common, episodic neurological disorder that affects 10–15% of the worldwide population, and it is classified by the World Health Organization (WHO) as one of the top 20 most debilitating diseases in the developed world, where it poses a significant personal and economic burden (Bigal and Lipton, 2009). Despite the considerable burden of migraines, the precise mechanism underlying migraine development has not been established. Among various theories that explain the pathophysiology of migraines, CSD is thought to have a fundamental role, particularly in the development of the aura that accompanies a migraine (Hadjikhani et al., 2001; Iwashita et al., 2013; Moskowitz et al., 2004). The typical features of a migraine include a headache of moderate or severe intensity that is characterized by a unilateral location, pulsating quality, aggravation of the migraine by routine physical activity, and association with nausea and/or photophobia. A subjective abnormal visual sensation due to painful stimulation of the trigeminal nerve was reported in a migraine patient (Drummond and Woodhouse, 1993). Therefore, we have considered the possibility that nociceptive stimulation of the trigeminal nerve may be able to induce CSD, and we observed in this study that we could induce CSD in animals by administrating capsaicin to the facial region.

Capsaicin is known to cause nociceptive stimulation and exert an excitatory effect on C-fibers via the transient receptor potential vanilloid subfamily member 1 (TRPV1) receptor, which has already been reported to localize to the sensory nerve (Shimizu et al., 2007). In this study, we have clarified the effect of sensory nerve nociceptive stimulation on CSD induction.

Experiments were performed on male Sprague-Dawley rats ($n = 34$; body weight, 250–270 g; 6–8 weeks old). All experimental procedures were approved by the Animal Welfare Committee of Keio University (No. 08033). All procedures were undertaken with the utmost caution to minimize the suffering of the animals.

After anesthetization with isoflurane (2.0% in room air with a flow rate of 400 mL/minute) via a concentration-controlled

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anesthesia unit (400; Univentor, Zejtun, Malta), 50 μl of 10 mM 8-methyl-N-vanillyl-*trans*-6-nonenamide (capsaicin; Sigma) solution was subcutaneously injected into the bilateral whisker pad or plantar region of each animal. During the experiment, the animals were divided into the following groups: animals treated with vehicle control (saline with 6% ethanol and 7% Tween-80) injected in the bilateral whisker pad for 4 days (control), animals injected with capsaicin in the bilateral whisker pad for 4 days (d4-face), animals injected with capsaicin in the bilateral whisker pad for 6 days (d6-face), animals injected with capsaicin in the bilateral plantar region for 4 days (d4-feet), or animals injected with capsaicin in the bilateral plantar region for 6 days (d6-feet). Furthermore, muscimol (1 mg/kg; Sigma) was administered to the tail vein in the animals injected with capsaicin in the bilateral whisker pad for 4 days (d4-face + m). In addition, bicuculline (Sigma) was administered intravenously at a dose of 0.5 mg/kg to the non-treated animals (bicuculline).

These animals were anesthetized with 2.0% isoflurane at least 12 h after the last capsaicin injection. Body temperature was maintained with a heating pad and thermocontroller (BWT-100; Bioresearch Center Co., Nagoya, Japan). Each animal was fixed to a head-holder (SG-3 N, modified to be flexible around the horizontal axis; Narishige Scientific Instrument Laboratory, Tokyo).

To measure DC potentials, an Ag/AgCl electrode (tip diameter = 200 μm, EEG-5002Ag; Bioresearch Center Co.) was inserted under the pia mater (4.5 mm posterior and 4 mm lateral to bregma) and fixed with dental cement. Ag/AgCl reference electrodes (EER-5004Ag; Bioresearch Center Co.) were placed in the subcutaneous tissue. DC potentials were amplified at 1–100 Hz and digitized at 1 kHz with a differential headstage and differential extracellular amplifier (Unekawa et al., 2012).

CSD was induced by adding a 10 μL drop of KCl solution, at various concentrations, into an additional posterior 2 mm-diameter hole with its center at the coordinates of 8 mm posterior and 4 mm lateral to the bregma. The dura in the hole was carefully removed. Initially, 0.1 M KCl was applied to the hole, and we observed for 10 min whether CSD was induced. If CSD was not induced at that concentration after 10 min of observation, we progressed to a higher concentration of KCl than what was previously used for the experiment. The concentrations of KCl which were used in the study were 0.3, 0.6, and 1.0 M. We measured the duration, which was defined as the interval between KCl application and the last CSD. We also counted the number of CSDs induced during the duration. The data are presented as the mean ± SD and were compared using a Kruskal–Wallis one-way analysis of variance followed by a Mann-Whitney test for multiple comparisons (SPSS for Windows, version 22; SPSS Inc., Chicago, IL, USA). The differences between the means were considered statistically significant at $p < 0.05$.

In the control group, CSD was induced at 1.0 M KCl in seven of eight animals and at 0.6 M KCl in one of eight animals (0.9 ± 0.1 M). The minimum KCl concentration required for inducing CSD was 0.5 ± 0.3 M in d4-feet, 0.3 ± 0.0 M in d6-feet, 0.2 ± 0.1 M in d4-face, and 0.1 ± 0.0 M in the d6-face group. In all animal groups treated with capsaicin, the minimum KCl concentrations required to induce CSD were lower than those required for the control animals. However, a significant difference was only observed in the d4-face and the d6-face groups ($p < 0.05$).

As shown in Table 1 and Fig. 1A, application of 0.1 M KCl did not induce CSD in the control, d4-feet, or d6-feet groups. In the d4-face group, one of four animals exhibited CSD, and in the d6-face group, all four animals experienced CSD. The duration of CSD in each group is described in Table 1.

When 0.3 M KCl was applied, no CSD was induced in the control group. Although all groups treated with capsaicin experienced CSDs, the number and duration of the CSDs were decreased in animals injected in the plantar region compared with animals injected in the face (Table 1 and Fig. 1B). A significant increase in the number and duration of induced CSDs was observed in the d4-face and d6-face groups compared with the controls ($p < 0.05$).

Table 1
The number and duration of CSDs in each group.

	N	The number of CSDs (times)	Duration of CSDs (minutes)
0.1 M KCl			
control	8	0	0
d4-feet	5	0	0
d6-feet	5	0	0
d4-face	4	1.3 ± 2.5	10.3 ± 20.5
d6-face	4	1.2 ± 0.5	5.3 ± 4.3
0.3 M KCl			
control	8	0	0
d4-feet	5	0.6 ± 0.5	0.8 ± 0.8
d6-feet	5	2.4 ± 2.6	16.6 ± 18.1
d4-face	4	5.0 ± 4.8	33.5 ± 31.9
d6-face	4	12.2 ± 2.5	90.2 ± 25.7
0.6 M KCl			
control	8	0.1 ± 0.3	0.3 ± 0.8
d4-feet	5	3.6 ± 3.0	19.0 ± 20.7
d6-feet	5	5.2 ± 2.8	25.0 ± 19.4
d4-face	4	12.5 ± 3.8	104.2 ± 35.2
d6-face	4	15.7 ± 2.2	95.5 ± 36.1
KCl			
control	8	6.6 ± 1.5	37.1 ± 12.4
d4-feet	5	6.4 ± 2.3	39.0 ± 11.6
d6-feet	5	6.4 ± 1.5	38.0 ± 6.8
d4-face	4	12.7 ± 5.4	89.5 ± 30.2
d6-face	4	18.5 ± 5.1	133.5 ± 73.1
d4-face + m	4	2.2 ± 1.8	15.2 ± 16.7
bicuculline	4	16.2 ± 7.2	92.7 ± 23.2

When 0.6 M KCl was applied, only one of eight animals experienced CSD in the control group (Table 1 and Fig. 1C). In both the d4-face and the d6-face groups, the number and duration of induced CSDs were significantly increased compared with the values in the controls ($p < 0.05$).

When 1.0 M KCl was applied, all animals in the control group experienced CSDs (Fig. 1D and Table 1). Representative data from each group for the DC potentials in response to application of 1.0 M potassium are shown in Fig. 2. The number and the duration of the CSDs in the d4- and d6-feet groups were not significantly different than the controls (Fig. 2A–C). In the d6-face group, the number of CSDs induced was significantly lower than that of the d6-feet group, and the duration of the induced CSDs was significantly increased compared with controls ($p < 0.05$).

When we administered muscimol to the facial region for 4 days (d4-face + m) to the animals treated with capsaicin, the number and duration of CSDs were significantly reduced compared with the d4-face group (Fig. 2D and F; $p < 0.05$). Conversely, when we administered bicuculline to the non-treated animals (bicuculline), the number and duration of the CSDs were significantly increased compared with the controls ($p < 0.05$), but was not significantly different compared with the d4-face and d6-face groups (Fig. 2A,D,E and G).

This study demonstrated that repetitive nociceptive stimulation of the trigeminal nerve in adult rats lowers the threshold required for the induction of CSD.

It has been reported that C-fiber activation via capsaicin administration leads to an increase in the excitatory receptive field size in the primary somatosensory cortex in both adult and neonatal rodents (Calford and Tweedale, 1991). Furthermore, depletion of peripheral sensory nerve fibers has been reported to reduce the density and distribution of gamma-aminobutyric acid (GABA) and its receptors, which is known to have inhibitory effects on neuronal transmission in brain regions (Micheva and Beaulieu, 1995a,b; Sheibani et al., 2010). $GABA_A$ receptor agonist has been reported to