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ARTICLE

Spreading depression and evoked potentials recorded in the somatosensory cortex of the rat

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ABSTRACT Cortical spreading depression (CSD) is associated with changes in the caliber of surface blood vessels; others have described it as a phenomenon which arises spontaneously and repetitively following acute cortical injury in animals, including both focal ischemia and trauma, while yet other researchers consider it to be an electrophysiological substrate of migraine aura, which may trigger headache. Our group is involved in research into both migraine and ischemia-induced pathophysiological states. It therefore appeared reasonable to include the study of CSD in the methodological repertoire utilized in our laboratory. We introduced two models of CSD induction: CSD evoked during continuous topical KCl application and CSD induced through a single KCl microinjection into the cortical tissue. This paper describes details of these two methods and of basic parameters of CSDs. **Acta Biol Szeged 54(2):161-164 (2010)**

KEY WORDS

cortical spreading depression,
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Cortical spreading depression (CSD), discovered by Leao in 1944, is a transient negative direct current (DC) potential shift that slowly propagates throughout the cortex irrespective of functional or vascular territories. The main event during the generation and propagation of CSD is the depolarization of a critical mass of brain tissue associated with a massive increase in extracellular K^+ and neurotransmitters, water influx into the cells and shrinkage of the extracellular space volume (Kraig and Nicholson 1978; Gardner-Medwin 1981; Somjen 2001).

Under experimental circumstances, various stimuli or functional states can trigger CSD, e.g. electrical stimulation, cortical trauma, high concentrations of excitatory amino acids or K^+. Different experimental models have been introduced, such as the recording of CSDs evoked during continuous topical KCl application (Ayata et al. 2006; Kudo et al. 2008), or the microinjection of KCl into the brain tissue (Richter et al. 2008, 2010).

In humans, CSD was for a long time mostly associated with the pathogenesis of migraine aura (Lauritzen 1994; Pietrobon and Striessnig 2003). Human imaging data have revealed the occurrence of a CSD-like event during a migraine attack (Woods et al. 1994; Hadjukhani et al. 2001). Recent research, however, demonstrated the occurrence of CSD with prolonged depression periods in the ECoG in patients with brain injury, brain hemorrhage and ischemia (Dohmen et al. 2008; Hartings et al. 2009).

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In *in vitro* studies, it has been shown that oxygen and glucose deprivation, for example, cause an anoxic depolarization similar to CSD (Jarvis et al. 2001). The question arises as to whether ischemia-related CSDs occur in the adult brain, and whether this is a relevant pathological mechanism via which to link hypoxia with a neuronal dysfunction.

During recent years, we have worked on the fields of both migraine and ischemia-induced pathophysiological states and the possibilities of neuroprotection (Robotka et al. 2008; Sas et al. 2008, 2010; Vamos et al. 2010). It seemed reasonable to introduce the study of CSD into the methodological repertoire used in our laboratory for the investigation of the pathomechanism of migraine and brain ischemia, and protection procedures.

We introduced two models of CSD induction: i) CSD evoked during continuous topical KCl application, ii) CSD induction through a KCl microinjection into the cortical tissue. The aim of this study was to adopt these methods and a) to quantify the basic parameters of the CSDs (the mean amplitude and the duration of half the amplitude), b) to determine the average CSD frequencies during continuous topical KCl application, and c) to study the relation between the evoked potentials (EPs) and CSDs.

Materials and methods

Animals

The experimental procedure used in this study adhered to the protocol for animal care approved by the Hungarian Health Committee (1998) and the European Communities Council

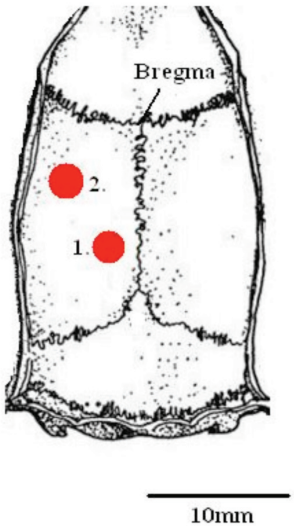

Figure 1. Schematic drawing showing the configuration of the experiment. 1: A hole above the occipital cortrex (-5;2 mm from the bregma) for the administration of KCl. 2: An exposed area in the parietal part of the skull beyond the somatosensory cortex (-3;5 mm fom the bregma) for the recording of the CSDs and SEPs.

Directives (86/609/EEC). Male Wistar rats (230-250 g) were used in the experiments. Every effort was made to minimize the number of animals used and their suffering. The animals were kept under 12-h light and 12-h dark conditions, with lights on at 6 a.m., and were raised with free access to water and food pellets. The room temperature was 22 ± 1°C.

Surgical procedures

All surgical procedures were carried out under deep anesthesia. During the experiments, the animals were anesthetized with an intraperitoneal injection of urethane (1.3 g/kg, i.p.), and their heads were then fixed in a stereotaxic head-holder (David Kopf. Instr.). On the left side, above the occipital cortex, a hole (diameter 1-2 mm) was made with a mini-drill. On the parietal part of the skull (just above the primary somatosensory area), the cortex was also exposed via another hole (diameter also 1-2 mm), with the same mini-drill (Fig. 1). The exposures were made under saline cooling. The dura mater and the underlying archnoidea were removed. After surgical preparation, the cortex was allowed to recover for 30 min under saline irrigation.

Recording of DC and evoked potentials

Ag/AgCl reference electrodes were fixed onto the skin of the neck. Intracortical DC potentials were recorded through use of a glass micropipette filled with 150 mM NaCl. This microelectrode was inserted into the primary somatosensory cortex to a depth of 1000-1200 µm corresponding to cortical layer V. In parallel with the DC potentials, EPs induced by whisker pad stimulation (4-6 V, 0.2 ms, 0.1 Hz), were recorded from the cortical surface with a ball-tipped silver electrode. The details relating to the stimulation and cortical recordings of EPs were published earlier (Farkas et al. 1999; Nagy et al. 2010). The distance between the intracortical (DC) and cortical surface electrodes was less than 1 mm. To elicit CSD, either continuous KCl application was carried out or 1 µl of KCl (3 M) was injected with a microinjector (CMA/100) into the cortex. For continuous KCl application, a cotton ball (2 mm in diameter) soaked with 3 M KCl was placed on the pial surface and kept moist by the addition of 5 µl of KCl solution every 15 min. The number of KCl-induced CSDs was counted for 2 h.

Data acquisition and evaluation

The amplified responses were fed into a computer via an interface. All data were recorded on a PC by using custom signal acquisition programs (Neurosys, Experimetria). As concerns the EPs, each point in Figure 2B represents a single somatosensory EP (SEP).

Results

In some experiments (6 rats), CSD was induced by a single microinjection of KCl (3 M, 1 µl) into the frontal pole of the occipital cortex (Fig. 1). This single injection induced 1-3 CSD waves, which were recorded in the primary somatosensory cortex, 3.6 mm apart from the site of the injection. The parameters of the CSD waves: the onset latency of the first wave: 66 ±12 s (n=6); maximum amplitude: 20±8 mV (n=13); and duration at half-maximum amplitude: 39±15 s (n=13) (Fig. 2A).

In parallel with the CSD, SEPs were recorded from the pial surface of the primary somatosensory cortex. Whisker pad stimulation evoked cortical responses with about 450-500 µV. The amplitudes of the evoked responses were stable for a long period, except during the CSD waves. When a CSD wave arrived, the amplitude of the SEP gradually decreased and in parallel the amplitude of the CSD wave increased. When a CSD wave was at its maximum, the amplitude of the SEP was close to zero µV, and it virtually disappeared. As the CSD amplitude decreased, the SEP amplitude increased in parallel. Between two CSD waves, the SEPs were fully recovered (Fig. 2A, B).

In another series of experiments, the continuous topical application of 3 M KCl evoked repetitive CSD in all the animals studied (n=3). Under the given experimental circumstances, the average CSD frequency was 36±3 CSD waves/2 h (Fig. 3).

Discussion

Although CSD was discovered more than 60 years ago (Leao 1944), our knowledge of its mechanism, and particularly its

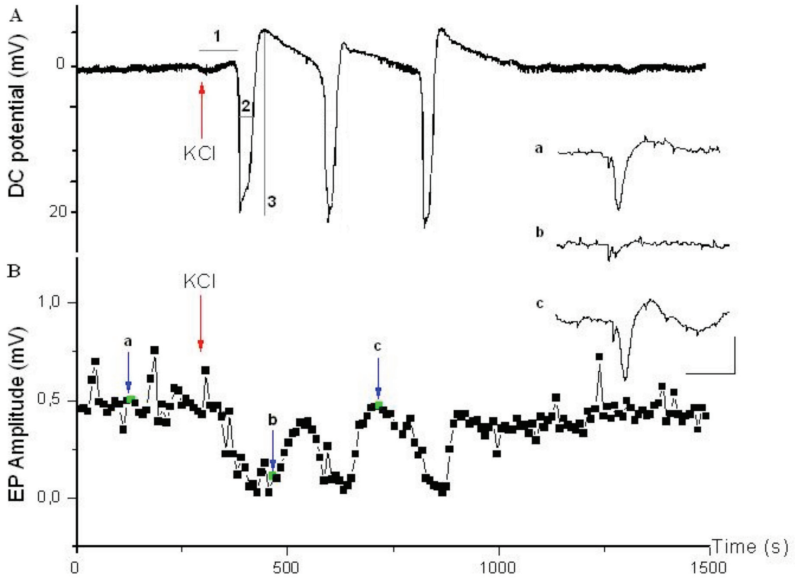

Figure 2. Cortical spreading depression (CSD) waves and somatosensory evoked potentials (SEPs) were recorded in parallel in the primary somatosensory cortex. A: CSD waves were induced with a single injection of KCl. The three characteristic parameters of the presented CSDs: (1): the onset latency of the first CSD wave: 76 s, (2): the duration at half maximum amplitude of the CSD: 33 s, (3): the maximum amplitude of the CSDs: 25 mV. B: Evoked potentials were recorded in parallel with the CSD waves. Each square in this graph represents single potentials. a, b, c: SEPs before, during and between CSD waves, respectively. Calibration in insert: 50 ms, 250 μV.

relation to the different pathological conditions such as migraine and ischemia, is rather limited. It is generally accepted that a migraine aura reflects the migration of CSD over the cortex. It has also been suggested that CSD may participate in the generation of head pain in migraine (Moskowitz et al. 1993).

A short-lasting interruption of the cerebral blood supply (up to 1 min) is known to cause a cessation of EEG activity, but this is usually not accompanied by a CSD wave. A prolonged interruption of the blood supply, however, causes a long-lasting negative DC shift in the cerebral cortex, which undergoes recovery only after release of the arterial occlusion. This phenomenon has been demonstrated both in the cortex (Leao 1947) and in the brainstem (Richter et al. 2010).

The precise molecular mechanisms of CSD are not known, but two pathological states, migraine aura and an ischemic condition of the brain, seem to be related to this phenomenon. Brain ischemia induces glutamate release from neurons and astrocytes (Rossi et al. 2000). In CSD, glutamate is thought to depolarize remote neurons and thereby cause propagation of the depolarizing wave (Somjen 2001). However, glutamate receptor blockers do not inhibit the onset and propagation of anoxic depolarization (Jarvis et al. 2001). At the same time, it has been suggested that the brainstem CSD induced by KCl administration can be explained by glutamatergic mechanisms (Richter et al. 2008).

These literature findings clearly demonstrate that CSD is somehow involved in such pathological conditions of the CNS as anoxic ischemia and migraine. During recent years, our research activity has focused on the pathomechanisms of migraine and ischemia-induced processes in the CNS, and mainly on the possibilities of neuroprotection in these conditions (Knyihár-Csillik et al. 2007, 2008; Robotka et al. 2008; Sas et al. 2008; Vámos et al. 2009, 2010). Although the symptoms, the progression and the outcome of stroke and migraine differ, there are some common pathophysiological mechanisms relating mainly to mitochondrial malfunctions (Sas et al. 2010).

Our recent studies on migraine and on ischemia-induced changes in the CNS increasingly require methodology involving excess glutamate, mass depolarization and glutamate

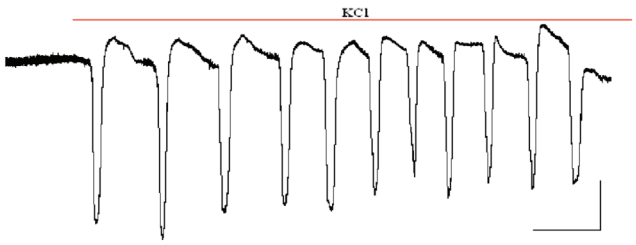

Figure 3. Representative 45 min DC potential recording from the primary somatosensory cortex during 2 h topical KCl application to the frontal part of the occipital cortex. Calibration scales indicate 5 min and 5 mV.

excitotoxiticy. CSD measurement appears to be the most appropriate such method.

In the course of these experiments, we introduced a method of recording CSD and characterized the main parameters of CSD waves (frequency, amplitude and duration). An extra feature of our approach is that the CSD waves are recorded in parallel with the evoked potentials.

In the near future, this combined method will be applied in our research on the pathomechanisms of migraine and glutamate excitotoxicity, and in the development of new neuroprotective kynurenic acid analogs.

Acknowledgement

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