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Increased calcium influx triggers and accelerates cortical spreading depression in vivo in male adult rats

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HIGHLIGHTS

- We topically applied ionophore A23187 in rat cortex to increase calcium influx.
- This ionophore (10–100 μM) dose-dependently accelerates spreading depression (CSD).
- Topical application of a much higher dose of this compound (2 mM) triggers CSD.
- Increased Ca^{2+} influx is suggested as a key element in the CSD induction mechanism.
- Data stimulate and justify further experiments on brain disorders related to CSD.

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ABSTRACT

Cortical spreading depression (CSD) is a depolarization wave associated with neurological disorders such as migraine, cerebral ischemia and traumatic brain injury. The mechanism of action of this phenomenon still remains unclear. Although it is suggested that extracellular K^+ accumulation contributes to CSD, other ions may play a relevant role in the mechanism of propagation of the wave. In this context, we hypothesize that Ca^{2+} may play an important function in the wave propagation. Our results demonstrate that enhancing Ca^{2+} influx into the cells by topical cortical application of the ionophore A23187 (10 μM, 50 μM and 100 μM solutions) increases the velocity of CSD propagation in a dose-dependent manner, and a much higher dose of this compound (2 mM) triggers CSD. In conclusion, increased Ca^{2+} influx can be a key element in the induction mechanism of the CSD, and should be assessed in further experimental strategies targeting brain disorders related to CSD.

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1. Introduction

Cortical spreading depression (CSD) is a reversible depolarization wave associated with failure of brain ion homeostasis, release of excitatory amino acids, increased energy metabolism and changes in cerebral blood flow that propagates across the brain gray matter at low velocity, usually in the range of 2–5 mm/min [1,2]. In normal brain CSD usually has to be induced by a perturbation of the brain homeostasis such as electrical, mechanical or chemical stimulation [1]. In neurological disorders such as migraine, cerebral ischemia and traumatic brain injury, CSD may play a key role as a pathophysiological mechanism in brain damage [2]. Clinical relevance of CSD in other neurological disorder and mechanisms of action in the trigger of this phenomenon still remains unclear [3].

Is has been suggested that extracellular K^+ accumulation may initiate CSD [3], making topical KCl application a useful model to trigger this phenomenon on in vivo models [4] and is the most reliable method in the context of reproducible results [5,6]. Although a change in K^+ homeostasis is important, other ions may be relevantly involved in the mechanism of propagation of the CSD wave. It is proposed that Ca^{2+} may play an important function in the wave propagation, since reversible changes in Ca^{2+} homeostasis are also observed in CSD [7]. Moreover, it is thought that release of K^+ into the extracellular space might be mediated by Ca^{2+} influx to the cell [3], though it is unclear whether Ca^{2+} is critical for initiation or propagation of CSD.

Based on these observations, it is possible that Ca^{2+} may play a role in the pathophysiology of CSD. In this context, a commonly used molecule to increase the intracellular concentration of Ca^{2+} is

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the ionophore A23187, which allows the influx of divalent ions into the cells, specifically Ca^{2+} [8,9]. Owing that Ca^{2+} may be playing a role in CSD physiopathology, in the present study we used this compound to stimulate extracellular Ca^{2+} influx and determine its effect on CSD propagation.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing 280–350 g were used in this study. The animals were housed in a room with controlled temperature (23 ± 2 °C) and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). The animals were handled in accordance with the standards of the Ethics Committee for Animal Research, of the Universidade Federal de Pernambuco, Brazil, which comply with the "Principles of Laboratory Animal Care" (NIH; Bethesda, USA).

2.2. Cortical topical ionophore A23187 treatment

Ionophore A23187 (Sigma, St. Louis, USA) was diluted in DMSO and solutions at (μM) 10, 50 and 100 were topically applied in three groups of male adult rats (n = 5, n = 6 and n = 5, respectively). This topical application was performed over the intact dura-mater, on two circular portions (3–4 mm diameter) of the cortical surface, where the recording electrodes were placed. After 1–2 h of baseline CSD recording, ionophore was applied during the last 15 min of the 30 min interval between two consecutive CSD-eliciting KCl-stimulations. At the end of the 15 min topical application, the treated region was dried out with a piece of cotton immediately before the next CSD episode was elicited. CSD propagation velocities recorded after topical application of ionophore were compared to mean values obtained in the same animals during the baseline period. As a rule, topical ionophore application was repeated three times (see Fig. 1A). After the third ionophore application, the treated cortical region was abundantly washed out with Ringer solution and the CSD recording continued during 1–2 h (recovery period). To determine the effect of ionophore at higher concentrations, 5 animals that recovered from 10 and 50 μM ionophore were topically treated with a 2 mM solution.

2.3. CSD recording

For the CSD recording, the rats were anesthetized i.p. with a mixture of 1000 mg/kg urethane plus 40 mg/kg chloralose, and three trephine holes were drilled on the right side of the skull. These holes were aligned in the frontal-occipital direction and were parallel to the midline. CSD was elicited at 30 min intervals by 1-min topical application of 2% KCl solution (approximately 270 mM) to the anterior hole (2 mm in diameter) drilled at the frontal region. The two other holes (3–4 mm in diameter) on the parieto-occipital region served as recording places. The direct-current (DC) slow potential change accompanying CSD were continuously recorded for about 7.5 h, using two Ag–AgCl agar-Ringer electrodes (one in each hole) against a common reference electrode of the same type placed on the nasal bones (see diagram in the lower part of Fig. 1B). The amplitude and duration of the negative DC potential change typical of CSD, as well as its propagation velocity, were calculated. The CSD velocity of propagation was calculated from the time required for a CSD wave to pass the distance between the two cortical recording points. During the recording period, rectal temperature was maintained at 37 ± 1 °C by means of a heating blanket. At the end of the session, the animal was killed with an overdose of anesthetic.

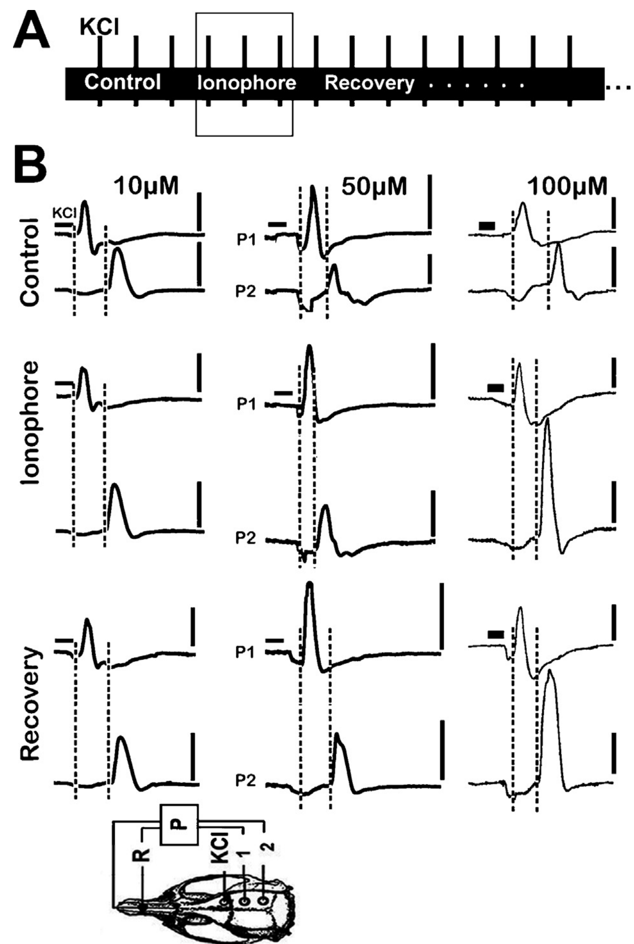

Fig. 1. (A) diagram of the protocol for ionophore topical cortical application during the CSD recording session. After a control (baseline) recording period, ionophore was applied three times (rectangle), and this was followed by a recovery period. The equidistant vertical dark lines indicate KCl stimulation at 30-min intervals, necessary to elicit CSD. (B) Slow potential change (P) recordings in adult rats (80–120 days old), showing the effect of topical applications of ionophore (10, 50 and 100 μM) on the latency for a CSD to cross the distance between the recording points 1 and 2 shown in the skull diagram. This diagram also shows the common reference electrode (R) on the nasal bones, and the stimulation point where KCl was applied for 1 min (horizontal black bars at the beginning of P1 traces). The vertical bars correspond to 10 mV (negative upward). The recovery from the topical molecule effect was reached 30–60 min after ionophore removal. Note the amplitude increase, in the 100 μM ionophore application (right column), on the recording point 2 (where ionophore was applied), but not on the point 1 (used as control).

2.4. Statistics

CSD amplitudes and durations before and after topical application of the ionophore were compared with the paired t-test. CSD velocities were compared using ANOVA followed by post hoc (Tukey) test. Differences were considered significant when $p < 0.05$

3. Results

3.1. Ionophore decreases latency and increases CSD velocity in male rats

To assess the effect of the Ca^{+2} influx on the CSD parameters, we applied the ionophore topically at different concentrations (10, 50 and 100 μM) during 15 min over the intact dura-mater at one of the trephine holes used for the electrophysiological recordings. Our results showed that 10 μM and 50 μM ionophore did not affect the amplitude and duration of the CSD, whereas 100 μM ionophore

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