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CORTICAL SPREADING DEPRESSION MODULATES THE CAUDATE NUCLEUS ACTIVITY

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Abstract—Cortical spreading depression (CSD) plays an important role in migraine with aura. The caudate nucleus has crucial functional interactions with brain regions likely to be important in migraine. The aim of the present *in vitro* study was to investigate the effect of CSD on the neuronal activity of the caudate. Intracellular recording was performed in the head of the caudate nucleus alongside of extracellular recording in Wistar rat somatosensory cortex. CSD was induced by local KCl injection. Changes in the membrane potentials of the caudate neurons began 1.2 ± 0.2 min after CSD. The neurons of the caudate nucleus depolarized first gradually and slightly then it depolarized abruptly at nearly the same point of time of the recovery of the cortical DC potential. Action potentials (APs) reappeared after the cortical DC shift returned to the baseline. Forty-five minutes after CSD, the caudate neurons showed lower frequency of APs and larger amplitude of depolarization prior to APs. The firing pattern of the caudate neurons evoked by injection of intracellular current pulses changed from slow adapting to fast adapting after CSD. Reduced neuronal activity in the caudate after CSD may be assumed to contribute to pain as well as changes in cognition and behavior in patients with migraine.

Key words: spreading depolarization, striatum, aura, dopamine, inhibition.

INTRODUCTION

Cortical spreading depression (CSD) is a pronounced depolarization of neurons and glia that spreads slowly across the brain tissue followed by dramatic changes in the distribution of micromilieu ions between intracellular and extracellular compartments (Leao, 1944; Somjen, 2001). There is unequivocal evidence that CSD occurs in the brain of patients suffering from migraine with aura, ischemic as well as hemorrhagic brain diseases and traumatic brain injury (Lauritzen et al., 2011; Dreier et al., 2012). It has been shown that CSD trigger epileptiform burst discharges in the human brain (Dreier et al., 2012; Eickhoff et al., 2014). The causative role of CSD in the generation of aura symptoms of migraine attacks is well established (Goadsby, 2007). An area of controversy surrounds whether CSD triggers the rest of the attack, including pain and postdrome. There is some evidence to suggest that CSD is also implicated in migraine pain (Gorji et al., 2004; Lambert et al., 2011; Bhaskar et al., 2013) as well as other premonitory and postdrome symptoms (Gorji, 2001). The potential interrelation of CSD and migraine has usually involved neocortical tissues and the possible roles in other brain regions, including the caudate nucleus were not fully investigated.

The caudate, originally thought to primarily be involved with the control of voluntary movement, is now known to be also an important part of cognitive and behavioral functions. The caudate nucleus has crucial functional interactions with brain regions likely to be important to migraine, such as areas associated with vision, the auditory and neighboring associative cortex, the thalamus and the amygdala (Oleshko and Maisky, 1993). The caudate nucleus plays a central role in cognitive processes, particularly executive functions, emotion and behavior (Grahn et al., 2008). Furthermore, the caudate nucleus seems to play an important role in both the sensory processing and suppression of pain (Wunderlich et al., 2011). The caudate nucleus stimulation modulated both sensory and motor components of the trigeminal system (Harper et al., 1979).

Characteristic features of spreading depression (SD) in the caudate nucleus were first described by Leao and Martines-Ferreira (Bures et al., 1974). Classical animal studies investigated the pattern of CSD propagation in the caudate and its neighboring structures. It has been shown that CSD propagates mostly to the caudate. Similarly, the wave of caudate SD penetrates to the

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, after-hyperpolarization; APs, action potentials; CSD, cortical spreading depression; DAPs, depolarization prior to APs; RMP, resting membrane potentials; SD, spreading depression.

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lateral nucleus of the amygdala and from there to the neocortex (Fifkova, 1964; Fifkova and Syka, 1964; Vinogradova et al., 1991, 2005). Because altered neural circuit function can be seen remote from the CSD initiation and propagation sites (Obrenovitch, 1999; Wernsmann et al., 2006; Dehbandi et al., 2008), using *in vitro* brain models, the effects of CSD on the neuronal activity of the caudate tissues were tested.

EXPERIMENTAL PROCEDURES

Slice preparation

Twenty adult Wistar rats (250–300 g) were decapitated under deep isofluran anesthesia and the brains were quickly transferred to ice-cold (4 °C) artificial cerebrospinal fluid (ACSF). The cerebellum was removed and a cut was made to divide the two cerebral hemispheres. Combined striatum–hippocampus–cortex slices containing the temporal cortex, the entorhinal cortex, the subiculum, the hippocampus as well as the striatum (500 μm) were cut in a nearly horizontal plane. The slices that were preincubated in ACSF contained (in mmol/l): NaCl 124, KCl 4, $CaCl_2$ 1.0, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26, and glucose 10 at 28 °C for 60 min. The ACSF was continuously equilibrated with 5% CO_2 in O_2, stabilizing pH at 7.35–7.4. After 30 min preincubation, $CaCl_2$ was elevated to 2.0 mmol/l. The slices were transferred to an interface recording chamber and perfused with ACSF at 32 °C. To assure oxygen supply, a warmed and humidified gas mixture of 95% O_2 and 5% CO_2 was conducted over the surface of the slices. During the experiments, the pH, the bath temperature, and the flow rate (1.5–2 ml/min; Schurr et al., 1985) remained constant.

Electrophysiological recordings

Intracellular recordings were performed in the head of the caudate nucleus using sharp micropipettes filled with 2 mol/l potassium methylsulfate (pH 7.4). The micropipette tips were merged for a long-time in ACSF. Two hundred ms square positive and negative current pulses were injected into the neurons, intending to determine the neuronal input resistance and discharge patterns. A constant positive or negative current was injected to the cells and the membrane potentials were elevated to −40 mV and decreased to −80 mV, respectively. The reference electrode and the connection to the microelectrode were symmetric Ag–Ag–KCl bridges. The microelectrodes were selected to have a resistance in the range between 80 and 160 MΩ. Simultaneous extracellular field potentials along with intracellular recordings were recorded with glass microelectrode (150 mmol/l NaCl; 2–10 MΩ) in the fifth layer of the somatosensory neocortex and I the head of caudate. The distance between intra- and extracellular recording microelectrodes in the caudate was approximately 0.5 mm and the distance between extracellular recording electrodes in the neocortex and caudate was approximately 5–6 mm. Neuron impalement was performed with a conventional high-performance, motor-driven microdrive. The potential of the intracellular electrode was referred to an extracellular micropipette electrode to ensure control of the true membrane potential during large shifts of extracellular potential. Extracellular recordings were obtained using a custom-made differential amplifier (with band-pass filters at 0.5–30 kHz, sampling rate 10 kHz, and 0.3–100 Hz) and the membrane potential fluctuations were obtained using a home-made active bridge mode amplifier (Jafarian et al., 2010; Gorji et al., 2011; filter 10 kHz). Traces were digitized by a Digidata 1200 (Axon Instruments, CA, USA) and the data were collected and analyzed by Axoscope 10 (Axon Instruments, CA, USA).

For each minute of experiments 20 action potentials (APs) were randomly selected for measurements. The amplitude of the APs was measured peak to nadir of the after-hyperpolarization (AHP) and the duration was measured by half-amplitude width duration. Intracellular recording data acceptable for inclusion in the study met the following criteria: recording stability without any sign of injury discharges, membrane potential more negative than −50 mV with a deviation of less than 5% during the first 15 min of recording.

Induction of CSD

A glass electrode filled with 3 M KCl or ACSF (control) was fixed in a special holder connected with plastic tube to a pressure injector and the tip inserted into the somatosensory neocortical slices (layer I–II). A high-pressure pulse was applied to inject in the tissue an amount of K^+ sufficient to induce CSD (tip diameter, 2 μm; injection pressure, 0.5–1.0 bar applied for 200–300 ms, two separate injections, 1–3 nl per pulse, 2–5 mm apart from nearby recording electrodes). The same amount of ACSF was applied in the slices in control experiments to exclude that the fluid injection per se may trigger CSD. CSD were evaluated with respect to their amplitudes, duration and velocity rates. Duration of DC potential fluctuation width was measured at its half-maximal amplitude. In each slice, only one SD was induced.

Experimental protocol

Intracellular recording was performed in the head of the caudate nucleus alongside of extracellular recording in the somatosensory neocortex (layer V). CSD was induced after at least 15 min of continuous intracellular recordings of the membrane potential in the caudate. Intracellular recordings were continued for at least 60 min after CSD initiation.

All data are given as mean ± SEM. The data were statistically processed and compared using Mann–Whitney Rank Sum test. Significance was established when the probability values were less than 0.05. The experiments were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the local ethics committee (Bezirksregierung Münster, AZ: 50.0835.1.0, G79/2002).