Experimental Neurology 224 (2010) 146–154

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

The relationship between sudden severe hypoxia and ischemia-associated spreading depolarization in adult rat brainstem in vivo ☆

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ARTICLE INFO

Article history:
Received 22 December 2009
Revised 19 February 2010
Accepted 2 March 2010
Available online 10 March 2010

Keywords:
Spreading depression
Hypoxia
Ischemia
DC potential
Laser Doppler flowmetry,
systemic blood pressure

ABSTRACT

Severe ischemia can induce spreading depolarization (SD) in the cerebral cortex, which is thought to contribute significantly to cerebral dysfunction. Whether the mature brainstem shows SD upon reduced oxygen supply has not been investigated although SDs may significantly influence brainstem functions. In anesthetized adult rats, we induced severe short-lasting hypoxia (SSH) by stopping artificial respiration for about 1 min or by ventilation with pure nitrogen for 1, 2 or 3 min, and milder hypoxia by ventilation with 6% O_2 in N_2 for 10 min. We measured DC potentials in the brainstem and cerebral cortex, systemic arterial blood pressure, heart rate and local blood flow at the brainstem or cerebral cortex surface. SSH lasting up to 1 min did not induce DC shifts in native brainstem but reduced heart rate, systemic blood pressure and blood flow in cortex and brainstem. Longer lasting SSH protocols both reduced systemic blood pressure and induced SD in the brainstem, but the magnitude of the cardiovascular response was not influenced by the simultaneous occurrence of SD. When neuronal excitability in the brainstem was artificially enhanced, SSH of 1 min evoked SD but again the magnitude of cardiovascular changes during SSH was not increased. SSH lasting 3 min evoked non-reversible sustained depolarization. SSH did not render the brainstem more excitable for classical SD evoked by local KCl application. Thus, sudden severe hypoxia/ischemia evokes SDs in the brainstem, but the occurrence of the so-elicited SD does not influence the immediate cardiovascular response to SSH.

Introduction

Spreading depression (SD) in cerebral cortex has been identified as a propagating transient negative direct current (DC) potential shift accompanied by transient disturbances of the ion homeostasis, water influx into cells and shrinkage of the extracellular space volume (Kraig and Nicholson, 1978; Gardner-Medwin, 1981; Nicholson, 1993; Somjen, 2001). This "spreading depression of electroencephalographic activity" was first described in the cerebral cortex of the rabbit by Leão (1944, 1947). In man, cortical SD phenomena were for a long time mostly associated with the pathogenesis of migraine aura (cf. Lauritzen, 1994; Pietrobon and Striessnig, 2003). However, in the last years, research of the Co-Operative Study on Brain Injury Depolarisations (COSBID) group revealed the occurrence of SDs with prolonged depression periods of the electrocorticogram in patients with acute brain injury, brain hemorrhage and ischemia (Dreier et al., 2006; Dohmen et al., 2008; Hartings et al., 2009).

While SD occurs in the cerebral cortex under different experimental and clinical conditions, the brainstem and the spinal cord were initially thought to be resistant to SD (Bureš et al., 1974). However, later SD could be observed in the spinal cord (Somjen and Czéh, 1989; Streit et al., 1995), and we were able to show that SD can be also evoked in the immature rat brainstem up to postnatal day 14 (Richter et al., 2003, 2005). Severe short-lasting hypoxia (SSH) of 30- to 60-s duration conditioned the brainstem for SD elicited by local application of KCl, although it did not induce potential shifts by itself. Long-lasting SDs in the brainstem caused breathing arrest in the spontaneously breathing rat pups. With maturation, the brainstem becomes resistant to SD (Richter et al., 2003). However, even the adult brainstem was rendered susceptible for KCl-evoked SD by superfusion with artificial cerebrospinal fluid in which 75% of the chloride were replaced by acetate and to which the potassium channel blocker tetraethylammonium chloride (TEA) together with 10 mM KCl was added (Richter et al., 2008).

In rat neocortical slices, Jarvis et al. (2001) showed that oxygen and glucose deprivation caused an anoxic depolarization, a phenomenon that is similar to SD. Just recently, Funke et al. (2009) showed that SSH generates SD in slices of the immature brainstem *in vitro*. Whether SSH can induce SD in the adult brainstem *in vivo* is not

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doi:10.1016/j.expneurol.2010.03.004

known. This question is of clinical relevance because some pathophysiological states such as headache, cluster headache or migraine after sleep apneas (Nobre et al., 2003; Rains and Poceta, 2006; Bigal and Gladstone, 2008; Mitsikostas et al., 2008) may be associated with or even caused by disturbances of brainstem oxygenation. Sudden unexplained death in epilepsy (SUDEP) (So, 2008) that is attributed to cardiac, respiratory and/or autonomic failure might be related to pathophysiological processes in the brainstem. The question arises, therefore, whether hypoxia/ischemia-related SDs occur in the adult brainstem and whether such SDs are a relevant pathophysiological mechanism to link hypoxia with neuronal dysfunction. In fact, the interdependence of breathing function and SD in the immature brainstem in rat pups raises the question, whether the putative occurrence of SSH-related SDs in the adult brainstem might be involved in the cardiovascular response evoked by SSH. Furthermore, we found previously that KCl-induced SDs in the adult brainstem temporarily increased heart rate, regional brainstem blood flow, and systemic blood pressure (Richter et al., 2008).

To address these questions, we recorded DC potentials during SSH in cerebral cortex and at different sites in the brainstem together with heart rate, systemic blood pressure and local blood flow. We concentrated on the trigeminal nucleus region, since this region was prone to generate SD by KCl after increasing neuronal excitability *in vivo* (Richter et al., 2008), and on the nucleus tractus solitarii (NTS) region, since in this area recently Funke et al. (2009) were able to elicit SD in adult rat slices *in vitro*. We compared data from native brainstem with those after conditioning the brainstem with TEA + KCl.

Actually, SD terminology changes from the classical term "spreading depression" that has been shown to be a special case of depolarization phenomena in the cerebral cortex to the more general term "spreading depolarization" (Hartings et al., 2009). For clarity, we use the classical term "SD" for KCl-induced spreading depolarizations and "sudden severe hypoxia/ischemia-associated spreading depolarization (SSH/I-SD)" for those induced by ventilation arrest, by ventilation with a lowered O_2 content or by ventilation with pure nitrogen.

Materials and methods

Animal preparation

The present study was approved by the animal protection committee and the regional government of Thüringen (Reg.-No. 02-040/06). Twenty male Wistar rats (body weight 300–450 g, aged older than 90 days) were anesthetized with 100 mg/kg sodium thiopentone i.p.; supplemental doses (20 mg/kg ip) were given as necessary to maintain areflexia. Through the cannulated trachea, the animals breathed spontaneously during surgery. A catheter (diameter 0.5 × 0.9 mm, Braun Melsungen, Germany) was inserted into the right femoral artery. Changes of systemic arterial blood pressure (ABP) were measured with the pressure transducer P23Db (Statham Instruments, Puerto Rico). Body temperature was kept at 37 °C using a feedback-controlled temperature constanter (L/M-80, List, Darmstadt, Germany). Another catheter was introduced into the femoral vein and allowed to supply the animals with 5% glucose or Tyrode solution. At the end of the experiments, the animals were sacrificed by an overdose of the anesthetic intravenously.

The head of the animal was fixed in a stereotaxic frame. The apical and parietal parts of the skull were exposed following a median incision. Over the left hemisphere, the dura mater was exposed in an area of 4 × 4 mm (2 mm posterior to bregma, 1–2 mm laterally from the midline) using a mini-drill under cooling with artificial cerebrospinal fluid (ACSF; in mM: NaCl 138.4, KCl 3.0, $CaCl_2$ 1.3, $MgCl_2$ 0.5, NaH_2PO_4 0.5, urea 2.2, glucose 3.4, warmed to 37 °C and equilibrated with 5% CO_2 in O_2) and kept moist with ACSF throughout preparation. The dura mater was opened. Incising the neck muscles and the ligamentum atlantooccipitale exposed the brainstem from the occipital bone to the first cervical vertebra. The dura mater and arachnoidea underneath were removed.

After the surgical preparation, the animals were paralyzed with pancuronium bromide (Pancuronium Organon, Organon GmbH, Oberschleißheim, Germany; 2–4 mg/kg/h i.v.) and artificially ventilated with room air that was enriched with O_2 (oxygen content 30–35%). Anesthesia by sodium thiopentone was maintained to the end of the experiment.

Recording of DC potentials

An Ag/AgCl reference electrode containing 2 M KCl was placed on the nasal bone. Intracortical DC potentials were recorded using a glass micropipette filled with 150 mM NaCl. This microelectrode was inserted into the parieto-temporal cortex to a cortical depth of 1000–1200 µm corresponding to cortical layer V. For DC recordings in the brainstem, two glass pipettes were glued together with a lateral tip separation of 1000 µm, but with all tips at the same depths to observe a propagation of depolarization waves. One electrode was a double-barreled glass pipette (one channel contained 1 M KCl, the other 150 mM NaCl), and the other one was a single-barreled pipette (containing 150 mM NaCl). The electrode pair was lowered into the brainstem in a region close to the caudal trigeminal nucleus (Sp. V) to a depth of 1200–1400 µm. To elicit SD, we injected an amount of about 0.5 µl 1 M KCl as in our previous study (Richter et al., 2008), with a pressure of 100 kPa for 1 s using a microinjector (pico-injector PLI-100, Harvard Apparatus, Holliston MA). In a subset of five animals, we introduced an additional single-barreled glass microelectrode (containing 150 mM NaCl) into the region of the nucleus tractus solitarii (NTS, approximate depth = 1.200–1.400 µm) of the contralateral brainstem side.

All electrodes were connected by Ag/AgCl wires to a custom built 4-channel-high impedance amplifier (Meyer, Munich, Germany). The signals were stored on PC. The animals were grounded through an Ag/AgCl electrode below the back skin.

Superfusion of the brain and conditioning for SD/SSH/I-SD

In 15 rats, we tested the susceptibility of the native brainstem to SSH/I-SD by inducing ventilation arrest, and we tested the ability of SSH to render the native brainstem susceptible for SD by KCl and compared this with the situation after enhancing neuronal excitability in the brainstem. For this, exposed brain areas were kept moist first with regular ACSF. To test whether the native brainstem is prone to SD, we stopped the superfusion with ACSF, injected once KCl and continued superfusion with ACSF 5 min later. After an interval of 20 min, we stopped the respiratory pump for 50–60 s to induce short-lasting hypoxia (SSH). We re-ventilated the animal at the latest when either the heart rate declined to ≤50% of baseline values or the cortical DC electrode indicated the beginning of a steep negative DC potential shift. Four minutes after SSH, we injected KCl to elicit SD in the brainstem. In three rats, we repeated this sequence consisting of testing for SD by KCl microinjection, 50–60 s respiratory arrest to induce SSH and a subsequent microinjection of KCl for trying to elicit SD three times at intervals of 1 hour each.

After having performed the abovementioned test sequence, we changed in these 15 animals the superfusate of the brainstem from regular to modified ACSF in which 100 mM of the chloride (75%) was exchanged by 100 mM acetate, and we added 10 mM tetraethylammonium chloride (TEA, Sigma, Seelze, Germany) and 10 mM KCl (acetate-ACSF + TEA + KCl). In our previous study, this superfusate conditioned the adult brainstem for SD evoked by KCl (Richter et al., 2008). After 60 min, the superfusion was stopped and a further KCl injection was made to elicit SD. Superfusion was continued either after 5 min if no DC shift had occurred or after the recovery from a DC