Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Oxygen and glucose deprivation (OGD)-induced spreading depression in the Substantia Nigra

*Rashika N. Karunasinghe, Janusz Lipski**

Department of Physiology and Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland 92019, New Zealand

ARTICLE INFO

Article history:
Accepted 14 June 2013
Available online 21 June 2013

Keywords:
Anoxia
Aglycemia
Brain slice
Rat
Hippocampus
Electrophysiology
Imaging

ABSTRACT

Spreading depression (SD) is a profound depolarization of neurons and glia that propagates in a wave-like manner across susceptible brain regions, and can develop during periods of compromised cellular energy such as ischemia, when it influences the severity of acute neuronal damage. Although SD has been well characterized in the cerebral cortex and hippocampus, little is known of this event in the Substantia Nigra (SN), a brainstem nucleus engaged in motor control and reward-related behavior. Transverse brain slices (250 μm; P21–23 rats) containing the SN were subject to oxygen and glucose deprivation (OGD) tests, modeling brain ischemia. SD developed in lateral aspects of the SN within 3.3±0.2 min of OGD onset, and spread through the Substantia Nigra pars reticulata (SNr), as indicated by fast-occurring and propagating increased tissue light transmittance and negative shift of extracellular DC potential. These events were associated with profound mitochondrial membrane depolarization ($\Delta\Psi_m$) throughout the SN, as demonstrated by increased Rhodamine 123 fluorescence. Extracellular recordings from individual SNr neurons indicated rapid depolarization followed by depolarizing block, while dopaminergic neurons in the Substantia Nigra pars compacta (SNc) showed inhibition of firing associated with hyperpolarization. SD evoked in the SNr was similar to OGD-induced SD in the CA1 region in hippocampal slices. In the hippocampus, SD also developed during anoxia or aglycemia alone (associated with less profound $\Delta\Psi_m$ than OGD), while these conditions rarely led to SD in the SNr. Our results demonstrate that OGD consistently evokes SD in the SN, and that this phenomenon only involves the SNr. It remains to be established whether nigral SD contributes to neuronal damage associated with a sudden-onset form of Parkinson's disease known as 'vascular parkinsonism'.

Abbreviations: LT, light transmittance; $\Delta\Psi_m$, mitochondrial membrane potential depolarization; OGD oxygen–glucose deprivation; Rh123, Rhodamine 123; SD spreading depression; SN Substantia Nigra; SNc Substantia Nigra pars compacta; SNr Substantia Nigra pars reticulata.

*Correspondence to: School of Medical Sciences, Faculty of Medical and Health Sciences, University of Auckland, 85 Park Rd, Grafton, Auckland 92019, New Zealand. Fax: +64 937 374 99.
E-mail address: j.lipski@auckland.ac.nz (J. Lipski).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.06.016

1. Introduction

'Spreading depression' (SD) was first described by Leão (1944) as a propagating suppression of neuronal activity in response to focal electrical or mechanical stimulation of brain tissue. Subsequent studies have demonstrated that under normoxic conditions this event does not normally lead to neural damage (Nedergaard and Hansen, 1988; Somjen, 2001), unless a large number of consecutive SD episodes are evoked (Pomper et al., 2005). SD can also occur as an early response during periods of compromised cellular energy, as for example in brain ischemia where it is often a near-terminal event, propagating from the ischemic core to surrounding penumbra. Therefore, under such conditions, SD can be regarded as an early indicator of neurological injury (eg. Back et al., 1996; Obeidat and Andrew, 1998; Takano et al., 2007).

In both normal and energy deprived states, SD can be initiated by factors which release potassium ions and/or glutamate (Grafstein, 1956; Somjen, 2001; Kager et al., 2002). This leads to a sudden drop of membrane potential in neurons and glia, further K^+ and glutamate release, and a self-propagating wave-like depolarization which spreads across susceptible brain tissue (Somjen, 2001; Kager et al., 2002). In neurons recorded both in vivo (Morlock et al., 1964; Sugaya et al., 1975) and in vitro (Grafstein, 1956; Tanaka et al., 1999), the fast depolarizing phase at the wavefront is associated with a burst of action potentials resembling a seizure discharge, and is followed by silencing of neuronal activity which reflects sodium channel inactivation (depolarizing block). These events lead to a dramatic impairment of ion homeostasis and cellular energy depletion (Mies and Paschen, 1984; Somjen, 2001; Carlson et al., 2012). During periods of brain ischemia, modeled in vitro by oxygen and glucose deprivation (OGD) (eg. Obeidat and Andrew, 1998; Tanaka et al., 1999; Grammer et al., 2008), the inability to restore ATP-dependent processes leads to persistent disruption of ionic gradients and prevents repolarization of affected cells. This may lead to activation of cell death cascades if reperfusion (or restoration of O_2 and glucose) is not resumed immediately after the onset of depolarization, particularly in submerged brain slices (eg. Tanaka et al., 1999; Müller and Ballanyi, 2003). In this condition, a deficit of intracellular ATP results not only from increased ATP utilization (Henrich and Buckler, 2008), but also from impaired mitochondrial function (eg. Bahar et al., 2000; Mané and Müller, 2012; Sonn and Mayevsky, 2012). In addition, the overall movements of electrolytes result in a fast-occurring cytoplasmic edema, which can further contribute to damage (eg. Obeidat and Andrew, 1998; Takano et al., 2007).

OGD-induced SD has been well characterized in the cerebral cortex and the CA1 hippocampal region, where the relatively selective vulnerability of pyramidal cells to ischemic damage is correlated with the early occurrence of SD (eg. Obeidat et al., 1998, 2000; Grammer et al., 2008; Gniel and Martin, 2010). In contrast to these forebrain regions, the mature brainstem appears relatively resistant to ischemic or anoxic SD (Ballanyi et al., 1996; Funke et al., 2009). Recently, Brisson and Andrew (2012) proposed that the decreasing rostro-caudal susceptibility gradient of the brain to ischemic damage is related to the propensity for SD induction, in a manner independent of vascular factors.

To our knowledge, ischemia- or OGD- induced SD has not previously been described in the Substantia Nigra (SN), a rostral brainstem (mesencephalic) nucleus which plays a crucial role in motor control and reward-related behavior. The SN, and in particular its pars compacta division, is a region containing dopaminergic neurons which degenerate in Parkinson's disease (PD; Hornykiewicz, 2001). SN damage has also been implicated in an unusual, sudden-onset form of the disorder known as vascular parkinsonism (VP; eg. Boecker et al., 1996; Vidailhet et al., 1999). We therefore aimed to evaluate the potential initiation and propagation of SD in the SN in response to OGD, as compared to the CA1 hippocampal region in rat brain slices.

2. Results

2.1. OGD-induced SD in the Substantia Nigra

Measurements of tissue light transmittance (LT) provide an index of volume changes which occur during periods of cellular stress, including OGD (Obeidat and Andrew, 1998; Aitken et al., 1999; Olson and Kreisman, 2005). We monitored changes in LT to assess the initiation and propagation of nigral SD in midbrain slices ($n=20$) obtained from P21–23 rats ($n=6$) in response to 10 min OGD. Soon after depletion of oxygen and glucose, LT slowly increased across the whole SN, indicative of progressive, global cell swelling. Within 3.3 ± 0.2 min, a secondary, more abrupt focal increase occurred in the Substantia Nigra pars reticulata (SNr) region (Fig. 1A1–A3). This rapid-phase response can be attributed to the sudden cytoplasmic edema developing at the expense of the interstitial volume at the SD wavefront (eg. Somjen, 2001; Lipski et al., 2006). The delay to this response correlated with the latency to a sudden negative shift in extracellular DC potential ($r=0.97$; Fig. 1A3–A4), considered to be the electrophysiological hallmark of SD (Leão, 1947; Herreras and Somjen, 1993). The SD wavefront consistently originated in the lateral part of the SNr (close to the Substantia Nigra pars lateralis, SNl; Paxinos et al., 2009), indicating that the threshold for SD initiation was lowest in this area (Fig. 1A2). Following initiation, the front of increased LT wave propagated ventro-medially at 0.9 ± 0.1 mm/min without significant slowing (Fig. 2B).

OGD consistently evoked abrupt and propagating increase in LT (ΔLT, $62\pm4\%$) and negative shift in extracellular DC potential (-1.0 ± 0.1 mV) in the SNr (Fig. 2A2, A3; Table 1). In contrast, the Substantia Nigra pars compacta (SNc) region showed smaller and more slowly developing changes in light transmittance (ΔLT $31\pm3\%$) and extracellular DC potential (-0.1 ± 0.03 mV), and the abrupt secondary increase of LT seen in the SNr was absent in that region (Fig. 1A1, A3–A4; Table 1). These data indicate that the SNc is not involved in the nigral SD response. OGD-induced changes in LT and extracellular DC potential in the SNr and SNc are illustrated in Fig. 1A1, A3, A4 (see also Fig. 2A2–A3 and Table 1).

Upon termination of OGD and reperfusion with standard ACSF, the elevated LT in the SNr region did not return to