Neuroscience 285 (2015) 303–311

SPECTROSCOPIC MEASUREMENT OF CORTICAL NITRIC OXIDE RELEASE INDUCED BY ASCENDING ACTIVATION

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Abstract—The transition from sleep to the awake state is regulated by the activation of subcortical nuclei of the brainstem (BS) and basal forebrain (BF), releasing acetylcholine and glutamate throughout the cortex and inducing a tonic state of neural activity. It has been suggested that such activation is also mediated by the massive and diffuse cortical release of nitric oxide (NO). In this work we have combined the spectroscopic measurement of NO levels in the somatosensory cortex of the cat through its marker methemoglobin, as well as two other hemodynamic markers (oxyhemoglobin – oxyHb – and deoxyhemoglobin – deoxyHb), together with the electrical stimulation of BS and BF – to induce an experimental transition from a sleep-like state to an awake-like mode. The results show an increase of NO levels either after BS or BF activation. The response induced by BS stimulation was biphasic in the three studied markers, and lasted for up to 30 s. The changes induced by BF were monophasic lasting for up to 20 s. The systemic blockade of NO production abolished the observed responses to BS whereas responses to BF stimulation were much less affected. These results indicate a crucial role for NO in the neuronal activation induced by the ascending systems.

Key words: hemodynamics, sleep, somatosensory cortex, spectroscopy.

INTRODUCTION

Arousal is the consequence of an orchestrated change in cortical and subcortical structures. The transition from sleep to the awake state is mainly induced by brainstem (BS) and basal forebrain (BF) neurons (Steriade et al., 1993a), which are part of the classic activating ascending system. As a result of such activation, the cortical release of acetylcholine (ACh) and glutamate (Glu) disrupts the sleep slow oscillatory activity and promotes a transition to the characteristic tonic state of the awake brain (for a review see Steriade et al., 1997). It has been suggested that nitric oxide (NO) is also an important actor in this activation, mainly through its release by cholinergic BF neurons sending axons to the cortex (Bickford et al., 1994; Cudeiro et al., 2000) and by cortical nitrergic neurons (Cudeiro et al., 1997).

Brain sleep mechanisms have been extensively studied using anesthetized cats as a model to reproduce the slow-wave sleep and its dynamics (Steriade et al., 1991a,b; McCormick and Bal, 1997). Also, under experimental conditions, the global cortical activity can be efficiently modified through microstimulation of either the peribrachial region (PBr; Moruzzi and Magoun, 1949; Francesconi et al., 1988; Hartveit et al., 1993; Uhlrich et al., 1995; Wolfe and Palmer, 1998), located in the BS, or the nucleus basalis of Meynert (NB; Metherate et al., 1992; Mariño and Cudeiro, 2003), located in the BF. The BS contains cholinergic, nitrergic and catecholaminergic neurons sending axons to BF and the thalamus (Steriade et al., 1988). BF neurons extensively release ACh throughout the cortex and, as indicated, probably NO, while thalamocortical neurons release Glu. In the anesthetized cat, the activation of such systems has been proved to be a useful tool to study the sleep–wake mechanisms (Steriade et al., 1991b; Li et al., 1999; Mariño and Cudeiro, 2003).

We have previously shown that the systemic and local blockade of NO synthesis (by 7-nitroindazole and L-NOArg respectively) reduces the tonic activation of cortical neurons after subcortical stimulation (Mariño and Cudeiro, 2003). Nonetheless, as far as we know, no direct measurements of that supposed massive NO release has been reported yet. Here, using intracortical spectroscopy in the somatosensory cortex (SSC), we have measured the *in vivo* dynamics of cortical NO levels during sleep-like slow oscillatory activity (induced by anesthesia) and after the activation induced by BS and BF regions, together with the measurements of two hemodynamic markers, oxyhemoglobin (oxyHb) and deoxyhemoglobin (deoxyHb). Our central hypothesis was that, during the intense activation induced by the ascending systems, there is a diffuse and acute production of neuronal NO in the cortex, thus collaborating in the transition from sleep to the wake state.

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Abbreviations: ACh, acetylcholine; BF, basal forebrain; BOLD, blood oxygenation level dependent; BS, brainstem; deoxyHb, deoxyhemoglobin; ECoG, electrocorticogram; Glu, glutamate; LFi, low-frequency index; metHb, methemoglobin; MR, magnetic resonance; NB, nucleus basalis; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; oxyHb, oxyhemoglobin; PBr, peribrachial region; SSC, somatosensory cortex.

http://dx.doi.org/10.1016/j.neuroscience.2014.11.028
0306-4522/

EXPERIMENTAL PROCEDURES

Experimental preparation

Experiments were performed on 4 adult cats of either sex. Anesthesia was induced with ketamine (15 mg/kg – Pfizer, Madrid, Spain) and xylazine (3 mg/kg – Sigma-Aldrich, Madrid, Spain) i.m. and maintained with isoflurane (1.5–2% for surgery, and 0.5–1% for maintenance, Abbot) in nitrous oxide (70%) and oxygen (30%). Animals were paralyzed with gallamine triethiodide (loading dose of 40 mg, maintenance 10 mg/kg/h i.v., SIGMA) and artificially ventilated; the end tidal CO_2 concentration was adjusted to 3.8–4.2%, body temperature was maintained at 37–38 °C, and heart rate was continuously monitored. Animals were suspended on a stereotaxic frame and the pressure points were infiltrated with lidocaine. The level of isoflurane was chosen to achieve a state of deep anesthesia and particular care was taken to keep a stable pattern of delta (1–4 Hz) slow oscillatory activity throughout the entire experiment. Three craniotomies were performed to insert optical fibers and bipolar tungsten electrodes (details below). All exposed cortical surfaces were bathed in saline to prevent desiccation. At the end of experiments animals were killed by an anesthetic overdose. All animal work was conducted according to national and international guidelines (Spanish Physiology Society and the European Communities Council Directive of November 24, 1986 (86/609/EEC)). The study was approved by the University of A Coruña Ethics Committee (CE-UDC30/1/09).

Electrophysiological and spectroscopy recordings

Electrical and spectroscopic measurements were obtained simultaneously in the primary SSC (S1; coordinates: anterior, 21–23; lateral, 8–10) at a depth of 1–1.5 mm. Electrocorticogram (ECoG) in S1 was continuously recorded and stored (Plexon Inc, Dallas, TX, USA) through a bipolar and concentric electrode at a sampling rate of 2 kHz and filtered on-line with a band-pass of 0.1–500 Hz analog filter (A-M Systems, model 1700).

The method to monitor NO lays in the fact that NO reacts with oxyHb to form methemoglobin (metHb). Thus, metHb levels are proportional to the concentration of NO and can be used as a surrogate marker of such substance (Feelisch et al., 1996; Kelm et al., 1997, González-Mora et al., 2002; Rivadulla et al., 2011). According to this, we used spectroscopy to record the levels of metHb as well as oxy- and deoxyHb. Spectroscopic signals were obtained using two optical fibers (model FCB-UV 100-3-2SMA; diameter 100 μm) arranged in parallel and attached to the electrode. Distance from the electrode tip to the fibers end (located slightly below, see Fig. 2E) was between 100 and 200 μm. Light in the range of 600–700 nm from a halogen lamp was passed through one of the optical fibers and the scattered light was collected by the other. Output was directed to a linear CCD detector (Ocean Optics, Eerbeek, Netherlands) via a compact built-in monochromator and sampled at a rate of 100 Hz with steps of 0.35 nm in the wavelength domain.

To convert the sample intensity recordings S to absorbance values the protocol was as follows: with the fibers located in the tissue, the emitted light was turned off and the dark intensity D was recorded. Next, light was turned on and the reference intensity R was recorded. Then, for a sample intensity at wavelength λ and recorded at time t, $S_\lambda(t)$, the corresponding absorbance, $A_\lambda(t)$, was calculated using the following equation:

$$A(t)_\lambda = -\log_{10}\left(\frac{S(t)_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right) \quad (1)$$

where D_λ and R_λ are the dark and reference intensities for a wavelength λ respectively. Because absorbance is a dimensionless parameter, the value is expressed in arbitrary units (AU).

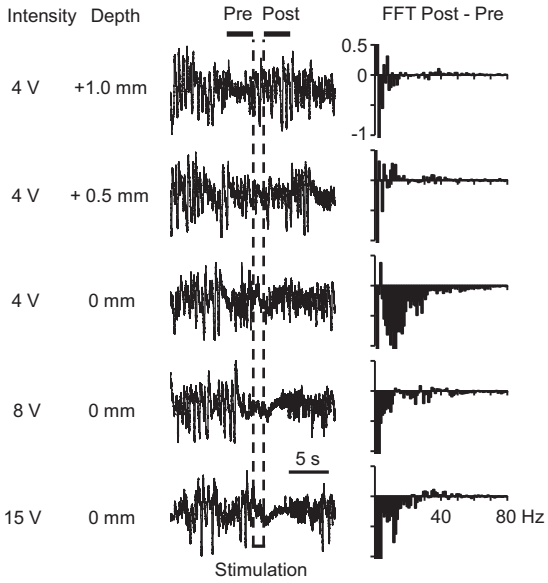

Fig. 1. Electrical stimulation effect obtained for different stimulation intensities and electrode distances from the stereotaxic position (0 mm) of the parabrachial region of BS. Both ECoG wave (left) and its power spectral density (right) reveal changes in low-and high-frequency components. The analysis included the 5 s prior stimulation (Pre), and the 5 s after stimulation (Post). See ''Experimental procedure'' section for details.

Subcortical stimulation and cortical activity modulation

The global cortical activity was efficiently modulated – as indicated by the ECoG recordings – by the combination of anesthesia and electrical stimulation. The continuous anesthetic administration maintained a slow oscillatory pattern within the delta range (Fig. 1, left part of the ECoGs). The temporal transition to an awake-like pattern was achieved through the application of electrical stimulation either in BS or BF (sequentially and randomly through electrical microstimulation at intervals of 2–8 min). Trains of rectangular cathodal shocks (0.05 ms, 0.1–1 mA) were delivered at a frequency of 50 Hz for a period of 2 s through bipolar electrodes (insulated except for 500 μm at the tip; contacts separated by 500 μm). Electrodes were positioned and held in place following a procedure published elsewhere (Mariño and Cudeiro 2003). It allowed us to precisely localize the subcortical loci