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Prooxidant *versus* antioxidant brain action of ascorbic acid in well-nourished and malnourished rats as a function of dose: A cortical spreading depression and malondialdehyde analysis

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ABSTRACT

Although ascorbic acid (AA) is an antioxidant, under certain conditions it can facilitate oxidation, which may underlie the opposite actions of AA on brain excitability in distinct seizure models. Here, we investigated whether chronic AA administration during brain development alters cortical excitability as a function of AA dose, as indexed by cortical spreading depression (CSD) and by the levels of lipid peroxidation-induced malondialdehyde. Well-nourished and early-malnourished rats received per gavage 30, 60, or 120 mg/kg/d of AA, saline, or no gavage treatment (naïve group) at postnatal days 7–28. CSD propagation and malondialdehyde levels were analyzed at 30–40 days. Confirming previous observations, CSD velocities were significantly higher in the early-malnourished groups than in the well-nourished groups. AA dose was important: 30 mg/kg/d AA decelerated CSD and reduced malondialdehyde levels, whereas 60 mg/kg/d and 120 mg/kg/d accelerated CSD and augmented malondialdehyde levels compared with the corresponding saline and naïve groups. Our findings reinforce previous suggestion that AA acts as an antioxidant in the brain when administered at low doses, but as a prooxidant at high doses, as indicated by CSD propagation and malondialdehyde levels.

1. Introduction

The mechanisms of excitability-related brain disturbances include active free radicals of oxygen (Braugher and Hall, 1989; Mori et al., 1990), which is certainly the basis for the growing use of antioxidants to protect the brain against excitability-induced damage (Murashima et al., 1998; Simeone et al., 2014). Ascorbic acid (AA) is one of the most studied antioxidants; it is highly concentrated in the adrenal gland and central nervous system (Miura et al., 2009), with postulated important action during brain development (Zalani et al., 1989). The physiological actions of AA include protection against oxidative stress, with possible protective actions in neurodegenerative diseases (Halliwell, 2006; Padayatty et al., 2003).

Regarding excitability-dependent brain phenomena, experimental evidence indicates that AA can exert biphasic modulating action (Oliveira et al., 2004). For example, the administration of 60 mg/kg/d of AA to rats increased the oxidative stress in the brain induced by bisphenol A, nonylphenol, and octylphenol (Aydogan et al., 2008), suggesting a prooxidant action of AA. Previously, our group demonstrated a similar prooxidant effect in weaned rats treated with 60 mg/kg/d of AA for three weeks (from postnatal days 7–28); compared with saline-treated controls, AA-exposed animals displayed significantly higher-velocity propagation of the brain excitability-related phenomenon known as cortical spreading depression (CSD) (Monte-Guedes et al., 2011).

CSD was first described as a reduction in the spontaneous electrical activity of the cerebral cortex in response to the mechanical, electrical, or chemical stimulation of one point on the cortical surface (Leão, 1944). The phenomenon is characterized by neuronal depolarization (Dreier, 2011), and has been electrophysiologically demonstrated in the brains of many vertebrate species

Abbreviations: AA, ascorbic acid; CSD, cortical spreading depression; sal, saline; DC, direct current; MDA, malondialdehyde; BCA, bicinchoninic acid.

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(Bureš et al., 1974) as well as in the human brain (Fabricius et al., 2008; Gorji and Speckmann, 2004). CSD is modulated by changes in brain excitability (Koroleva and Bures, 1980), and is influenced by the production of reactive oxygen species in brain tissue (El-Bachá et al., 1998). Related to neuronal excitability, CSD has been associated with relevant neurological diseases such as migraine (Bhaskar et al., 2013; Rogawski, 2008), brain ischemia (Busija et al., 2008), and epilepsy (Costa-Cruz et al., 2006; Guedes and Cavalheiro, 1997). Different CSD velocities of propagation along the cortical tissue, which indicate different brain susceptibilities to CSD, are associated with the manipulation of pharmacological, environmental, and nutritional conditions (see Guedes, 2011 for a review).

Malnutrition can disrupt the electrophysiological organization of the nervous system both in laboratory animals (Chen et al., 1997; Morgane et al., 1978) and in humans (Grantham-McGregor, 1995; Levitsky and Strupp, 1995). We previously demonstrated that early malnutrition increases CSD propagation (Rocha-de-Melo et al., 2006), but no information is available regarding the interaction between malnutrition and AA effects on CSD.

In the present work, we addressed two questions via electrophysiological recording of CSD in the brains of weaned young rats subjected to malnutrition during lactation. First, how does the administration of different doses of AA during brain development affect CSD propagation? Second, how is this effect influenced by malnutrition early in life? We demonstrated CSD deceleration and acceleration following the administration of low and high doses of AA, respectively.

2. Material and methods

2.1. Animals

Wistar rat pups ($n = 96$) born from distinct dams were randomly distributed at birth to form litters with eight pups per nurse and assigned to two nutritional groups according to the mother's dietary conditions. The well-nourished group ($n = 53$) was suckled by dams fed a commercial laboratory chow diet (Purina do Brazil LTDA) containing 23% protein. The malnourished group ($n = 43$) was suckled by dams fed a regional basic diet containing 8% protein. This diet mimics the diet of low-income human populations of Northeastern Brazil (Teodósio et al., 1990). After weaning at post-natal day 21, the pups were fed the chow diet.

Both nutritional groups were subdivided into five groups denominated according with the treatment they received, from postnatal days 7–28, per gavage: the saline group (Sal; $n = 9$ Well-nourished rats and 9 Malnourished rats) received saline via gavage. For the AA-treated groups, rats received 30 mg/kg/d (AA-30; $n = 15$ Well-nourished rats and 9 Malnourished rats), 60 mg/kg/d (AA-60; $n = 6$ Well-nourished rats and 6 Malnourished rats), or 120 mg/kg/d (AA-120; $n = 12$ Well-nourished rats and 9 Malnourished rats). AA was purchased from Sigma, St. Louis, MO, USA. A fifth "naïve" group ($n = 11$ Well-nourished rats and 10 Malnourished rats) received no gavage.

Animals were reared in polypropylene cages (51 cm × 35.5 cm × 18.5 cm) in a room maintained at 22 ± 1 °C with a 12:12 h light/dark cycle (lights on at 6:00 AM) with free access to food and water. They were weighed at postnatal days 7, 14, 21, and 28. All experiments were carried out at the Universidade Federal de Pernambuco (Brazil) in accordance with the guidelines of the Institutional Ethics Committee for Animal Research (approval protocol no. 23076.013959/2012-79), which comply with the "Principles of Laboratory Animal Care" from the National Institutes of Health of the United States. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. Recording of CSD

At 30-40 postnatal days, the animals were subjected to a 4-h CSD recording session, as previously described (Lima et al., 2013). Briefly, under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, i.p.; both purchased from Sigma, St. Louis, Mo, USA) three trephine holes (2–3 mm in diameter) were made on the right side of the skull, parallel to the midline. The first hole (on the frontal bone) was used to apply the stimulus to elicit CSD. The other two holes (on the parietal bone) were used to record the propagating CSD wave. Rectal temperature was continuously monitored and maintained at 37 ± 1 °C by a heating blanket. During the recording session, CSD was elicited at 20-min intervals by applying, for 1 min, a cotton ball (1–2 mm diameter) soaked in 2% KCl solution (approximately 0.27 M) to the anterior hole drilled at the frontal region. The direct current (DC)-potential change typical of CSD was recorded at the two parietal points on the cortical surface with a pair of Ag–AgCl agar-Ringer electrodes that were connected to a digital data acquisition system (EMG Systems, São Paulo, Brazil). A common reference electrode of the same type was placed on the nasal bones. For all CSD episodes, we calculated the amplitude and duration of the negative slow potential shifts of the CSD waves, as previously reported (Lima et al., 2013). The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the two recording electrodes. In the measurement of CSD velocities, the initial point of each DC negative rising phase was used as the reference point.

In each CSD recording session, three or four animals per group were recorded simultaneously. After finishing the recording session, the still-anesthetized animals were decapitated. The brains were removed rapidly and carefully, rinsed in ice-cold saline, and dried on filter paper. The pooled cortical tissue of 3–4 animals was homogenized in a 0.9% (w/v) NaCl solution (1:10) at 4 °C, centrifuged for 10 min at 1000 × g at 4 °C, and the supernatants were stored at −80 °C for the analysis of lipid peroxidation, as recently reported in another publication from our group (Cardoso et al., 2012).

2.3. Analysis of lipid peroxidation

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) levels using a thiobarbituric acid-reactive substances-based method (Ohkawa et al., 1979). The MDA levels were determined in 36 of the well-nourished rats (Sal, $n = 7$; naïve, $n = 6$; AA-30, $n = 10$; AA-60, $n = 5$; AA-120, $n = 8$) and 39 of the malnourished rats (Sal, $n = 8$; naïve, $n = 11$; AA-30, $n = 8$; AA-60, $n = 6$; AA-120, $n = 6$). The reaction was developed by sequential addition of 40 μL of 8.1% sodium dodecyl sulfate, 300 μL of 20% acetic acid (pH 3.5), and 300 μL of 0.8% thiobarbituric acid solutions to the 100-μL homogenate aliquot in a boiling water bath for 30 min. Experiments were carried out in triplicate. After cooling the tubes with tap water, 300 μL of n-butanol were added to the sample. The tubes were centrifuged at 2500 × g for 10 min, and the organic phase was read at 532 nm using a plate reader. Total protein concentrations were determined with the BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

2.4. Statistical analysis

Intergroup differences were compared using two-way analysis of variance (ANOVA) including nutritional status (well-nourished and malnourished) and treatment (saline, naïve, AA-30, AA-60, and AA-120) as factors. ANOVA was followed by a Holm–Sidak post-hoc test when indicated. Differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Body weight

The body weights of the five well-nourished groups and five malnourished groups are presented in Fig. 1. During days 7–28, all malnourished groups had lower body weights than the corresponding well-nourished groups ($p < 0.05$). No weight difference associated with AA treatment was observed.

3.2. CSD recording

Fig. 2 contains typical electrophysiological CSD recordings (slow DC potential change) of five well-nourished and five malnourished rats that were representative of the five treatment groups. Under normal conditions (Sal and naïve groups in Fig. 2), 1-min stimulation with 2% KCl at one point of the right frontal cortex elicited a single CSD wave that propagated without interruption and was recorded by the two electrodes located more posterior on the surface of the parietal cortex (Fig. 2). Recording of the slow potential change confirmed the presence of CSD after KCl application. The electroencephalographic changes caused by CSD always recovered after a few minutes (Fig. 2), and we maintained a 20-min interval between subsequent KCl stimulations. In the majority of AA-120 animals (58.3% and 90% of the well-nourished and malnourished rats, respectively), a single KCl stimulation elicited two CSD episodes (Fig. 2), which may be indicative of increased cortical sensitivity due to the AA-120 treatment.

3.3. CSD parameters

ANOVA showed a significant main effect of nutritional status on the CSD velocity of propagation ($F[1,94] = 89.46$; $p < 0.001$). Post-