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Research Report

Succinate causes oxidative damage through *N*-methyl-D-aspartate-mediated mechanisms

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Abstract

In this study we investigated whether succinate, the accumulating substrate in succinate dehydrogenase (SDH) deficiencies and SDH inhibitor intoxication, causes lipoperoxidation and protein carbonylation, and if NMDA receptors are involved in the succinate-induced oxidative damage. Adult male mice (30–40 g) received an intracerebroventricular injection of succinic acid (0.7, 1.0 and 1.7 μmol/5 μl) or 0.9% NaCl (5 μl) and had their exploratory behavior assessed in an open field for 10 min. Succinate (0.7 and 1.0 μmol/5 μl) decreased locomotor activity behavior and increased thiobarbituric acid reactive substances (TBARS) and protein carbonylation in the forebrain. Conversely, 1.7 μmol of succinate did not alter locomotor activity or oxidative damage parameters. The involvement of NMDA receptors in the succinate-induced increase of total protein carbonylation content and exploratory behavior inhibition was assessed by co-administrating MK-801 (7 nmol/2.5 μl icv), a noncompetitive NMDA receptor antagonist, with succinate (1 μmol/2.5 μl icv). The co-administration of MK-801 protected against succinate-induced increase of total protein carbonylation and decrease of locomotor activity. These results suggest the involvement of NMDA receptors in these effects of succinate, which may of particular relevance for succinate-accumulating conditions, such as SDH inhibitors intoxication and inherited SDH deficiencies.

Theme: Disorders of the nervous system
Topic: Neurotoxicity

Keywords: Locomotor behavior; Free radical; MK-801; Succinate dehydrogenase; Malonate; 3-Nitropropionic acid

1. Introduction

Succinate is the endogenous substrate of succinate dehydrogenase (SDH), which is active in the tricarboxylic acid cycle and the electron transport chain during ATP synthesis [1,16]. This dicarboxylic acid accumulates in some inborn errors of the metabolism, such as complex II deficiency, malonic and methylmalonic acidemias. Succinate accumulation in malonic and methylmalonic acidemias is probably due to the competitive inhibition of SDH by malonate [14,16,26] and methylmalonate [11,14,24,38,41]. In fact, it has been shown that succinate accumulation reaches nearly 150 μM in the urine of malonic acidemic patients [31], and cerebral concentrations range between 0.5 and 8.3 mM in the white matter of patients with complex II deficiency [6]. Interestingly, white matter cerebral damage in these patients proved to be more extensive in those presenting intermediate

Abbreviations: ANOVA, analysis of variance; AP5, D-2-amino-5-phosphonovaleric acid; ATP, adenosine triphosphate; DHBA, dihydroxybenzoic acid; DNPH, 2,4-dinitrophenylhydrazine; fEPSPs, field excitatory post-synaptic potentials; icv, intracerebroventricular; MK-801, (+)-5-methyl-10,11-dihydroxy-5-dibenzo (a,b) cycloheptene-5,10-imine or dizocilpine; NMDA, *N*-methyl-D-aspartate; SDH, succinate dehydrogenase; 3-NPA, 3-nitropropionic acid; SDS, sodium dodecyl sulfate; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde

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cerebral succinate concentrations (0.5–1.3 mM) than in those presenting high cerebral succinate concentrations (3.7–8.3 mM).

A recent study from Roehrs et al. [33] may have shed some light on this apparent paradox. In that study it was demonstrated that succinate, at low concentrations (0.3–1 mM), increases neuronal post-synaptic excitatory potentials (fEPSPs) in hippocampal slices from adult rats by NMDA-mediated mechanisms. Conversely, high concentrations of succinate (3–10 mM) decrease fEPSPs and reverse the increase of fEPSPs induced by low concentrations of succinate [33]. The excitatory action of succinate was confirmed in vivo, since it induced convulsions in mice, which were prevented by the co-administration of MK-801 (an NMDA receptor antagonist). The authors suggested that succinate might be acting as a partial NMDA agonist, or at two distinct sites with opposite electrophysiological implications. The findings by Roehrs et al. [33] explain why high concentrations of succinate (above the mM range) attenuate or reverse the neurotoxic action of malonate and methylmalonate [3,10,17,34,42] but causes convulsions, at comparatively low doses, by NMDA-mediated mechanisms, in mice.

The activation of NMDA receptors is though to be associated with generation of reactive species [21,27,28,37,39,43], which have been recognized as important mediators of tissue injury in several neurodegeneration models [2,15,20,35,36], including those in which succinate accumulation has been demonstrated [19] or is a strong possibility, such as competitive SDH inhibitors exposure [13,23,24]. In line with this view, intrastriatal malonate increases 2,3 and 2,5-DHBA, 3-nitrotyrosine and peroxynitrite generation [25] and methylmalonic acid increases lipoperoxidation [13,23,24]. However, until the present moment, no study has addressed whether succinate accumulation causes oxidative damage per se. Therefore, in this study we investigated whether succinate causes lipoperoxidation and protein carbonylation, and if NMDA receptors are involved in the succinate-induced oxidative damage.

2. Materials and methods

2.1. Chemicals

All reagents were acquired from Sigma (St. Louis, MO, USA), except thiobarbituric acid (TBA), which was obtained from Merck (Darmstadt, Germany). Succinate and other solutions injected were prepared in 0.9% NaCl (saline).

2.2. Animals

Adult male Swiss albino mice (30–40 g), maintained in a 12-h dark/12-h light cycle at controlled temperature (22 ± 1 °C), with free access to tap water and standard laboratory chow (Guabi, Santa Maria, RS, Brazil) were used. All experimental protocols were conducted in accordance with National and International legislation (guidelines of the Brazilian College of Animal Experimentation (COBEA) and U.S. Public Health Services Policy on Humane Care and Use of Laboratory Animals-PHS Policy), and with the approval of the Ethical Committee for animal research of the Federal University of Santa Maria (protocol number 0120256/2002-19). The number of animals used was kept to a minimum by using planned statistical analyses at predefined stages of the experiments. Adequate measures were taken to minimize pain and discomfort.

2.3. Behavioral effects of succinate on mice

Free hand intracerebroventricular (icv) injections into the lateral ventricles of the conscious mice were made using a 30-gauge needle attached to a 10-μl Hamilton syringe (3 mm of the needle tip exposed) according to Clark et al. [8] by an experimenter who was not aware of the pharmacological treatment. The site of injection was an imaginary line drawn through the anterior lobe of the ears and from an imaginary midsagittal line, and the whole injection procedure was completed within 5–10 s, in order to minimize discomfort and pain. Immediately after behavioral evaluation the animals were decapitated and had the site of the intracerebroventricular injection (icv) confirmed by needle track verification with a PZO MST131 stereomicroscope. Only data from animals with the needle track aiming the lateral ventricle were considered. Animals were injected (icv) with 5 μl of succinate (0.7, 1.0 and 1.7 μmol) or 0.9% NaCl and, immediately thereafter, individually placed in a round open-field (35 cm of internal diameter), which had its floor divided into 10 areas of equal size. During 10 min the number of areas crossed, the number of rearing responses and the total time spent in immobility were recorded manually. The involvement of NMDA receptors in the succinate-induced neurochemical and behavioral alterations was assessed by co-injecting the animals with 7.0 nmol MK-801 and 1.0 μmol succinate in 2.5 μl plus 2.5 μl volumes in the same syringe, separated by an air bubble. The animals were immediately transferred to the open field and observed for 10 min, as described above.

2.4. TBARS assay ex vivo

Immediately after the behavioral evaluation, the animals were killed by decapitation and had their forebrain removed. Tissues were homogenized in 10 volumes (w/v) of 10 mM Tris–HCl, pH 7.4, containing sodium dodecyl sulfate (SDS, 0.10%—final concentration) using a glass homogenizer and TBARS content was estimated in a medium containing 0.2 ml of brain homogenate, 0.1 ml of 8.1% SDS, 0.4 ml of acetic acid buffer (500 mM, pH 3.4) and 0.75 ml of 0.81% thiobarbituric acid (TBA). The mixture was finally made up