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Research Report

Effect of δ-aminolevulinic acid treatment on *N*-methyl-D-aspartate receptor at different ages in the rat brain

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Abstract

We report here the effects of the chronic treatment with the oxidant agent δ-aminolevulinic acid (ALA) on the *N*-methyl-D-aspartate (NMDA) receptors in 4-, 12- and 24-month-old male Wistar rats. ALA was administered daily for 15 days (40 mg/kg i.p). The study was performed by membrane homogenate binding and autoradiography, using tritiated 5-methyl-10, 11-dihydro-5*H*-dibenzo(a,d)cycloheptan-5,10-imine maleate ([^{3}H]MK-801). [^{3}H]MK-801 binding was significantly decreased in most areas studied (cortex and hippocampus) at all ages in treated rats with respect to their controls. Furthermore, Western blot assays were performed using antibodies against the NMDA receptor NR2A subunit, which is widely distributed in the brain, mainly in cortex and hippocampus. In cortex but not in hippocampus, the ALA treatment induced significant decreases in the amounts of NR2A subunit in 12- and 24-month-old animals. We conclude that chronic treatment with ALA is able to induce NMDA receptor decreases in an age-independent way and that NR2A subunit seems to be involved in these decreases in cerebral cortex, but not in the other structures studied.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Excitatory amino acid receptors: structure, function, and expression

Keywords: NMDA receptor; Aging; [^{3}H]MK-801; Brain; δ-Aminolevulinic acid

1. Introduction

The damage inflicted by oxygen free radicals, called oxidative stress, seems to play an important role in aging [18,19,37,39,40], particularly in the central nervous system [17,37]. Thus, increases in the free radical generation during normal aging process have been suggested to occur [15,27] resulting in an accelerated rate of accumulated damage and associated pathophysiology in advanced age [39]. The brain is considered to be at particular risk for free radical damage because of high concentrations of easily oxidized polyunsaturated fatty acids and its high metabolic activity. Since the brain utilizes large amounts of oxygen, the generation of damaging reactive oxygen species is also elevated as the result of oxidative stress [6].

The ionotropic glutamatergic NMDA receptor has been reported to play an important role in neuronal development, normal synaptic transmission, learning and memory, and in various acute and chronic neuropathologic syndromes [7,28,30]. Deficits in NMDA-receptor-mediated glutamatergic neurotransmission have been described as biochemical mechanisms of age-associated deficits in cognitive functions [36]. Some evidence indicates that oxidative damage in the brain mediated by NMDA receptors may be related to reactive astrocytosis. Astrocytosis is a sequential morphological change of astrocytic reaction to tissue damage and is associated with the regulation of antioxidant defense mechanisms to reduce

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oxidative damage [20]. Reactive astrocytosis is a well known phenomenon that occurs in the normal aging process of the brain [43] and in response to many CNS pathologies, such as stroke, trauma, growth of a tumor, or neurodegenerative diseases [35]. Reactive glia may cause glutamate release and direct excitotoxicity to neurons [1]. Excessive stimulation of NMDA receptors by glial-provoked glutamate has been suggested to synergize with glutamate release from ischemic neurons, whose loss of NMDA receptors can be reversed by antioxidant treatment [4]. Activation of NMDA receptors has been reported to cause the formation of nitric oxide, a free radical gas, through nitric oxide synthase activation [49], which directly relates NMDA receptors to oxidative stress [39,44].

We have previously reported an NMDA receptor increase following treatment with the antioxidant agent vitamin E, but we found that the increases were age-independent [26]. To contrast these results, we studied the NMDA receptor response to treatment with the oxidant agent δ-aminolevulinic acid (ALA) at the same three ages: young (4-month-old), medium-aged (12-month-old) and aged animals (24-month-old) used in the previous report [26].

ALA is a heme precursor overproduced in various porphyric disorders [3,38,41] that has been reported to undergo enolization at pH 7.0–8.0 and subsequent iron-catalyzed oxidation, with the formation of reactive oxygen species [38] including the superoxide anion radical, hydrogen peroxide, the hydroxyl radical and the δ-aminolevulinic acid enoyl radical [6]. This behavior accounts for the reported ALA-induced oxidative damage to mitochondria, liposomes, DNA and proteins [10,12]. In addition, ALA administration to rats has been reported to generate free radicals in the brain [6] and to trigger oxidative damage to brain lipids and proteins [10], and it has been assayed as a prooxidant agent [11,33]. Moreover, ALA has been reported to inhibit glutamate uptake and stimulate the release of this excitatory neurotransmitter from rat synaptosomes [5,29]. Furthermore, glutamatergic mechanisms have been reported to underlie the ALA-induced convulsions [13].

NMDA receptors are heteromeric ligand-gated ion channels involved in glutamatergic transmission. Seven NMDA subunits (NR1, NR2A-D, NR3A-B) are known, allowing the existence of a heterogeneous population of receptor proteins with distinct physiological and biochemical properties [22,45]. NMDA receptors contain separate binding sites for the endogenous agonist glutamate (NMDA site) and glycine (a strychnine-insensitive site), both of which must be occupied for receptor activation. The ion channel is permeable to Ca^{+2}, Na^{+} and K^{+} and contains binding sites for Mg^{+2} and MK-801 (dizocilpine) (5-methyl-10, 11-dihydro-5*H*-dibenzo(a,d)cycloheptan-5,10-imine maleate), a non-competitive NMDA receptor antagonist widely used for characterizing this receptor [8,25,32,34]. MK-801 binding distribution shows a good correlation with NR2A subunit distribution [46], and hence we carried out a complementary Western blot study of this subunit expression to determine whether it was responsible for the differences in the [^{3}H]MK-801 binding studies.

To our knowledge, the present report is the first study on the changes in NMDA receptors that occur after chronic treatment with δ-aminolevulinic acid in rats at different ages using these methods.

2. Materials and methods

2.1. Animals

The animal experiments reported here were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Male Wistar rats aged 4, 12 and 24 months were paired and housed under a controlled light–dark cycle at a constant temperature (22–23 °C) with free access to food and water for 1 week prior to treatment with δ-aminolevulinic acid. For Western blotting assays, groups of 6 rats of each age were injected intraperitoneally with 40 mg/kg of δ-aminolevulinic acid once a day over 15 days. Thus, the three experimental groups were designated 4 MA (4-month-old ALA-treated rats), 12 MA (12-month-old ALA-treated rats) and 24 MA (24-month-old ALA-treated rats). Another three groups of six animals of each age (4 M, 12 M and 24 M) were used as controls and were injected with purified soy oil used as carrier (20% INTRALIPID®, Pharmacia).

A similar set of 6 groups (6 animals each) was used for membrane binding assays, and another similar set (6 groups, six animals) was used for autoradiographic assays. Identical treatment was carried out for each set of animals.

All animals were sacrificed by decapitation. The brains to be used in autoradiography were rapidly removed and rapidly frozen in liquid nitrogen. Those for use in the Western blotting and membrane binding assays were removed, and the cerebral cortex and hippocampus were dissected out on ice and immediately frozen in liquid nitrogen until used.

2.2. Membrane homogenates

To obtain membrane homogenates for membrane homogenate binding assays, the procedure was carried out as described previously [8]. Thus, cerebral cortex and hippocampus were allowed to thaw at 4 °C, minced and homogenized in 15 volumes of ice-cold 5 mM Tris–ClH buffer, pH 7.4 using a Polytron (PT10/35) homogenizer at a setting of 6 for 10 s (twice). The whole process was carried out at 4 °C. Homogenates were centrifuged at 48,000 × *g* for 20 min at 4 °C. The supernatant was discarded, and the pellet was washed twice under the same conditions and frozen for 24 h. Then, the pellet was resuspended in the