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Research Paper

Possible involvement of membrane lipids peroxidation and oxidation of catalytically essential thiols of the cerebral transmembrane sodium pump as component mechanisms of iron-mediated oxidative stress-linked dysfunction of the pump's activity

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ABSTRACT

The precise molecular events defining the complex role of oxidative stress in the inactivation of the cerebral sodium pump in radical-induced neurodegenerative diseases is yet to be fully clarified and thus still open. Herein we investigated the modulation of the activity of the cerebral transmembrane electrogenic enzyme in Fe^{2+}-mediated in vitro oxidative stress model. The results show that Fe^{2+} inhibited the transmembrane enzyme in a concentration dependent manner and this effect was accompanied by a biphasic generation of aldehydic product of lipid peroxidation. While dithiothreitol prevented both Fe^{2+} inhibitory effect on the pump and lipid peroxidation, vitamin E prevented only lipid peroxidation but not inhibition of the pump. Besides, malondialdehyde (MDA) inhibited the pump by a mechanism not related to oxidation of its critical thiols. Apparently, the low activity of the pump in degenerative diseases mediated by Fe^{2+} may involve complex multi-component mechanisms which may partly involve an initial oxidation of the critical thiols of the enzyme directly mediated by Fe^{2+} and during severe progression of such diseases; aldehydic products of lipid peroxidation such as MDA may further exacerbate this inhibitory effect by a mechanism that is likely not related to the oxidation of the catalytically essential thiols of the ouabain-sensitive cerebral electrogenic pump.

Introduction

Reports have shown that the activity of Na^+/K^+-ATPase in many tissues and organs of mammalian systems can be profoundly inhibited under conditions of oxidative stress related diseases. Thus the pathophysiology, molecular and biochemical mechanisms underlying the reduced activity of the electrogenic enzyme in several free-radical linked degenerative and metabolic pathologies have been an area of intense study [1–5]. Although Na^+/K^+-ATPase is widely expressed in quite a number of tissues, the transmembrane protein is mainly expressed in brain and neuronal cells. Consequently, dysfunction of the pump has been characterized by neuronal hyperexcitability, depolarization and swelling [6]. Hence, the malfunctioning of the cerebral sodium pump has been associated with some specific neurological disorders such as diabetes, Alzheimer's disease, epilepsy and bipolar disorder among others. In these neurological pathologies, constant depolarization across the cell membrane induces an imbalance in the amount of neurotransmitters that are released within the cell [3,4,7–10].

More importantly, it has been observed that neurological disorders associated with low activity of the cerebral sodium pump have also been characterized by increased oxidative stress indices in the brain of such human or disease models. Thus there is an inverse relationship between extent of oxidative stress mediated damage and the activity of the Na^+/K^+-ATPase in these neurological dysfunctions [11]. However, earlier investigators have attempted to unravel the underlying mechanisms that participate in free radicals-induced dysfunction of the pump. In this regard, emerging data led to some speculative conclusions.

Firstly, it was suggested that reactive oxygen species (ROS) may evoke an inhibition on the enzyme indirectly. Herein, the ROS may attack lipids in the membrane that anchor the transmembrane

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enzyme and consequently leading to an altered membrane microviscosity, which ultimately result in loss of the enzyme function [12,13]. On the other hand, ROS have been shown to directly attack and eventually modify amino acid residues of the enzyme thus leading to loss of activity of the transmembrane protein [14]. Yet some authors have speculated that aldehydic products which arise from the lipid peroxidation process may also interact with the transmembrane enzyme ultimately leading to its inactivation [15,16].

Despite these findings and observations however, the precise biochemical and molecular events that characterize free radical-induced dysfunction of the cerebral enzyme appear complex and far from being completely understood. Partly, the complexity arises from the multifactorial agents and factors that mediate oxidative stress. Therefore, in order to better understand the molecular events associated with oxidative stress-induced inactivation of the cerebral pump, the study of the participation of these agents and factors must be holistic, all inclusive and total. Evidently, unravelling these complex mechanism(s) is thus still open.

One of the most potent neurotoxic agent that exhibit prooxidant effect under in vitro and in vivo conditions is iron especially Fe^{2+}. In fact, reports have shown that impaired iron metabolism is an initial cause of some neurodegeneration mediated by ROS [17]. Moreso, several common genetic and sporadic neurodegenerative disorders have been associated with dysregulated iron homeostasis in the central nervous system (CNS) [17]. In general, iron accumulation causes neurodegeneration chiefly by inducing the formation of free radicals which eventually damage cellular macromolecules such as lipids, DNA and proteins. Furthermore, iron-induced oxidative stress is particularly dangerous because it can cause further iron release from iron-containing proteins such as ferritin, heme-proteins, and Fe–S clusters, forming a destructive intracellular positive-feedback loop that exacerbates the toxic effects of brain iron overload [17].

The harmful and deleterious effect of iron especially in the brain has been the prime interest of researchers. Primarily, this is because the mammalian brain is highly susceptible to free radical attack due to high oxygen tension and a high content of polyunsaturated fatty acids in cell membrane phospholipids [18]. Evidences suggest that iron invasion is an initial cause of neuronal cell death and axonal degeneration [19–21]. Moreso, a cardinal pathology associated with several common sporadic neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease has been associated with iron accumulation. Findings have shown that under in vitro models relating to these pathologies, there is a strong coincidence between localized disturbed iron homeostasis and neuronal cell death in the brain [19,20,22–26]. Furthermore, several in vivo studies of iron regulatory proteins using transgenic rodents [27,28] have indeed suggested that iron may be a pathogenic factor for neurodegeneration. Indeed experimental evidences have suggested that iron chelators [20,29,30] and genetic manipulation resulting in low iron levels [29,31,32] are neuroprotective strategies against these iron-induced neurodegenerative diseases. Other central nervous system (CNS) disorders that have been proposed to be associated with disturbed iron homeostasis include the Freiderich's Ataxia [26], Restless Leg Syndrome [33], Ischaemic/Haemorrhagic Stroke [34] and Multiple Sclerosis [25].

From the foregoing, it is apparent that iron-mediated ROS production may be a suitable model in unravelling the precise mechanisms of ROS-mediated dysfunction of the ouabain-sensitive electrogenic sodium pump. Really, some authors have studied iron-induced oxidative stress models to study the inactivation of the cerebral pump's activity. In this regard, Rauchova and colleagues observed that iron-induced altered membrane fluidity thus leading to the inhibition of the Na^+/K^+-ATPase activity at the initial process of lipid peroxidation. However, as lipid peroxidation progresses, the inhibition of the transmembrane enzyme strongly correlates only with the production of thiobarbituric acid reactive species (TBARS) and conjugated dienes (CD) [11]. However, their study is largely inconclusive and thus still open. Primarily, their study did not take into consideration the fact that the transmembrane enzyme is a sulphydryl protein and thus the possibility of a direct or indirect interaction between iron and the enzyme was not clarified and hence the need for the present study.

Materials and methods

Chemicals

Adenosine triphosphate (ATP), α-tocopherol, cysteine, reduced glutathione, dithiothreitol, thiobarbituric acid (TBA), were obtained from Sigma (St. Louis, MO). All other chemicals which are of analytical grade were obtained from standard commercial suppliers.

Animals

Male adult Wistar rats (200–250 g) from our own breeding colony were used. Animals were kept in separate animal cages, on a 12-h light: 12-h dark cycle, at a room temperature of 22–24 °C, and with free access to food and water. The animals were used according to standard guidelines on the Care and Use of Experimental Animal Resources.

Preparation of tissue homogenate

Rats were decapitated under mild ether anaesthesia and the cerebral tissue (whole brain) was rapidly removed, placed on ice and weighed. The brain was immediately homogenized in cold 10 mM Tris–HCl, pH 7.4 (1/10, w/v) with 10 up-and-down strokes at approximately 1200 rev/min in a Teflon–glass homogenizer. The homogenate was centrifuged for 10 min at 4000*g* to yield a pellet that was discarded and a low-speed supernatant (S1).

Incubation systems for TBARS and sodium pump assays

Aliquot of S1 was used for the assays of thiobarbituric acid reactive substances (TBARS) as well as Na^+/K^+-ATPase activity. For both assays the reaction mixture contained 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris–HCl, pH 7.4, $FeSO_4$ (final concentrations range of 1–100 μM), with and without α-tocopherol (final concentration, 1–100 μM) and with and without dithiothreitol (final concentration, 2 mM), with or without malondialdehyde (MDA) (1–100 μM) and 100–180 μg protein in a final volume of 500 μl. The reaction was initiated by addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. The reaction mixture was incubated at 37 °C for 30 min. At the end of the incubation time period, tubes were assayed for sodium pump activity and TBARS production.

Assay of sodium pump

The reaction system for the assay of the activity of cerebral Na^+/K^+-ATPase was essentially the same as described above under the section “incubation systems for TBARS and sodium pump”. However, at the end of the incubation time period (30–60 min), the reaction was stopped by addition of 5% trichloroacetic acid. Released inorganic phosphorous (P_i) was measured by the method