REDISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN RESPONSE TO CEREBRAL ISCHEMIA IN RAT BRAIN

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD), a cytoplasmic enzyme, plays a protective role during oxidative stress in eucaryotic cells, since they provide coenzymes and substrates to the primary antioxidant enzymes. The redistribution of G6PD in the hippocampus was studied post-ischemia (PI). There was a characteristic localisation of G6PD in pyramidal cell layers of the rat hippocampus. In hippocampus CA1 cells were stained weakly whereas CA3 cells showed strong histochemical staining. Ischemia induced up-regulation of G6PD in the hippocampus was in a specific manner. First, the activity increased in the whole hippocampus (at 4 hours PI) which persisted 6 hrs PI in CA1 area. However G6PD activity decreased in the CA3 area & dentate gyrus. At 10 & 24 hrs PI, activity decreased in CA1 area but normalised in CA3 area & dentate gyrus compared to controls. This suggests that the sensitive CA1 neurons are transiently capable of generating an anti-oxidative arsenal to cope with the oxidative stress in the first few hours PI. We can conclude that the brain contains inducible endogenous mechanisms that are capable of enhancing the ability of neurons to withstand lethal ischemic challenge.

KEY WORDS

Glucose-6-phosphate dehydrogenase, cerebral ischemia, hippocampus, oxidative stress, selective vulnerability.

INTRODUCTION

Brain tissue is most vulnerable to ischemia as compared to other organs. A stoppage of oxygen supply for 4-5 min. is sufficient to do irreparable damage to the human brain. The mechanisms that give rise to ischemic brain damage have not been fully determined but there is mounting evidence that destructive mechanisms associated with ischemia are initiated by activation of glutamate receptors resulting in elevated intracellular Ca^{2+} and reactive oxygen formation (1). The brain damage due to a cerebral ischemia is divided into three stages. In first stage of induction secondary to ischemia there is release of glutamate leading to activation of NMDA receptors (2). This is followed by stage of amplification during which raised intracellular levels

of Ca^{2+} take place, exciting neighboring neurons. In the last stage of expression there is generation of oxygen free radicals, activation of various proteases and phospholipases which cause neuronal death (3).

Mammalian brain is most vulnerable to oxidative stress because of its high rate of oxidative metabolism, intensive production of reactive oxygen metabolites, relative low antioxidant capacity and low repair mechanism (4,5). Oxidative stress is believed to be implicated in a variety of human degenerative disorders of the CNS, including Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis and in pathological conditions such as ischemia (6,7). The cytotoxic oxygen species are mainly hydrogen peroxide (H_2O_2), superoxide anions (O_2^-) and hydroxyl radicals ($\text{OH}^\bullet$). The different brain areas are endowed with enzymatic and nonenzymatic defense system to participate in the cellular defense against reactive oxygen species (ROS). Among the antioxidant enzyme system Glutathione peroxidase, catalase and Superoxide dismutase primarily constitute the antioxidant system. Other enzymes

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such as G6PD, 6-phosphogluconate dehydrogenase by virtue of their ability to produce NADPH along with Glutathione reductase are conventionally considered as supporters of the antioxidant systems. G6PD is a cytoplasmic enzyme that affects the production of reduced form of cytosolic nicotinadenosine dinucleotide phosphate coenzyme (NADPH) by controlling the step from Glucose-6-Phosphate to 6-phosphogluconate in the pentose phosphate pathway (8,9). This enzyme is highly conserved during evolution and plays multiple roles in the cell. Until recently, the role of this housekeeping enzyme in the cell response to the oxidative stress was limited to human erythrocytes that lack any other NADPH producing route. However, recent observations have shown that the G6PD also plays a protective role against reactive oxygen species in eucaryotic cells that possess alternative routes for the production of NADPH and that G6PD expression is upregulated by oxidants through a mechanism acting mainly on the rate of transcription of this gene (10,11). The aim of present study is to observe G6PD activity at different time intervals after global transient ischemia in the rat model.

MATERIAL AND METHODS

Cerebral ischemia: Transient forebrain ischemia was performed in male wistar rats (250-300g) according to Smith et al (12). Cerebral ischemia was induced by clamping both common carotid arteries lowering the mean arterial BP to 40 mm Hg. After 10 min. the infusion of blood and the removal of the clamps restored the blood pressure. Arterial pH, pCO₂, pO₂, arterial blood pressure and plasma glucose concentration were determined 15 min. before and 15 min. after the induction of ischemia.

Experimental Design: Animals (15 rats) were divided into two groups: controls (n=3) and control ischemic rats (12 rats). In group 2 (n=3), animals were sacrificed at different time interval post ischemia i.e. at 4hr, 6 hr, 10 hr and 24 hr.

G6PD Histochemical staining: Rats were killed by decapitation under deep anesthesia using chloral hydrate. Brains were rapidly removed and put in 30% sucrose solution overnight at 4°C. Unfixed cryostat sections (12 µm thick) of rat brain were prepared using -20° C microtome cryostat and collected on glass slides. The sections were air dried and processed for G6PD activity. The

demonstration of G6PD activity on cryosections was done by incubating for 45 -60 minutes at 37° C in a medium containing 0.1 M Tris buffer (pH 7.2), 2.5 mg / ml NADP sodium salt, 2 mg / ml glucose 6 phosphate, 1 mg / ml Nitro blue Tetrazolium (NBT). Control slides were processed with the above mixture in the absence of substrate (13). To ensure comparable histochemical staining, sections were processed together at the same time under same conditions. The intensity of histochemical staining for G6PD was determined by measuring the optical density (OD), using an image analysis system (Zeiss, IBAS). The optical density was expressed in arbitrary units corresponding to grey levels. For background we measured areas which were not stained for G6PD. The optical density of each area was related to the background value by the formula: $OD \text{ area} - OD \text{ background} / OD \text{ background} \times 100$ (14).

Statistical Analysis: Statistical analysis was performed by using the student -t- test.

RESULTS

All sections after staining, when examined showed G6PD enzymatic activity localised in the cerebral cortex, cerebellum, olfactory bulbs and hippocampus exhibited a special pattern of enzymatic activity distribution (Fig. 1-A). G6PD activity was more expressed in pyramidal cells of Ammon's horn than in granular cells of dentate gyrus (Fig. 1-A). Moreover, there was remarkable difference in the G6PD distribution within Ammon's Horn itself moving from CA1 to CA3 subfield (Fig. 2A-B and Fig. 3). The pyramidal cells of the CA3 region were more stained than the neurons in the CA 1 area. The histochemical staining is localised in cytosol and dendrites (Fig. 2-A).

A significant increase in G6PD activity was observed at 4-hrs post ischemia in the whole hippocampus (Fig. 1-B). At 6 hrs after ischemia, a marked increase in G6PD activity was detected in the hippocampal CA1 subfield and a decrease in the dentate and CA3 subfields (Fig. 1C, 2C, 3). There was no change in G6PD activity in other brain areas such as cerebral cortex or striatum (not shown). At 10 and 24 hrs post ischemia, the G6PD activity was decreased in the CA1 area (Fig. 2D) and normalized in dentate and CA3 subfields compared to controls.

DISCUSSION

G6PD is considered as supporter of the primary

antioxidant enzymes. It is a ubiquitous enzyme that supplies the cell with NADPH required for a variety of reductive reactions and biosynthetic processes. The identification of G6PD in response to experimental ischemia highlights the potential importance of this reaction and its products in the tissue response to this insult. But the bimodal reaction of G6PD activity i.e. its increase at 4 hrs and decrease at 10-hrs post ischemia in the CA1 subfield is striking. The progressive fall in G6PD activity observed at 10 and 24 hrs postischemia may be due to inhibition of protein synthesis and accelerated proteolysis and structural disintegration. Further, the generation of highly reactive oxygen species with their potent damaging effects on lipids, proteins and DNA leads to increase G6PD activity. This induction of G6PD could be a part of protective response to generate local antioxidative defense system.

During oxidative stress, change in the levels of various lipid peroxide products and antioxidant enzymes occurs. Seif-El-Nasr et al. (15) have shown increase in lipid peroxide products-malondialdehyde, glutathione and phospholipids along with antioxidant enzymes - superoxide dismutase, lactate dehydrogenase after cerebral ischemia. G6PD is also a part of an inducible mechanism of cell response to oxidative stress. Salvemini et al. (11) have demonstrated that G6PD expression is enhanced by oxidative stress induced

by agents that either increase the intracellular O₂ concentration or decrease the GSH pool. The mechanism regulating G6PD expression appears to affect the rate of transcription initiation.

From our study, it seems that there is a good correlation between selective vulnerability of hippocampus and the distribution of G6PD. At speculative level we suggest that low G6PD activity in CA1 area decreases their capacity to sustain the antioxidant enzymes to neutralize hydrogen peroxide formed during the ischemic challenge. The relatively high activity of G6PD in the CA3 areas explains the role of it in the production of high concentration of NADPH, which is involved in the reduction of the aldehydes, derived from the metabolism of the neurotransmitters (9,13). It also sustains the antioxidant enzymes to cope with the hydrogen peroxide production under ischemic conditions. Akai et al (16) have also demonstrated that CA1 cells were weakly immunostained whereas CA3 pyramidal cells were strongly reactive for SOD, an antioxidant enzyme. The differences in the intensity of SOD immunostaining reactions may relate to the variations in the susceptibility of the hippocampus to ischemia.

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Fig. 1: Histochemical demonstration of G6PD activity in unfixed cryostat sections from hippocampus in controls showing the striking difference in activity between the cells in the CA3 & CA1 subfields (A); 4 hrs post ischemia showing increased G6PD activity in the whole hippocampus (B) and 6 hrs post ischemia showing remarkable decrease of G6PD activity in dentate gyrus and CA3 subfields (C). Scale = 50 mm.

Fig. 2: Histochemical demonstration of G6PD activity in unfixed cryostat sections from hippocampus in controls showing the striking difference in activity between the cells in the CA3 subfield (A) and the CA1 subfield (B) .C: shows a detail of the CA1 subfield 6 hrs post ischemia (White arrow). D: shows markedly decreased G6PD activity 24 hrs post ischemia. Scale = 30 mm.

Fig. 3: Optical density analysis of histochemical staining for G6PD in the rat hippocampus of controls, at different time intervals after ischemic insult.

$p < 0.05$ in controls and $p < 0.01$ in 6, 10 and 24 hours postischemia. Statistical analysis by Student t-test.

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