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Research Report

Transient oxygen–glucose deprivation causes immediate changes in redox activity in mouse brain tissue

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ABSTRACT

Redox activity is an important property of living cells, and decreases in redox activity are likely to be an upstream event in ischemic brain injuries. In this study, immediate changes in redox activity caused by ischemic injury were investigated in oxygen–glucose deprivation (OGD) treated mouse brain tissue. Adult mouse brain slices were subjected to 10 min or 15 min OGD treatments and were immediately stained with an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) staining procedure. After 10 min OGD, the redox activity decreased in the lateral globus pallidus (LGP), medial globus pallidus (MGP), pyramidal cell layer of hippocampus CA1 ($CA1_{PL}$) and the granular layer of the cerebellum ($cereb_{GL}$). After 15 min OGD, decreases also occurred in the substantia nigra (SN) and several other areas of the brain stem. Hoechst 33342 was used to confirm that changes in redox activity occurred before morphological alterations in the cellular nuclei — morphological changes were not observed even after a 60 min OGD. The results presented here indicate that functional ischemic vulnerability exists in several brain regions, and will be helpful for systematic research on mammalian brain injury caused by transient metabolic stress.

1. Introduction

The mitochondrion is a primary target of ischemic injury. Mitochondrial redox activity is used to describe the cellular and tissular injury caused by ischemic stress (Connelly et al., 2000; Lipton, 1999; Mathews et al., 2000). In a large number of patients, development of obvious and complicated clinical symptoms in the initial stages of reperfusion treatment after transient ischemic injury suggests that changes in mitochondrial function in various brain areas have already taken place and may not be easy to reverse (Lipton, 1999). However, immediate changes in redox activity in these brain areas following transient ischemic injury have still not been demonstrated. In ischemic brain tissues, changes in redox activity have been detected by TTC (2,3,5-triphenyltetrazolium chloride) staining, but functional changes have not been found

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Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OGD, oxygen–glucose deprivation; TH, tyrosine hydroxylase; LGP, lateral globus pallidus; MGP, medial globus pallidus; SN, substantia nigra; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; $CA1_{PL}$, the pyramidal cell layer of the hippocampus CA1; $cereb_{GL}$, the granular layer of the cerebellum

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earlier than histomorphological changes such as attenuated staining of microtubule-associated protein 2 in the striatum after middle cerebral artery occlusion, and decreases in cell density in the hippocampus CA1 in gerbils after global cerebral ischemia (Kuroiwa et al., 1996; Popp et al., 2009). Immediate changes in redox activity following transient oxygen–glucose deprivation (OGD) have been detected in the hippocampus using a MTT assay and mitochondrial endogenous fluorescence; however, the location of these changes in the brain tissues cannot be identified by these methods (Brongholi et al., 2006; Kann and Kovacs, 2007; Shiino et al., 1998). We have previously demonstrated an immediate decrease in redox activity in the substantia nigra (SN) of the mouse brain following 20 min OGD at room temperature using an improved MTT-staining method (Chen et al., 2007). Here, in order to understand the complex clinical symptoms that emerge in the initial stages of reperfusion treatment after transient ischemic injury from the point of view of mitochondrial function, we have analyzed ischemic vulnerability by testing redox activity using a MTT-staining procedure following 10 min or 15 min OGD treatments in different anatomical structures of the mouse brain.

2. Results

2.1. *Mapping brain tissues with MTT staining*

An improved MTT staining method was used to investigate the change in redox activity in mouse brain tissues at 31 °C. Fig. 1 shows representative photographs of MTT-stained slices after perfusion with ACSF for 15 min (CT), or OGD solution for 10 or 15 min, and cresyl violet-stained CT slices. In MTT-stained slices, the change in redox activity can be estimated from the gray scale, and regions with a lower gray scale than their corresponding CT were considered to be regions where the redox activity was decreased by the OGD treatments. Table 1 lists the changes in redox activity of several regions which are considered to be related to ischemic clinical symptoms.

The mapping of redox activity in control brain slices shown in Fig. 1 indicates that metabolic rates vary according to region in the mammalian brain under normal conditions *in vivo*. However this inference requires further experimental confirmation.

2.2. *10 or 15 min OGDs cause alterations in tissue redox activity in the basal ganglia*

With the exception of the caudate–putamen nucleus (CPu), the main nuclei of the basal ganglia, including the lateral globus pallidus (LGP), and substantia nigra compacta (SNc), and the output nuclei of the substantia nigra reticulata (SNr) and medial globus pallidus (MGP), had significantly lower redox activities than the CT. After a 10 min OGD treatment, the LGP showed the most significant reduction in crystal density; its reduction was severe especially in the central part of serial coronal slices, with an average decrease of 50.3±4.5% compared to the CT (Fig. 2). After a 15 min OGD treatment, further decreases in redox activity were observed in the LGP. The SN, including both the SNr and the SNc, did not display significant alterations in redox activity in the 10 min OGD group, but displayed a visible reduction in redox activity just lower than that of the LGP in the 15 min OGD group. This decrease was severe especially in the rostral part of serial coronal slices (Fig. 3). A decrease in redox activity in the SNr region was visible from images of MTT-stained tissues and could also be detected in the SNc (Fig. 3 A–C) after double-staining of the SN with MTT and a TH antibody which allows visualization of dopaminergic neurons.

2.3. *10 or 15 min OGDs cause immediate alterations in tissue redox activity in the cerebellum*

In the cerebellum, the granular layer ($cereb_{GL}$) showed a significant reduction in crystal density in both the 10 min and 15 min OGD groups, and analysis of micrographs indicated that the redox activity of the $cereb_{GL}$ showed a significant decrease of almost the same extent in these two groups (Fig. 4).

2.4. *10 or 15 min OGDs cause immediate alterations in tissue redox activity in the hippocampus*

Alterations in crystal density were not so significant in the hippocampus after both 10 and 15 min OGD treatments. However, analysis of the images indicated alterations in redox activity in some subregions of the hippocampus. Redox activity in the pyramidal cell layer of CA1 ($CA1_{PL}$) decreased to almost the same extent (26%) in both the 10 min and 15 min OGD groups.

2.5. *Changes in tissue redox activity occur before morphological alterations in the nucleus*

In order to confirm that the change in redox activity was earlier than morphological alterations in cellular nuclei, slices were loaded with Hoechst 33342, a DNA-specific dye, after OGD treatments. OGD treatments of 10 min and 15 min were lengthened to 15 min and 60 min respectively, since, in other reports, changes in the nuclei occurring after short-term ischemic injury often occur after a delay (Kuroiwa et al., 1996). However, from the fluorescence images of Hoechst 33342, no obvious differences in the morphology of cellular nuclei were observed between the CT and the 15 or 60 min OGD-treated slices which included all the regions investigated using MTT staining described above. Fig. 5 shows an example of Hoechst 33342-stained nuclei in the $CA1_{PL}$.

3. Discussion

This research is the first to examine the tissue redox activity caused by 10 or 15 min OGD in various anatomical structures of brain which are considered to be important in cognition, perception and movement. These treatment durations are longer than those used in a gerbil global cerebral ischemic model which were able to cause delayed injury in hippocampus $CA1_{PL}$ (Kuroiwa et al., 1996) and shorter than those used in a rat middle cerebral artery occlusion model which were able to cause infarct in the striatum (Christensen and Diemer,