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Research Report

Simultaneous expression of glutathione, thioredoxin-1, and their reductases in nerve transected hypoglossal motor neurons of rat

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ABSTRACT

Anti-oxidative stress responses are crucial for the survival of nerve-injured motor neurons. Herein, we examined changes in expression of glutathione reductase (GSHr), thioredoxins (TRX1 and TRX2), and thioredoxin reductases (TRXr1 and TRXr2), important constituents of anti-oxidative pathways, following rat hypoglossal nerve transection. RT-PCR and in situ hybridization demonstrated that GSHr, TRX1, and TRXr1 mRNAs were significantly up-regulated during the first few weeks in nerve-injured motor neurons, while TRX2 and TRXr2 mRNAs were unchanged throughout 8 weeks after nerve transection. The up-regulation of GSH, GSHr, TRX1, and TRXr1 proteins in injured neurons was confirmed by immunohistochemical analysis. Western blotting also demonstrated up-regulation of GSHr, TRX1, and TRXr1 in injured neurons. These data suggest that the two major redox systems, GSH/GSHr and TRX1/TRXr1, are simultaneously activated in injured neurons, and likely provide protection of injured neurons against oxidative stress.

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1. Introduction

Neurons are vulnerable to damage from oxidative stress, with failure of protective mechanisms against oxidative stress considered to lead to neuronal death, including many adult-onset neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) (Bossy-Wetzel et al., 2004; Fukui and Moraes, 2008). Nerve-injured neurons are also exposed to various stresses including oxidative stress (Raivich and Makwana, 2007). However, nerve-injured neurons in the peripheral nervous system (PNS) are capable of surviving and regenerating. As neurons in the central nervous system (CNS) are under a different environment, expression of antioxidant molecules in response to PNS nerve injury may contribute to a reduction in oxidative stress and maintenance of neuronal integrity.

The fine regulation of the intracellular redox state is essential for limiting neuronal cell death induced by oxidative stress. The redox environment within neural cells is controlled by multiple redox systems. The glutathione (GSH)-mediated redox system is a major cellular redox component, and plays a critical role in regulating redox-dependent cellu-

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Abbreviations: GSH, glutathione; GSHr, glutathione reductase; TRX, thioredoxin; TRXr, thioredoxin reductase; SOD, superoxide dismutase

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lar functions. Additionally, the thioredoxin (TRX) system contributes significantly to the redox environment by reducing inter- and intra-chain protein disulfide bonds, as well as by maintaining the activity of important antioxidant enzymes such as peroxiredoxins (Mansur et al., 1998; Masutani et al., 2005; Kondo et al., 2006). The sum of these effects determines overall cellular function and integrity (Maher, 2006). In this respect, it is feasible that the major antioxidant systems, including GSH and TRX, play vital roles in the mechanisms allowing injured neurons to cope with oxidative stress. Critical processes which regulate these antioxidant functions include the ability to synthesize GSH and TRX, and to keep them reduced via glutathione reductase (GSHr) and thioredoxin reductase (TRXr). Mammalian cells contain one GSH and GSHr coupling system and two major TRX and TRXr coupling systems, the cytosolic TRX1/TRXr1 and the mitochondrial TRX2/TRXr2 (Mustacich and Powis, 2000). However, little is known about alterations of those reductases and their expression profiles in response to nerve injury.

In the present study, we examined the expression profiles of molecules associated with the major redox systems in response to nerve injury, and revealed that two major redox systems, GSH and TRX, were simultaneously induced in injured motor neurons.

2. Results

To clarify whether GSH/GSHr and TRX/TRXr are involved in nerve regeneration, we initially examined the mRNA expression profiles for GSHr, TRX1, TRX2, TRXr1, and TRXr2 at 3 and 7 days after hypoglossal nerve transection using RT-PCR. There was a significant increase in GSHr, TRX1, and TRXr1 mRNA expression at 7 days after axotomy, but no change in TRX2 and TRXr2 mRNA expression (Fig. 1A). The relative mRNA signal intensities for GSHr, TRX1, and TRXr1 were increased 1.5-, 1.3-, and 1.6-fold, respectively, in the injured side compared with that in the control side at 7 days after hypoglossal nerve injury (Fig. 1B–D).

To confirm these alterations and to further identify the cell types expressing GSH/GSHr and TRX/TRXr, in situ hybridization histochemistry (ISH) was performed using radioactive probes. Both film and emulsion autoradiography produced similar results to RT-PCR data (Fig. 2A). To determine the cell types expressing GSHr, TRX1, and TRXr1 mRNA, an emulsion autoradiogram was observed under bright-field illumination after Nissl counter staining. The silver grains indicating hybridization signals for GSHr, TRX1, and TRXr1 mRNA were accumulated on large cells, but not on the surrounding glial cells (Fig. 2B), suggesting induced expression on nerve-injured motor neurons. The hybridization signals on the hypoglossal nuclei of the film autoradiogram were measured to determine the mRNA expression profiles during recovery up to 8 weeks (Fig. 2C). Expression of GSHr mRNA was significantly up-regulated from 3 days after nerve injury, and peaked at 7 days. TRX1 and TRXr1 mRNA were markedly up-regulated on the injured side from 3 days after hypoglossal nerve transection, which remained elevated for 4 weeks after nerve injury (Fig. 2C).

By Western blotting, the levels of GSHr, TRX1, and TRXr1 proteins were increased by 1.3-, 1.9-, and 1.3-fold, respectively, at 7 days after axotomy in the injured side compared with the control side of hypoglossal nuclei (Fig. 3A; Student's *t*-test, $p<0.05$). Immunohistochemical staining of GSH, GSHr, TRX1, and TRXr1 proteins was also increased in injured motor neurons, with a prominently cytoplasmic expression pattern (Fig. 3B). The increased GSH, GSHr, TRX1, and TRXr1 immunoreactivity was initially detected in the ipsilateral side at 1 day after surgery, but markedly increased to a peak at 5 to 7 days. Collectively, these results suggest that GSH, GSHr, TRX1, and TRXr1 are transcriptionally and translationally up-regulated in injured motor neurons.

3. Discussion

The present study demonstrated that both the GSH and TRX redox systems were induced in nerve-injured motor neurons, and that they seem crucial for motor neuron survival and regeneration. Interestingly, GSHr and TRXr1 were simultaneously induced along with GSH and TRX1 in response to nerve injury, likely due to the fact that most, if not all, of the functions of GSH and TRX1 depend on their reductase activity. These coupled inductions between GSH-GSHr and TRX1-TRX1r are advantageous for promoting and maintaining their enzymatic activities against oxidative stress in nerve-injured motor neurons. It is generally assumed that these two major thiol-containing systems function in redox control, and support enzyme systems for elimination of peroxides. Each system has also been shown to have other distinct functions; GSH, for instance, is well suited for redox control of monothioles in proteins, whereas TRX is a two-electron reductant that can directly reduce sulfenic acids in proteins (Jones, 2008). In fact, the GSH and TRX systems were previously shown to have unique functions in the control of the transcription factor Nrf-2, with cytoplasmic activation of Nrf-2 being regulated by glutathione and DNA binding by TRX1 (Hansen et al., 2004). As such, although it is unclear whether these systems have similar or unique functions in the control of redox systems, it is likely that they function synergistically to protect motor neurons from various oxidative stresses.

In the present study, both cytoplasmic TRX1 and TRXr1 were up-regulated, while there was no change in expression of the mitochondrial TRX2 and TRXr2, suggesting that the cytoplasmic TRX system may play a greater role in the mechanisms underlying suppression of the oxidative stress response to nerve injury. Recently, it was demonstrated that mice lacking TRXr1 exhibit cerebellar defects, while no obvious histopathology was observed in mice lacking TRXr2, suggesting that TRXr1, but not TRXr2, plays a critical role in neuronal development and function (Soerensen et al., 2008). By contrast, mice with cardiac cell-specific inactivation of the TrxR2 gene developed fatal dilated cardiomyopathy, and died shortly after birth (Conrad et al., 2004), while mice with a heart-specific deletion of TRXr1 developed normally, with no obvious phenotypes (Jakupoglu et al., 2005). Thus, these data suggest that TRXr2 is crucial for heart development and function, while TRXr1 activity is required for neuronal development and

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