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Research Report

Transient down-regulation and restoration of glycogen synthase levels in axotomized rat facial motoneurons

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ABSTRACT

In adult rats, transection of the facial nerve causes a functional down-regulation of motoneurons and glial activation/proliferation. It has not been clear how energy-supplying systems are regulated in an axotomized facial nucleus. Here we investigated the regulation of molecules involved in glycogen degradation/synthesis in axotomized facial nuclei in rats. Immunoblotting revealed that the amounts of glycogen phosphorylase in the contralateral and ipsilateral nuclei were unchanged for the first 14 days, whereas the amount of glycogen synthase in the axotomized facial nuclei was significantly decreased from days 7–14 post-insult. A quantitative analysis estimated that the glycogen synthase levels in the transected nucleus were reduced to approx. 50% at 14 days post-injury. An immunohistochemical study showed that the injured motoneurons had decreased expressions of glycogen synthase proteins. The glycogen synthase levels in the axotomized facial nucleus had returned to control levels by 5 weeks post-insult, as had the cholinergic markers. The immunohistochemical study also revealed the recovery of glycogen synthase levels at the later stage. The glycogen phosphorylase levels in the injured nucleus were not significantly changed during weeks 3–5 post-insult. Taken together, these results demonstrated that the injured facial motoneurons transiently reduced glycogen synthase levels at around 1–2 weeks post-insult, but restored the levels at 4–5 weeks post-insult.

Abbreviations: ABC, avidin biotin peroxidase complex; anti-GP1 antibody, anti-glycogen phosphorylase antibody recognizing liver-, muscle- and brain-type GP; anti-GP2 antibody, anti-glycogen phosphorylase antibody recognizing brain-type GP; CD11b, cluster of differentiation 11b; ChAT, choline acetyltransferase; CNS, central nervous system; GFAP, glial fibrillary acidic protein; GLUT, glucose transporter; GP, glycogen phosphorylase; GS, glycogen synthase; HRP, horseradish peroxidase; Iba1, ionized Ca^{2+} binding adapter molecule 1; NF, neurofilament; NR3B, N-methyl-D-aspartate receptor 3B subunit; PBS, phosphate-buffered saline; VAchT, vesicular acetylcholine transporter.

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1. Introduction

Axotomy of the adult rat facial nerve results in motoneuronal and glial changes in the ipsilateral nucleus (Moran and Graeber, 2004). In the injured facial motoneurons, transient reductions are observed in the levels of choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAchT) and m2 muscalinic acetylcholine receptor (Ichimiya et al., 2013), suggesting that the motoneurons are functionally depressed. In contrast, ramified microglia around the injured motoneuron cell bodies transform into activated microglia, and they start to proliferate with enhanced levels of proliferating cell nuclear antigen and cyclins A/D (Yamamoto et al., 2010). Astrocytes in lesioned rat facial nucleus did not proliferate (Graeber et al., 1998), but they promoted the expression of glial fibrillary acidic protein (GFAP) for several weeks (Graeber and Kreutzberg, 1988), suggesting that the astrocytes maintain the functionally active state for long time.

These motoneuronal alterations and glial responses have been thought to be closely associated with energy-supply systems, because these events require a significant amount of energy for protein synthesis/DNA replication. In fact, the glucose influx into transected rat facial nucleus (Kreutzberg and Emmert, 1980; Ito et al., 1999) and that into transected rat hypoglossal nucleus (Smith et al., 1984) was found to increase. In sciatic nerve injury, glycogen phosphorylase (GP), which has the ability to catalyze the degradation of glycogen to glucose, has been reported to be elevated in injured motoneurons (Woolf et al., 1984). These findings suggest that the glucose supply is facilitated in the insulted nucleus/motoneurons. In addition to this issue, we were further inclined to identify the changes in molecules related to the glucose-supplying system. We found no reports of studies in which the levels of glucose metabolism-related proteins were quantitatively determined in injured nuclei/motoneurons.

In the present study, we analyzed the amounts of GP for glycogen degradation and glycogen synthase (GS) for glycogen synthesis in the injured and control rat facial nuclei. Although the levels of GP were not significantly changed between control and injured nuclei for 5 weeks, the GS levels of the injured facial motoneurons were found to reduce temporarily at days 7–14 after axotomy, and then return to normal levels by 5 weeks post-insult, accompanied by functional recovery.

2. Results

2.1. *Neuronal response and glial activation in the transected rat facial nucleus*

First, we investigated the response of motoneurons and glial cells to facial nerve injury in the axotomized rat facial nucleus. The immunoblot analysis showed that functional markers for motoneurons ChAT (EC 2.3.1.6) and VAchT in the operated nucleus were down-regulated between days 3 and 14 post-insult (Fig. 1(A), upper panel). However, no significant differences in the levels of neurofilament (NF) between the contralateral and ipsilateral nuclei were found (Fig. 1(A), upper panel).

The amounts of the astrocytic marker GFAP on the transected side (R) were recognized to be slightly higher than those on the control side (L) from days 5 to 14 post-axotomy (Fig. 1(A), lower panel). The levels of ionized Ca^{2+} binding adapter molecule 1 (Iba1) protein on the axotomized side (R) began to increase at day 3, peaked at day 5, and thereafter decreased gradually (Fig. 1(A), lower panel). There was no significant difference in the levels of actin between the control and transected facial nuclei (Fig. 1(A), lower panel).

Since the profiles of ChAT, VAchT, GFAP, Iba1 and actin (Fig. 1(A)) agree well with our previous results (Yamamoto et al., 2010; Ichimiya et al., 2013), these facial nucleus samples were used for the analyses as described below.

2.2. *Changes of enzymes for glycogen degradation/synthesis*

Hypothesizing that glycogen degradation and/or synthesis is affected in the transected facial nucleus, we compared the levels of GP (EC 2.4.1.1), which is essential for glycogen degradation, and those of GS (EC 2.4.1.11) which catalyzes glycogen synthesis between the transected and control sides.

We carried out the immunoblotting for GP by using two types of antibodies. The first determination was done using anti-GP1 antibody (GP1), which can recognize liver-, muscle- and brain-type GP. The results indicated that there was no significant differences in the levels of GP in either nuclei up to day 14 (the values relative to the contralateral side: 101.8±14.5%, 97.7±3.7%, 88.9±10.5%, 104.1±7.3%, and 92.4±15.7% at 1, 3, 5, 7 and 14 days post-insult, respectively; Fig. 1(B)). The second analysis using anti-GP2 antibody (GP2), which can recognize brain-type GP, also showed no significant difference in the levels of GP between the transected and contralateral nuclei (valued relative to the contralateral side: 103.0±10.3%, 106.9±5.8%, 108.3±10.5%, 89.0±10.9%, and 94.8±5.7% at 1, 3, 5, 7 and 14 days post-insult, respectively; Fig. 1(B)). The results of these quantitative analyses demonstrated consistently that the amounts of GP did not change in the injured rat facial nucleus during the first 2 weeks post-insult.

In contrast, the amounts of GS in the injured nucleus began to decrease at day 7 post-insult (Fig. 1(C)). The quantification revealed that GS levels in the transected side were reduced to approx. one-half at 14 days post-injury (values relative to the contralateral side: 103.9±15.4%, 95.1±10.7%, 82.5±7.3%, 75.0±4.8%, and 46.9±6.9% at 1, 3, 5, 7 and 14 days post-insult, respectively; Fig. 1(C)).

A simultaneous analysis of three rats reinforced that GS levels in the transected facial nucleus declined at 14 days post-insult (value relative to the contralateral side, 43.0±6.1%; Fig. 1(D)). In contrast, no significant difference in PG levels was observed in the same samples (value relative to the contralateral side, 97.8±9.1%; Fig. 1(D)). The results indicate that the step of glycogen synthesis is affected in the axotomized facial nucleus.

2.3. *Identification of glycogen synthase-suppressing cells*

Since the expression of GS protein decreased significantly in the injured nuclei, we determined immunohistochemically