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BRAIN RESEARCH

Research Report

The sodium-driven chloride/bicarbonate exchanger NDCBE in rat brain is upregulated by chronic metabolic acidosis

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ABSTRACT

Acid extruders in neurons prohibit intracellular pH from falling very far below normal. Our recent report suggests that the acid-extruding sodium/bicarbonate transporter NBCn1 (Slc4a7) in rat brain is upregulated by chronic metabolic acidosis. In this study, we examined whether the Na^+-driven Cl/HCO_3 exchanger NDCBE (Slc4a8) is also upregulated by similar systemic acid loads. Immunoblot revealed NDCBE protein (130 kDa) expressed in a variety of rat brain regions. In the hippocampus, NDCBE was localized to CA1–CA4 pyramidal neurons and dentate gyrus granular neurons determined by immunoperoxidase immunohistochemistry. The staining was dispersed in cell bodies and dendrites. NDCBE protein expression was then compared between rats in chronic metabolic acidosis and control rats. Immunoblot of crude plasma membrane fractions from the hippocampus showed a slight increase in NDCBE in acidotic rats ($p=0.05$). However, the expression in CA3 pyramidal neurons was significantly increased, determined by immunohistochemistry and quantitative analysis. The increase was also observed in other neurons including entorhinal cortical neurons, posterior cortical neurons, and outer stellate cells in cerebellum. The staining in choroid plexus epithelia was unaffected by chronic metabolic acidosis. These data demonstrate that the Na^+-driven Cl/HCO_3 exchanger NDCBE is upregulated by chronic acid loads in a cell-specific manner.

1. Introduction

In most cells, the steady-state intracellular pH (pH_i) is determined by the balance between acid extruders and acid loaders. Acid extruders raise pH_i by removing acid or taking up base, while acid loaders lower pH_i by removing base or taking up acid. The sodium/bicarbonate transporters are acid-extruders that move HCO_3^- into the cytosol and buffer intracellular H^+ (Romero et al., 2004). Two different groups of acid-extruding HCO_3^- transporters are present in the CNS (Chesler, 2003): Na/HCO_3 cotransporters (Chen et al., 2008b;Cooper et al., 2005;Damkier et al., 2007;Majumdar et al., 2008), which normally move Na^+ and HCO_3^- into the cells, and Na^+-driven Cl/HCO_3 exchanger NDCBE (Chen et al., 2008a), which moves these ions in exchange for internal Cl^-. Na/HCO_3 cotransporters are further divided into electrogenic transporters (NBCe1 and NBCe2) and electroneutral transporters (NBCn1 and NBCn2/NCBE). In general, neurons possess NBCn1, NBCn2/NCBE, and NDCBE, whereas astrocytes possess NBCe1 and NBCe2.

Previous studies suggest that acid-extruding HCO_3 transporters in the brain are stimulated in response to acidity. NBCe1 in astrocytic cultures is upregulated by acidic media (Giffard et al., 2000). NBCe1 in gerbil hippocampus is upregulated by seizure, which lowers pH_i (Kang et al., 2002). NBCn1 in

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the neonatal rat cortex is upregulated by chronic respiratory acidosis (Kanaan et al., 2007). We also reported that NBCn1 in cultured hippocampal neurons from embryonic rats is upregulated after acidic incubation (Cooper et al., 2009) and that the transporter in rat hippocampus is upregulated by chronic metabolic acidosis (Park et al., 2010). The reason for these upregulations is probably to compensate H^+ load in acidic cells. The excessive load of H^+ caused by acidosis/acidification may drive neurons and glial cells to enhance HCO_3^- influx via Na/HCO_3 transporters.

Despite these reports, it is not known whether the functionally similar NDCBE is also affected by acidosis or acidification. NDCBE has been considered as the major acid-extruding HCO_3^- transporter in neurons (Baxter and Church, 1996; Schwiening and Boron, 1994); thus, predicting that NDCBE would respond to acidic pH more substantially than NBCs. In this study, we examined the effect of systemic acid load on NDCBE protein expression in rat brain. We examined NDCBE expression in rat brains and then compared its levels in different brain regions of control rats and rats in chronic metabolic acidosis by immunoblot, immunohistochemistry, and quantitative analysis of the staining. Our data show that NDCBE1 is upregulated in multiple neurons in a cell type-specific manner.

2. Results

2.1. *NDCBE is expressed in many different regions of rat brains*

We performed immunoblot to validate the polyclonal antibody against the N-terminal 45 amino acids (position 239–283) of human NDCBE. These residues are relatively unique to NDCBE among SLC4 bicarbonate transporters. Fig. 1A shows an immunoblotting result of membrane preparations from rat hippocampus. The NDCBE antiserum recognized an immunoreactive band with the expected molecular weight of 130 kDa for NDCBE. Preimmune serum also did not detect the band. The immunoreactive band also disappeared when the antiserum was preabsorbed with the MBP/NDCBE fusion protein containing the immunogenic amino acids. Fig. 1B shows immunocytochemistry of cultured neurons prepared from rat embryonic hippocampus (Cooper et al., 2005). Tetramethylrhodamine fluorescence of NDCBE was dispersed in neurons. Strong nuclear labeling is non-specific. Preabsorbing the antiserum with the MBP/NDCBE fusion protein showed negligible immunofluorescence in the cytosol of the cell body and dendrites.

We then performed immunohistochemistry of NDCBE in rat hippocampus (Fig. 1C). DAB-labeled NDCBE stainings were found throughout CA1–CA4 pyramidal neurons and dentate gyrus granular neurons. Neurons in the hilus were also stained. The staining in CA3 neurons was prominent in cell bodies and weak in proximal dendrites in stratum lucidum. The image at a higher magnification (100×) showed the staining being dispersed in the cytosol of the cell body. Controls without the primary antibody showed no staining. In other experiments, we compared NDCBE staining patterns with NBCn1 staining patterns in rat brain. The two proteins showed different staining patterns (see Supplementary content), despite that the two proteins share amino acids by more than 65%.

To determine NDCBE protein expression among different regions in the CNS, we probed a rat membrane blot containing different brain regions with the NDCBE antiserum (Fig. 2). NDCBE was strongly detected in olfactory bulb, cerebellum, posterior cortex, and frontal cortex. The protein was also moderately detected in the hippocampus.

2.2. *NDCBE in CA3 pyramidal neurons is upregulated by chronic metabolic acidosis*

We previously showed that NBCn1 in rat brain is upregulated by chronic metabolic acidosis (Park et al., 2010). To examine whether NDCBE is also upregulated, we performed immunoblot of membrane preparations from the hippocampus of normal *versus* acidotic rats. Chronic metabolic acidosis was induced by feeding rats with 0.4 N HCl-containing chow for 7 days. The arterial pH was pH 7.18 for acidic rats and 7.32 for control rats ($p<0.05$; $n=10$ for each). The arterial HCO_3^- level was 16.3 mM for acidotic rats and 24.2 mM for control rats ($p<0.05$), and P_{CO2} was 44 and 46 mm Hg, respectively. The reason is unclear, but a similar HCl-feeding protocol results in insignificant P_{CO2} change (Seshadri et al., 2006). Other plasma/urine chemistries of these animals are described previously (Park et al., 2010). Immunoblot showed that NDCBE protein expression was negligibly increased in acidotic rats (Fig. 3A). Semiquantitative measurements of NDCBE normalized to β-actin revealed negligible increase ($p=0.05$; Fig. 3B). Thus, chronic metabolic acidosis appears to cause negligible effect on the global expression of NDCBE in the hippocampus.

We then performed immunohistochemistry and compared NDCBE immunostainings in the hippocampus of control rats *versus* acidotic rats (Fig. 4A). We found that the staining in CA3 pyramidal neurons was substantially increased, while those in CA1 neurons and dentate gyrus granular neurons remained unaffected. The upregulation in CA3 neurons primarily occurred in the cell body. The neuronal staining in the hilus also appears to be increased. Quantitative measurements of the staining showed a 2.5-fold increase ($p<0.05$) in CA3 neurons of acidotic rats (Fig. 4B).

In parallel experiments, we analyzed NDCBE stainings in cerebellum and posterior cortex. The staining in cerebellar granule cells was unaffected by chronic metabolic acidosis, whereas the staining in posterior cortical neurons was increased by 1.7-fold ($p<0.05$).

2.3. *NDCBE response to chronic metabolic acidosis varies depending upon brain regions*

The above observation on NDCBE upregulation in selective neurons led us to test whether NDCBE response to chronic metabolic acidosis varies among different brain regions. Fig. 5 shows quantitation of NDCBE immunostainings in other brain regions. Acidotic rats exhibited increased NDCBE stainings in outer stellate neurons in cerebellum, entorhinal cortical neurons, and thalamic neurons. The staining in olfactory bulb was decreased ($p<0.05$). The nature of this decrease is unclear. In addition, NDCBE expression in choroid plexus epithelia remained unaffected.