GABA-Mediated Trophic Effect on Oligodendrocytes Requires Na-K-2Cl Cotransport Activity


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Wang, Hao, Yiping Yan, Douglas B. Kintner, Christian Lytle, and Dandan Sun. GABA-mediated trophic effect on oligodendrocytes requires Na-K-2Cl cotransport activity. J Neurophysiol 90: 1257–1265, 2003. First published April 2, 2003; First published April 2, 2003; 10.1152/jn.01174.2002. The Na-K-2Cl cotransporter isoform1 (NKCC1) is present in many animal cells where it plays prominent roles in regulating cell volume and maintaining intracellular Cl⁻ concentration ([Cl⁻]i) above electrochemical equilibrium. We show here that NKCC1 is present and active in cultured oligodendrocytes. Expression of NKCC1 in the rat spinal cord increased during development from postnatal day 6 through 21 in parallel with that of myelin basic protein. In cultured oligodendrocytes, 39% of the total K⁺ ([86Rb⁺]i) influx represented NKCC1 activity. Activation of GABA_A receptors with muscimol produced a reduction in intracellular Cl⁻ content, cell shrinkage, and a stimulation of NKCC1 activity. Muscimol also triggered an increase in intracellular Ca²⁺, which depended on NKCC1 activity. Survival of oligodendrocytes following withdrawal of growth factors was enhanced by muscimol and this effect also required NKCC1 activity. Our results suggest that NKCC1 functions in oligodendrocytes to maintain [Cl⁻]i above electrochemical equilibrium and that NKCC1 is required for GABAergic trophic effects.

**INTRODUCTION**


In various types of immature neurons, activation of γ-aminobutyric acid-A (GABA_A) receptors initiates a depolarizing efflux of Cl⁻ that triggers an influx of Ca²⁺ (Ben Ari et al. 1989). This Ca²⁺ then activates processes of maturation and differentiation (Ben Ari et al. 1997; Marty et al. 1996). GABA also triggers a depolarizing efflux of Cl⁻ in cultured oligodendrocytes (Kettenmann et al. 1991). Re-accumulation of intracellular Cl⁻ after depletion is blocked by removal of extracellular Na⁺ or inhibition of NKCC1 activity (Hoppe and Kettenmann 1989a,b). Thus NKCC1 may be important in maintaining the high [Cl⁻]i in oligodendrocytes. While the trophic effect of GABA on neurons is now well established (Fukuda et al. 1998; Ikeda et al. 1997; Marty et al. 1996), the effect of GABA on oligodendrocyte survival and growth remains uncertain.

Here we report that NKCC1 is abundantly expressed in oligodendrocytes and that its activity is regulated by GABA_A receptors. GABA-mediated oligodendrocyte survival depends on NKCC1 activity.

**METHODS**

**Materials**

Eagle’s balanced salt solution (EBSS), Leibovitz’s L-15 medium (L-15 medium), and Dulbecco’s modified Eagle’s medium (DMEM) were from Gibco (Grand Island, NY). Deoxyribonuclease I (DNase), poly-l-ornithine, platelet-derived growth factor (PDGF), and N1 medium were from Sigma (St. Louis, MO). Mouse anti-2'-3’-cyclic nucleotide 3’-phosphodiesterase monoclonal antibody (CNPase) was from Chemi-Con International (Temecula, CA). N-glycanase was from Glyko (Novato, CA). Fura-2-acetoxymethyl ester (AM) was from Molecular Probes (Eugene, OR). Rip monoclonal antibody was from Developmental Studies Hybridoma Bank (Iowa City, IA).

**Enriched oligodendrocyte culture**

Spinal cords were removed from 7- to 10-day-old rat pups (Sprague-Dawley) and transferred to a 60-mm dish containing ice-cold L-15 medium. Under a dissecting microscope, the meninges and nerve roots were removed. The spinal cords were cut into 1-mm³ pieces and incubated in EBSS containing 0.25 mg/ml trypsin and 50 μg/ml DNase at 37°C for 30 min with shaking. The enzymatic activity of trypsin was stopped by adding fetal bovine serum (FBS). After centrifugation, the tissues were resuspended with L-15 medium (10% FBS and 50 μg/ml DNase) and triturated. Cell suspension was centrifuged at 30,000 g at 4°C in 29% Percoll as described before (Grever et al. 1999). The cell pellet was suspended in DMEM supplemented with N1 medium, 10 ng/ml biotin, 0.5% FBS, and 50 μg/ml DNase and triturated. Cell suspension was centrifuged at 30,000 g at 4°C in 29% Percoll as described before (Grever et al. 1999). The cell pellet was suspended in DMEM supplemented with N1 medium, 10 ng/ml biotin, 0.5% FBS, and 10 ng/ml PDGF. In each well, 2 × 10⁴ (96-well plate), 6 × 10⁴ (24-well plate), and 12 × 10⁴ (6-well plate) cells were plated. The plates were coated with poly-l-ornithine (0.1 mg/ml). Cultures were maintained in 5% CO₂ atmosphere at 37°C and reseeded every 2 days with DMEM containing freshly prepared PDGF. More than ninety percent of cells in culture were oligodendrocytes, analyzed with immuno-flow cytometry. All animal procedures were conducted in strict compliance with the NIH guidelines.

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overnight with a primary antibody. The blots were then rinsed.

Immunofluorescence staining

Cultured cells grown on poly-l-ornithine-coated chamber slides were rinsed with PBS (pH 7.4) and fixed with 4% paraformaldehyde in PBS for 40 min at room temperature. After rinsing, cells were incubated with blocking solution (10% goat serum, 0.4% Triton X-100, and 1% bovine serum albumin in PBS) for 1 h. Cells were incubated with a primary antibody in blocking solution overnight at 4°C. After rinsing, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated or Texas red conjugated secondary antibodies for 1 h. The images were captured by a laser-scanning confocal microscope (Bio-Rad MRC 1000, Bio-Rad, Hercules, CA), as described before (Su et al. 2000).

Gel electrophoresis and immunoblotting

Cultured oligodendrocytes on six-well plates were washed with ice-cold PBS (pH 7.4) containing 2 mM EDTA and protease inhibitors as described previously (Sun and Murali 1999). Cells were scraped from the plate and lysed in PBS by 30 s of sonication at 4°C using an ultrasonic processor (Sonics and Materials, Danbury, CT). Rat spinal cord, Orem, UT) was treated with blocking solution (10% goat serum, 0.4% Triton X-100, and 1% bovine serum albumin in PBS) for 40 min at room temperature. After rinsing, cells were incubated with blocking solution (10% goat serum, 0.4% Triton X-100, and 1% bovine serum albumin in PBS) for 40 min at room temperature. After rinsing, cells were incubated with a primary antibody in blocking solution overnight at 4°C. After rinsing, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated or Texas red conjugated secondary antibodies for 1 h. The images were captured by a laser-scanning confocal microscope (Bio-Rad MRC 1000, Bio-Rad, Hercules, CA), as described before (Su et al. 2000).

Assay for NKCC1 activity

NKCC1 activity was measured as bumetanide-sensitive 86Rb influx, using 86Rb as a tracer of K+(Sun and Murali 1999). Cultured oligodendrocytes on 4–5 days in vitro (DIV 4–5) were equilibrated for 10–30 min at room temperature with an isotonic HEPES-buffered MEM (290 mOsm). The concentrations of components in HEPES-MEM were described before (Su et al. 2000). Cells were incubated for 10 min in HEPES-MEM with or without 10 μM bumetanide. The cells were exposed to 2 μCi/ml of 86Rb in HEPES-MEM for 3 min at room temperature, in either the presence or the absence of 10 μM bumetanide. 86Rb influx was terminated by rinsing cells with ice-cold 0.1 M MgCl2. Cells were extracted in 1% SDS, and radioactivity in cell lysates was analyzed by liquid scintillation counting (1900CA, Packard, Downers Grove, IL). 86Rb influx rate was calculated as the

![Image](https://example.com/image.png)

**FIG. 1.** Expression of Na+-K+–2Cl⁻ cotransporter isoform I (NKCC1) in cultured oligodendrocytes. A. Rip antibody staining (1:100). B. NKCC1 staining (with NT antibody (polyclonal antiserum) against the N-terminus of NKCC1, 1:200). C. double images of A and B. D. anti-2',3'-cyclic nucleotide 3'-phosphodiesterase monoclonal antibody (CNP) staining (1:100). E: NKCC1 staining (with NT antibody, 1:200). F: double images of D and E. G: NKCC1 staining (with T4 antibody against C-terminus of NKCC1, 1:100). H: bright-field image. I: negative control in which a primary antibody was omitted.
slope of $^{86}\text{Rb}$ uptake over time and expressed in nanomoles of $^{86}\text{Rb}$ per milligram of protein per minute. Six determinations were obtained in each experiment throughout the study, and protein content was measured in each sample by the bicinchoninic acid method (Smith et al. 1985).

**Intracellular Cl\(\textsuperscript{-}\) content measurement**

Cells on 96-well plates were preincubated in HEPES-MEM containing \(36\text{Cl} (0.4 \mu\text{Ci/ml})\) for 30 min at room temperature. The cells were then incubated in HEPES-MEM containing \(36\text{Cl} (0.4 \mu\text{Ci/ml})\) in the presence of either bumetanide (10 \(\mu\text{M}\)), muscimol (10 \(\mu\text{M}\)), muscimol plus bicusculine (10 \(\mu\text{M}\)), or muscimol plus bumetanide for 20 min. Intracellular \(36\text{Cl}\) content measurement was terminated by two washes with a washing buffer, as described previously (Su et al. 2002). Radioactivity of cellular extract in 1\% SDS was analyzed by liquid scintillation counting. In each experiment, specific activities (counts/\(\mu\text{mol/min}\)) of \(36\text{Cl}\) were determined for each assay condition and used to calculate intracellular Cl\(\textsuperscript{-}\) content (\(\mu\text{mol/mg protein}\)).

**Measurement of relative cell volume changes in a single cell**

Relative cell volume changes were estimated using video-enhanced differential interference contrast (DIC) microscopy, as described in our previous study (Su et al. 2002). Oligodendrocytes cultured on poly-L-ornithine-coated coverslips were placed in an open-bath imaging chamber (Warner Instruments, Hamen, CT; bath volume 40 \(\mu\text{l}\)) on the stage of a Nikon TE 300 inverted epifluorescence microscope. Cells were equilibrated with an isotonic HEPES-MEM (312 mOsm) for 15 min at room temperature (Su et al. 2002). Cells were exposed sequentially to isotonic HEPES-MEM, HEPES-MEM plus muscimol (10 \(\mu\text{M}\)), muscimol plus bicuculline (10 \(\mu\text{M}\)), and hypertonic HEPES-MEM (480 mOsm). Between each treatment, cells were re-equilibrated with isotonic HEPES-MEM. Cells were visualized using a Nikon 60\(\times\) Plan Apo oil immersion objective lens and cell images were recorded every minute as described previously (Su et al. 2002). The mean cross-sectional area (CSA) was calculated after tracing the perimeter of the cell body with MetaMorph image-processing software (Universal Imaging, Downingtown, PA).

The control CSA values were obtained when cells were exposed to isotonic HEPES-MEM only. Relative changes of mean cross-sectional area (CSAr) were calculated as experimental CSA/control CSA. Hypertonic buffer of 480 mOsm was prepared by holding the salt concentrations constant and adding 165 mM sucrose.

This approach to estimate cell volume changes can be criticized because it does not consider the cell volume changes in the z-axis. Thus we may underestimate the actual cell volume changes.

**Cell survival assay**

Calcein-AM and propidium iodide were used to determine survival of oligodendrocytes. Cells were grown in DMEM with supplements

![Figure 2](http://jn.physiology.org/Downloadedfrom)
(N1, Biotin, PDGF) for 2–3 days. Cells were then cultured for 18 h in either DMEM plus supplements, DMEM plus 30 μM muscimol, DMEM plus 30 μM muscimol and 10 μM bicuculline, or DMEM plus 30 μM muscimol and 10 μM bumetanide, respectively. To assay the effect of 25 mM [K⁺]o on survival of oligodendrocytes, cells were cultured in DMEM plus 25 mM KCl, DMEM plus 25 mM KCl, and 1 μM nifedipine for 18 h. Nifedipine is stable in culture medium for ≥30 h (Franklin et al. 1995). Muscimol and bicuculline are stable in cultures for 6 days (Franklin et al. 1995). Images to the sum of calcein-AM- and propidium iodide positive cells. Mortality was calculated as the ratio of propidium iodide positive cells 1000 cells were counted in each condition in a blind manner. Cell imaging chamber containing HEPES-MEM at room temperature. The chamber was mounted on the stage of the Nikon TE 300 inverted epifluorescence microscope. Oligodendrocytes were excited every 10 s at 340 and 380 nm and the emission fluorescence at 510 nm was recorded. The ratio of 340/380 was used to indicate the [Ca 2+]i as described before (Gryniewicz et al. 1985; Su et al. 2000) Images were collected and analyzed with MetaFluor image-processing software.

Statistics

Throughout the study, statistical significance was determined by analysis of variance (ANOVA; Bonferroni/Dunn) at a confidence of 95% (P < 0.05).

RESULTS

Localization of NKCC1 in oligodendrocytes by immunofluorescence staining

As shown in Fig. 1, A and D, oligodendrocytes in primary cultures were identified by immunocytochemical detection of the oligodendrocyte-specific proteins Rip and CNP (Jhaveri et al. 1992). Oligodendrocytes in DIV 2–3 culture exhibited a “spider’s web”-like morphology with many slender and branched processes (Fig. 1, A–G, H). NKCC1 was detected in the cell body and processed with polyclonal antibody NT antiserum against the N-terminus of human NKCC1 (Fig. 1, B and E) and monoclonal antibody T4 (Fig. 1G), respectively. Colocalization of Rip and NKCC1 or CNP and NKCC1 was found in oligodendrocytes (Fig. 1, C and F).

Development-dependent expression of NKCC1 in oligodendrocytes from rat spinal cord

Expression of NKCC1 in spinal cords and cultured oligodendrocytes was also evaluated by immunoblotting. As shown in Fig. 2A, expression of NKCC1 in rat spinal cord varied over the course of postnatal development. In the rat spinal cord, NKCC1 was low at P1 yet progressively increased from P6 through adulthood (Fig. 2, A and C). A development-dependent pattern of expression was found for MBP. In contrast, levels of β-tubulin did not change significantly from P1 to adulthood (Fig. 2, A and C). NKCC1 was also detected in cultured oligodendrocytes (Fig. 2B). Deglycosylation of the native 146-kDa NKCC1 protein in oligodendrocytes with N-glycanase resulted in a core protein of approximately 135 kDa.

GABA_A receptor activation stimulates NKCC1 activity

In cultured oligodendrocytes (DIV 4–5) under control conditions, NKCC1 activity (bumetanide-sensitive 86Rb influx rate) was 9.1 ± 1.8 nmol/mg protein/min (Fig. 3A). This component constituted 39.3% of the total 86Rb influx and required the simultaneous presence of extracellular Na⁺ (Fig. 3A) or Cl⁻ (data not shown). Activation of the GABA_A receptor with muscimol stimulated NKCC1 activity approximately 50% (Fig. 3B). This stimulation was blocked by the GABA_A receptor antagonist bicuculline.

GABA_A receptor activation modulates intracellular 36Cl content in oligodendrocytes

As shown in Fig. 4A, cells were preequilibrated in HEPES-MEM with 36Cl (0.4 μCi/ml) for 0–50 min. A steady-state level of intracellular 36Cl was obtained by a
30-min incubation and maintained during the 50-min equilibration. Thus in the rest of the study, a 30-min preincubation was performed. After a 30-min equilibration with $^{36}$Cl, changes of intracellular Cl$^-$ content were measured when cells were exposed to HEPES-MEM containing $^{36}$Cl (0.4 $\mu$Ci/ml) with or without 30 $\mu$M muscimol for 20 min. Activation of the GABA$_A$ receptor with muscimol reduced intracellular Cl$^-$ content from 0.70 $\pm$ 0.05 to 0.48 $\pm$ 0.03 $\mu$mol/mg protein ($P < 0.05$, Fig. 4B). This response was blocked by bicuculline (Fig. 4B). Inhibition of NKCC1 activity with bumetanide had no significant effect on resting levels of Cl$^-$ content or on loss of Cl$^-$ evoked by muscimol (Fig. 4B).

**Activation of GABA$_A$ receptors leads to oligodendrocyte shrinkage**

We determined whether activation of the GABA$_A$ receptor causes shrinkage in oligodendrocytes. Figure 5, A and B, illustrates that muscimol caused CSAr to decrease in oligodendrocytes gradually over 10 min (0.95 $\pm$ 0.01; $P < 0.05$). This decrease is reversible and CSAr recovered to the basal levels in isotonic HEPES-MEM. In contrast, in the presence of both muscimol and the GABA$_A$ receptor inhibitor bicuculline, no significant changes in CSAr were found (1.00 $\pm$ 0.01, $P > 0.05$). The same oligodendrocytes remained responsive to a subsequent hypertonic challenge (0.91 $\pm$ 0.01, $P < 0.05$) and then returned to the basal cell volume levels when they were returned to isotonic HEPES-MEM (1.00 $\pm$ 0.01).

**GABA$_A$ receptor activation modulates oligodendrocyte $[Ca^{2+}]_i$**

Intracellular free Ca$^{2+}$ ($[Ca^{2+}]_i$) was monitored using the Ca$^{2+}$-sensitive dye fura-2. Muscimol evoked a transient increase in $[Ca^{2+}]_i$ (Fig. 6A). The increases in intracellular Ca$^{2+}$ in the first and second exposures of 100 $\mu$M muscimol were similar (0.106 $\pm$ 0.006 and 0.100 $\pm$ 0.003, respectively, Fig. 6A). This muscimol-induced response was blocked not only by the GABA$_A$ receptor antagonist bicuculline, but also by the NKCC1 inhibitor bumetanide (Fig. 6B and C). The average change of intracellular Ca$^{2+}$ induced by 100 $\mu$M muscimol was 0.106 $\pm$ 0.006. In the presence of bicuculline or bumetanide, the average change was 0.008 $\pm$ 0.005 or 0.006 $\pm$ 0.008, respectively (Fig. 6D, $P < 0.05$).
GABA<sub>A</sub> receptors affect oligodendrocyte survival

Activation of GABA<sub>A</sub> receptors in immature neurons leads to Ca<sup>2+</sup> influx and this is thought to play an important role in cell maturation during development (Ben Ari et al. 1997; LoTurco et al. 1995). Whether GABA-mediated Ca<sup>2+</sup> influx has a similar trophic effect on oligodendrocytes remains unknown. Survival of oligodendrocytes in vitro depends on the presence of growth factors (Noble et al. 1988; Raff et al. 1988). Consistent with these early reports, we found that removal of PDGF and other supplements led to cell death (Fig. 7). In contrast, activation of GABA<sub>A</sub> receptors with muscimol significantly reduced mortality following removal of growth factors. Whereas bumetanide did not affect basal cell death (22.8 ± 1.3%) or cell death induced by removal of PDGF and other supplements (Fig. 7), it prevented the effect of muscimol. Thus the improved survival of oligodendrocytes with the GABA<sub>A</sub> receptor activation appears to require NKCC1 activation.

High [K<sup>+</sup>]<sub>i</sub>-mediated cell survival

In neurons, the mechanism by which GABA<sub>A</sub> receptor activation improves survival is believed to involve Ca<sup>2+</sup> entry through depolarization-activated Ca<sup>2+</sup> channels. Membrane depolarization produced by raising extracellular potassium ([K<sup>+</sup>]<sub>e</sub>) is known to have a trophic effect in neurons (Franklin et al. 1995). This maneuver had a similar effect in oligodendrocytes (Fig. 8). Raising [K<sup>+</sup>]<sub>e</sub> to 25 mM prevented cell death induced by removal of PDGF and other supplements, but not in the presence of the L-type Ca<sup>2+</sup> channel antagonist nifedipine (1 μM). Neither nifedipine nor bicuculline had any effects on the basal levels of cell mortality in DMEM-containing supplements (Fig. 8). Cell mortality was not affected when NKCC1 activity was blocked by bumetanide in the presence of 25 mM [K<sup>+</sup>]<sub>e</sub> (Fig. 8). Measurements of intracellular Ca<sup>2+</sup> revealed that 25 mM [K<sup>+</sup>]<sub>e</sub> increased [Ca<sup>2+</sup>]<sub>i</sub>, through a mechanism blocked by nifedipine but not by bumetanide (Fig. 9, A–C). Taken together, our data suggest that a rise of intracellular Ca<sup>2+</sup> plays a role in the preservation of oligodendrocyte survival.
Ca\(^{2+}\) mediated by either muscimol or elevated [K\(^{+}\)], plays an important role in oligodendrocyte survival.

**DISCUSSION**

The results presented here establish that NKCC1 is expressed in the spinal cord and cultured oligodendrocytes. The expression of NKCC1 in developing spinal cords was found to parallel with spinal cord myelination. NKCC1-mediated \(^{86}\)Rb influx represented 39% of the total \(^{86}\)Rb influx in oligodendrocytes. The role of NKCC1 in oligodendrocytes is not understood. NKCC1 may serve to maintain [Cl\(^{-}\)]\(_{i}\) above electrochemical equilibrium in oligodendrocytes and thereby play a pivotal role in GABAergic functions. GABA-mediated depolarization depends on a relatively high [Cl\(^{-}\)]\(_{i}\). We found that NKCC1 activity was significantly stimulated in response to activation of GABA\(_{A}\) receptors. Given that NKCC1 is biochemically stimulated in response to decreases in [Cl\(^{-}\)]\(_{i}\) and cell volume (Lytle 1997), GABA may boost NKCC1 activity following Cl\(^{-}\) efflux, a reduction in [Cl\(^{-}\)]\(_{i}\), and subsequent cell shrinkage. In accord with this hypothesis, activating GABA\(_{A}\) receptors brought about a decrease in oligodendrocyte Cl\(^{-}\) content and cell shrinkage.

However, concurrent inhibition of NKCC1-mediated Cl\(^{-}\) influx and activation of GABA-induced Cl\(^{-}\) efflux did not cause additive effects on a decrease in [Cl\(^{-}\)]\(_{i}\). The mechanisms

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**FIG. 7.** GABA-mediated survival of oligodendrocytes. Cells (DIV 3) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) without supplements, DMEM plus 10 \(\mu\)M bumetanide, DMEM plus 30 \(\mu\)M muscimol, DMEM plus 30 \(\mu\)M muscimol and 10 \(\mu\)M bicuculline, or DMEM plus 30 \(\mu\)M muscimol and 10 \(\mu\)M bumetanide for 18 h. Control cells were cultured in DMEM plus supplements. Live cells were determined with calcein-AM. Dead cells were stained with propidium iodide. Summarized cell mortality data were shown. Data are means ± SE, \(n = 3–4\). * \(P < 0.05\) vs. DMEM plus supplements, \# \(P < 0.05\) vs. DMEM without supplements.

**FIG. 8.** High [K\(^{+}\)]\(_o\)-mediated survival of oligodendrocytes. Cells (DIV 3) were cultured for 18 h in DMEM without supplements, DMEM plus 25 mM KCl, DMEM plus 25 mM KCl and 1 \(\mu\)M nifedipine, or 25 mM KCl plus bumetanide (10 \(\mu\)M). Control cells were cultured in DMEM plus supplements for 18 h. Live cells were determined with calcein-AM. Dead cells were stained with propidium iodide. Cell mortality data were shown. Data are means ± SE, \(n = 3\). In the case of bumetanide, data are mean of 10 cells from one culture. * \(P < 0.05\) vs. 25 mM KCl-induced 1st peak.

**FIG. 9.** Changes of intracellular Ca\(^{2+}\) by high [K\(^{+}\)]\(_o\). A and B: cells were exposed to either HEPES-MEM (5 mM KCl), HEPES-MEM containing 25 mM KCl, 25 mM KCl plus 1 \(\mu\)M nifedipine, or 25 mM KCl plus 10 \(\mu\)M bumetanide. 25 mM KCl was obtained by replacing NaCl in HEPES-MEM with equimolar KCl. Data are representative examples of 3 experiments. Summarized data were shown in C. Data are mean ± SE, \(n = 3\). In the case of bumetanide, data are mean of 10 cells from one culture. * \(P < 0.05\) vs. 25 mM KCl-induced 1st peak.
underlying this observation are not clear. This may suggest that other Cl⁻ transport mechanisms (such as Na⁺-dependent and independent Cl⁻/HCO₃⁻ transporters and/or Cl⁻ channels) may conduct Cl⁻ influx and prevent a further loss of intracellular Cl⁻.

GABAAergic neurons develop earlier than glutamatergic neurons and GABA induces depolarization in immature neurons (Kaila 1994). This GABA-induced depolarization elevates [Ca²⁺], via activation of voltage-dependent Ca²⁺ channels and contributes to several aspects of the CNS development, including gene expression (Bading et al. 1993; Vaccarino et al. 1992) and neuronal growth and differentiation (Ben Ari et al. 1997; LoTurco et al. 1995). Our study suggests that GABA-mediated Ca²⁺-influx likewise exerts a trophic effect on oligodendrocytes. We found that activation of GABA_A receptors triggered a rise in intracellular Ca²⁺ through L-type voltage-gated Ca²⁺ channels and promoted oligodendrocyte survival following withdrawal of PDGF and other supplements. Inhibition of NKKC1 blocked this trophic effect. These data further support the view that NKKC1 activity is required for GABA_A receptor function in oligodendrocytes.

A functional link between the GABA_A receptor and L-type voltage-gated Ca²⁺ channels has been suggested in oligodendrocytes (Kirchhoff and Kettenmann 1992). We found that activating these channels by membrane depolarization (high [K⁺]_o), also promotes survival of oligodendrocytes. These findings are consistent with high [K⁺]_o-mediated trophic effects in developing neurons (Collins and Lile 1989; Franklin et al. 1995; Koike et al. 1989).

In the current study, 30–100 μM muscimol was used. We believe that the effects mediated by 30–100 μM muscimol are physiologically relevant. The in vivo ambient concentration of GABA in the extracellular space is 0.8–2.9 μM, which is sufficient to activate GABA_A receptors (Lerma et al. 1986). GABA in the GABAergic synaptic cleft can reach 0.5–1.0 mM (Maconoche et al. 1994). GABA released from neuronal growth cone or neurites could increase the local GABA concentration above the ambient concentration range and mediate the concentration of neuron and glial interaction in vivo. It has been hypothesized that activation of nearby glial GABA receptors could be the means for oligodendrocyte precursors to detect migrating neurites. Thus GABA may play a role in oligodendrocyte maturation (Kettenmann et al. 1991). Our findings imply that GABA release from the nearby neurons can exert trophic effects on oligodendrocytes by activating GABA_A receptor-mediated Cl⁻ efflux and triggering opening of voltage-dependent Ca²⁺ channels. However, these actions require the function of NKKC1, which maintains the intracellular Cl⁻ above the Cl⁻ equilibrium potential, and enable GABA to cause membrane depolarization. Therefore NKKC1 may play an important role in oligodendrocyte development.

The GABA_A receptor is a heteropentamer drawn from a repertoire of α₁–6, β₁–3, γ₁–3, δ, ε, π, θ, and ρ₁–3 subunits in the CNS (Lambert et al. 2001). Various subunit combinations influence the physiological and pharmacological properties of the receptor. Currently, the subunit combination of GABA_A receptor in oligodendrocytes is not clear. The pharmacological studies of the GABA_A receptor in oligodendrocytes indicate that GABA activates a Cl⁻ conductance in a dose-dependent manner (Kettenmann et al. 1991). The GABA response in oligodendrocyte precursor cells is blocked by picrotoxin and bicuculline. Pentobarbital and flunitrazepam increase the GABA-induced currents but β-carbolines act as inverse benzodiazepine agonists (Kettenmann et al. 1991).

In summary, our results demonstrate that NKKC1 is expressed at relatively high levels in oligodendrocytes where it helps maintain [Cl⁻]_i above electrochemical equilibrium in opposition to GABA-induced Cl⁻ efflux. Our data also suggest that NKKC1 activity may be important in oligodendrocyte development in association with GABA_A receptor function.

**DISCLOSURES**

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