Supporting Information

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SI Materials and Methods

Cell Culture and Transfection Procedures. For electrophysiological experiments, mixed cortical neuronal/glia cultures were prepared from embryonic day (E) 16 Sprague–Dawley rats (Charles River Laboratories). Cortices were dissociated with trypsin, and the resultant cell suspension, adjusted to 670,000 cells per well, was plated on glass coverslips in six-well plates as described (1). Nonneuronal cell proliferation was inhibited after 2 wk in culture with 1–2 μM cytosine arabinoside, and cultures used at 3 to 4 wk in vitro. For biochemical experiments, neuronal-enriched cultures (2) were prepared from E18 rats and seeded onto culture plates at a density of 470,000 cells per well. Cytosine arabinoside was applied 5 d after plating, and cultures were used at 12–14 d in vitro. For neuronal transfection, cells were treated for 5 h in serum-free medium with 2 μL of Lipofectamine 2000 (Invitrogen), 50 μL of OptiMEM (GIBCO), and 1.5 μg of DNA per well. For Chinese hamster ovary (CHO) cell transfections, cells were plated on coverslips in 24-well plates at a density of 5.6 × 10^4 cells per well for electrophysiological measurements or in 100-mm dishes at a density of 1.7 × 10^5 cells per dish for biochemical studies. Cells were treated for 3–4 h in serum-free medium with a total of 1.2 μL of Lipofectamine and 0.28 μg of DNA per well for electrophysiology, or 55.1 μL of Lipofectamine and 7.334 μg of DNA per dish for biochemistry. Following transfection, cells were maintained in F12 medium containing 10% (vol/vol) FBS at 37 °C, 5% CO_2 for 24 h before experimentation.

Drug Treatments and Antibodies. The apoptotic stimulus for electrophysiological and biochemical experiments was 10 min exposure to 30 μM 2,2'-dithiodipyridine (DTDP) at 37 °C, 5% CO_2. For electrophysiology, the DTDP-containing solution was removed before 3-h incubation in fresh medium containing 10 μM butyryl-carbonyl-aspartate-fluoromethyl ketone (BAF), a cysteine protease inhibitor that maintains cell viability without affecting K⁺ currents. Myristoylated CaMKIIα tide (1 μM; EMD) was used to inhibit CaMKII; thapsigargin (1 μM; Sigma Aldrich) was used to deplete endoplasmic reticulum Ca²⁺ stores. N,N,N',N'-tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN, 3 μM) and butyronitrate (BAPTA-AM) (100 μM) were used to chelate Zn²⁺ and Ca²⁺, respectively. Antibodies were purchased from the following suppliers: rabbit anti-p38, anti-phospho-p38, anti-CaMKII, and anti-phospho-CaMKII from Cell Signaling Technologies; rabbit anti-syntxin from Abcam; rabbit antioxidized-CaMKII were either a gift from M. Anderson (University of Iowa Carver College of Medicine, Iowa City, IA) or purchased from Millipore; mouse anti-Kv2.1 from NeuroMab; mouse anti-phospho-tyrosine from BD Biosciences; mouse anti-CaMKII from Santa Cruz Biotechnology. Immunofluorescence-purified serum directed at phosphorylated S800 of Kv2.1 was prepared in our laboratory (3); and rabbit anti-phospho-Kv2.1/603 antibodies were a gift from J. Trimmer (University of California, Davis).

Electrophysiological Measurements. Current recordings were performed on eGFP-positive cotransfected neurons or CHO cells by using the whole-cell patch clamp configuration technique as described (4). The intracellular (electrode) solution contained 100 mM K-glucuronate, 11 mM EGTA, 10 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂ × 2H₂O, 10 mM Hepes; pH adjusted to 7.2 with concentrated KOH; and 2.2 mM ATP and 0.33 mM GTP were added and the osmolality was adjusted to 280 mOsm with sucrose. The extracellular solution contained 115 mM NaCl, 2.5 mM KCl, 2.0 mM MgCl₂, 10 mM Hepes, 10 mM d-glucose; 0.25 μM tetradoxotin; pH adjusted to 7.2. Measurements were obtained under whole-cell voltage clamp with an Axopatch-1D amplifier and pClamp software (Molecular Devices), using 2–3 MΩ recording electrodes. Electrodes were pulled from 1.5-mm borosilicate glass (Warner Instruments) with a model P-97 mechanical pipette puller (Sutter Instruments). Series resistance was partially compensated (80%) in all cases. Currents were filtered at 2 kHz and digitized at 10 kHz with a Digidata 1440A Digitizer (Molecular Devices). K⁺ currents were evoked with incremental 10-mV voltage steps to +80 mV from a holding potential of −80 mV. To determine current density values, steady-state current amplitudes were measured 180 ms after the initiation of the +10-mV step and normalized to cell capacitance.

Viability Assays. Viability in transfected neurons was assessed by using a luciferase reporter assay (5, 6). Twenty-four hours after transfection with a luciferase reporter plasmid and any other plasmids of interest, cultures were treated overnight with either vehicle or microglia (50,000 cells per mL) (7) plated directly on top of neurons and activated by IFN-γ and lipopolysaccharide (8). Cells were then assayed for luciferase-mediated luminescence by using a SteadyLite luciferase assay system (PerkinElmer). The luciferase reagent was added directly to cells and luminescence measured after 10 min by using a Wallac 1420 96-well microplate reader (PerkinElmer Life Sciences). Cell viability is proportional to luciferase luminescence, confirmed by cell counts in GFP cotransfected sister coverslips (6).

Electrophoresis and Immunoblotting. Protein samples from equal amounts of cell lysate were separated on 7.5% or 10% SDS/PAGE by using the Mini Protean 3 System (Bio-Rad). Separated protein bands were transferred onto 0.2-μm nitrocellulose membranes (Bio-Rad), blocked with 1% BSA in PBS with 0.05% Tween 20 (PBST) at room temperature for 1 h, and probed with appropriate primary antibodies diluted in PBST. Blots were then incubated with infrared fluorescent goat secondary antibodies at room temperature for 1 h, visualized by using the Odyssey Imaging System (LI-COR Biosciences), and quantified by using infrared fluorometry.

Immunoprecipitation. Protein was harvested by washing cell culture dishes with ice-cold PBS followed by a short incubation with 1% [3-(cholamidopropyl) dimethylammonio]-1-propanesulphonate (CHAPS) buffer. Protein A/G agarose bead slurry (Santa Cruz) was added to the samples and rocked at 4 °C for 1 h to preclar nonspecific protein binding. Samples were then incubated overnight at 4 °C with the appropriate immunoprecipitating antibodies. Following another incubation with the bead slurry, the protein samples were prepared/de-natured by the addition of sample preparation buffer (625 mM Tris, 25% glycerol, 2% SDS, 0.01% bromophenol blue, and 5% β-mercaptoethanol) and heated at 95 °C for 5 min before SDS/PAGE and immunoblotting.

Intraneuronal Zn²⁺ and Ca²⁺ Imaging. To assess changes in intracellular free Zn²⁺, the Zn²⁺-sensitive fluorescent reporter FluoZin-3 (Molecular Probes) was used. FluoZin-3 responds robustly to small changes in Zn²⁺, is insensitive to high Ca²⁺ and magnesium, and is unaffected by low intracellular pH or oxidants (9). Further, Zn²⁺ signals from surrounding astrocytes are minimal under these conditions, because these cells appear to buffer intracellular Zn²⁺ more effectively than neurons (10). Intracellular
Ca\textsuperscript{2+} levels were measured by using the ratiometric, fluorescent Ca\textsuperscript{2+} dye Fura-2 (Teflabs). Coverslips were loaded with either FluoZin-3 AM or Fura-2 AM (5 μM, 30 min) and immediately transferred to a recording chamber (Warner) mounted on an inverted epifluorescence microscope. Using a computer controlled monochromator (Polychrome II; TILL Photonics) and CCD camera (IMAGO; TILL Photonics), images were acquired every 10 s by exciting cells with 485 nm light (520 nm emission) for Zn\textsuperscript{2+} imaging, or alternately with 340 and 380 nm light (510 nm emission) for Ca\textsuperscript{2+} imaging. After background subtraction, fluorescence was expressed as the difference between the average baseline fluorescence just before treatment and the average peak fluorescence during treatment (∆F) for 10–20 neurons per coverslip.


**Fig. S1.** CaMKII methionine oxidation by hydrogen peroxide. Representative immunoblot from protein samples were obtained from neuronal cultures under control conditions or after 10-min exposure to 100 μM H\textsubscript{2}O\textsubscript{2}. Blots were probed with antibodies specific for oxidized M281/282 CaMKII or total CaMKII.

**Fig. S2.** Immunoblot from Kv2.1-expressing CHO cells cotransfected with CaMKII or CDKS + p35 demonstrates that unlike CDKS, CaMKII does not elicit a phosphorylation-induced molecular weight shift of Kv2.1. Membranes were probed with antibodies specific for Kv2.1 or p-Kv2.1S603, a known target of CDKS used to confirm the effect of the cyclin-dependent kinase on Kv2.1 (1).

Fig. S3. Src expression is sufficient to induce the phosphorylation of Kv2.1 residues Y124 and S800 by Src and endogenous p38, respectively. (A) CHO cells were cotransfected with Kv2.1 and either Src or p38, and Kv2.1 was immunoprecipitated. Membranes were probed with antibodies specific for p-Kv2.1S800, p-tyrosine, or Kv2.1 to serve as a loading control. (B) Cells were cotransfected with Kv2.1 and either Src, p38, or both. Immunoprecipitation of Kv2.1 was followed by probing with antibodies specific for p-S800 or p-tyrosine.

Fig. S4. Zn$^{2+}$ chelation prevents the DTDP-induced enhancement of K$^+$ currents in neurons. Representative whole-cell K$^+$ currents and corresponding mean ± SEM current densities (at +10 mV) recorded from cortical neurons after exposure to 3 μM TPEN ($n = 7$) or 3 μM TPEN + 30 μM DTDP ($n = 9$). Currents were evoked by a series of voltage steps from −80 mV to +80 mV in 10-mV increments. Calibration: 10 nA, 25 ms.
Fig. S5. CaMKIIK42R-expressing neurons are resistant to DTDP-induced toxicity (60 μM, 10 min) compared with vector-expressing cells (mean ± SEM, n = 3; *P < 0.05; paired t test). Viability was assessed 24 h following exposure by using a luciferase expression assay (1).