Supporting Information

Hinson et al. 10.1073/pnas.1109980108

SI Materials and Methods

Cell Culture. Cells used for immunofluorescence studies were grown on glass coverslips coated with poly-D-lysine/laminin (astrocytes) or poly-L-lysine (HEK293), purchased from Biocoat, BD Biosciences. Cells used for Western blot studies were grown directly on tissue culture dishes.

Immunostaining. Oocytes were rinsed in ND96 (96 mM NaCl; 2 mM KCl; 1 mM $MgCl_2$; 1.8 mM $CaCl_2$; 5 mM Hepes, pH 7.5; osmolarity ~195 ± 5 mOsm), fixed in 4% paraformaldehyde in PBS (1 h at 22 °C) and then exposed to 100 mM glycine in PBS (30 min at 22 °C). After 16 h in 30% sucrose (at 4 °C), oocytes were mounted in Optimal Cutting Temperature (OCT) media, snap-frozen, and sectioned (10 μm). Sections were rehydrated in PBS and immunostained. Confocal images were captured using a Zeiss LSM510 confocal microscope with a 63× water immersion lens. Human brain tissue was fixed in 10–15% formalin, embedded in paraffin, sectioned, deparaffinized, and immunostained by avidin–biotin or alkaline phosphatase/anti-alkaline phosphatase technique after incubating for 16 h at 4 °C with mouse antimyelin proteolipid protein MAb IgG (Serotec) and rabbit anti-AQP4 C terminus IgG (Sigma-Aldrich). As controls, we omitted primary antibody.

Oocyte Injection. Prepared oocytes were injected using capped human AQP1, AQP4-M1, and AQP4-M23 (± EGFP) cRNA synthesized using linearized cDNA template and an mMessage mMachine kit (Ambion). Reaction product concentrations and quality were assessed by UV absorbance and gel electrophoresis, respectively. Oocytes were injected with 50 nL of cRNA (0.2 ng/nL) or water and held at 16 °C in OR_3 media (1).

Hypotonic Stress Assay. Oocytes injected with cRNA or vehicle (water) were incubated for 4 d at 16 °C prior to assay, rinsed in sterile ND96 buffer, exposed (4 h) to purified control-IgG or neuromyelitis optica (NMO)-IgG (2.5 mg/mL) at 22 °C or on a wet ice bath (at <4 °C), and transferred to distilled water of corresponding temperature. Lysis time was observed by dissecting microscope. Fold increase in lysis time was calculated by comparing incubations in control-IgG and NMO-IgG and expressed as the average of five or more independent experiments.

Oocyte Membrane Isolation. At 4 °C, rinsed oocytes were homogenized in buffer A (83 mM NaCl; 1 mM $MgCl_2$; 10 mM HEPES, pH 7.5); debris was sedimented (1,000 × *g* for 10 min); supernatant membranes were pelleted (1,000 × *g* for 20 min), washed twice, and resuspended (in 50 mM Hepes, pH 7.4; 2 mM EDTA); and aliquots were stored at −20 °C.

Mammalian Membrane Preparations. Membranes were prepared from homogenized cells using homogenization buffer (HB) (7.5 mM sodium phosphate, pH 7.0; 0.25 M sucrose, 5 mM EDTA, 5 mM EGTA with protease inhibitors). After clearing debris (1,000 × *g* for 10 min), supernatant membranes were sedimented (200,000 × *g* for 30 min at 4 °C), resuspended in HB, and snap-frozen. For Western analysis, thawed membranes were sedimented by centrifugation (20,000 × *g* for 30 min at 4 °C) and solubilized in 5% SDS, 20 mM Tris (pH 8.0), and 5 mM EDTA (at 37 °C for 30 min).

Western Blot. Proteins were resolved by electrophoresis (15% polyacrylamide, 22 °C), transferred to nitrocellulose (2), blocked, exposed (1 h) to AQP4-IgG (mouse Mab7, 1:2,000) or rabbit anti-actin IgG (1:2,000), and washed and probed with horseradish peroxidase-conjugated IgG specific for mouse or rabbit IgG. Astrocyte membrane proteins (5 μg) were resolved on 4.0% acrylamide/2.0 M urea stacking gel and 12% acrylamide/4.0 M urea resolving gel (3, 4) to separate AQP4 isoforms, transblotted to PVDF membrane, and blocked and exposed to probe IgGs. We detected bound IgG autoradiographically with SuperSignal West Pico Chemiluminescence (Pierce).

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