Supporting Information

Zhou et al. 10.1073/pnas.1302167110

SI Experimental Procedures

cDNAs and Mutagenesis. The muscarinic receptor 1 (M1) cDNA was a gift from Hailin Zhang (University of Hebei, Shijiazhuang, China). green fluorescent protein fusion with PH domain of phospholipase C (GFP-PH-PLC) was a gift from Patrick Verstreken (Katholieke Universiteit Leuven, Leuven, Belgium). Danio rerio voltage-sensitive phosphatase (Dr-VSP) was a gift from Yasushi Okamura (Okazaki Institute for Integrative Bioscience, Okazaki, Japan). Voltage-gated potassium channel (Kv)subfamily 7 (KCNQ)1, KCNQ2, and KCNQ3 were gifts from D. Mackinnon (State University of New York, Stony Brook, NY), M. Sanguinetti (University of Utah, Salt Lake City, UT) and M. Shapiro (University of Texas Health Science Center, San Antonio, TX), respectively. Point mutations were introduced by using the Quick Change II site-directed mutagenesis kit (Stratagene) and verified by DNA sequencing. CHO cell culture, transient transfection, and electrophysiological recording were carried out according to the published protocols (1, 2).

Fluorescence Measurements of Phosphatidylinositol 4,5-Bisphosphate Hydrolysis. CHO cells were grown on glass coverslips and transfected with GFP-PH-PLC plasmid. The cells were perfused with the same extracellular solution as that used during electrophysiological recording and were imaged using a 40× objective. Images acquired using Nikon Eclipse Ti were analyzed using Nikon Elements BR3.2. Constant regions within the profile of each cell were designated as membrane and cytoplasm individually (excluding the nucleus). The ratio of average fluorescence intensity of membrane to cytoplasm was calculated and plotted.

Hippocampus Neuron Culture and Recording in Neurons. The neuronal culture method was adopted and modified from the protocol (3). Briefly, hippocampal neurons were dissected and isolated from embryonic day 18 (E18) C57BL mice (4–6). The tissue was digested with papain (Worthington Biochem) and resuspended into single cells. The single-cell suspension was plated on poly-D-lysine–coated coverslips. The neurons were maintained in culture medium consisting of neurobasal medium with B-27 supplement (Invitrogen), penicillin, streptomycin, and 2 mM L-glutamine. Cultured neurons on day 12 to day 15 were used for electrophysiological recording. Whole cell recording conditions were the same as those used in transfected cells.

Oocyte Preparation and Macropatch Recording. *Xenopus laevis* oocytes were prepared and injected following previously reported protocols (7). mRNA for each channel was made using Ambion's mMESSAGE mMACHINE T7 kit. The mRNA was injected at 10–30 ng/oocyte and recordings were performed 3–7 d later. For macropatch recordings electrodes with resistances 1.5–2 MΩ were filled with filter ND96 solution containing 2 mM KCl, 91 mM NaCl, 1 mM $MgCl_2$, 5 mM NaOH, and 5 mM Hepes (pH 7.4 with NaOH). Internal (bath solution) was a high-potassium solution containing 96 mM KCl, 5 mM EDTA, and 10 mM Hepes (pH 7.4 with KOH). Before establishing an inside-out patch, the bath solution was switched to recording solution, which contains 60 mM KCl, 5 mM $EGTAK_2$, 5 mM KF, 0.1 mM Na_3VO_4, 10 mM $K_4P_2O_7$, and 10 mM Hepes (pH 7.4). Recordings were performed using the EPC10 (Heka) amplifier and Pulse software (Heka) at room temperature. Solutions were applied using the DAD12 (ALA Instruments) fast superfusion system.

Data and Statistical Analysis. Patch-clamp data were processed using Clampfit 9.2 (Molecular Devices), and then analyzed in Graphpad Prism 5 (GraphPad Software). Dose–response curves were fitted with the Hill equation: $E = E_{max}/(1 + (EC_{50}/C)^P)$, where EC_{50} is the drug concentration producing half of the maximum response, and P is the Hill coefficient. Voltage-dependent activation curves were fitted with the Boltzmann equation, $G = G_{min} + (G_{max} - G_{min})/(1 + \exp(V - V_{1/2})/S)$, where G_{max} is the maximum conductance, G_{min} is the minimum conductance, $V_{1/2}$ is the voltage for reaching 50% of maximum conductance, and S is the slope factor. Data are presented as means ± SE. Significance was estimated using paired two-tailed Student's *t* tests.

1. Xiong Q, Sun H, Li M (2007) Zinc pyrithione-mediated activation of voltage-gated KCNQ potassium channels rescues epileptogenic mutants. *Nat Chem Biol* 3(5):287–296.
2. Xiong Q, Sun H, Zhang Y, Nan F, Li M (2008) Combinatorial augmentation of voltage-gated KCNQ potassium channels by chemical openers. *Proc Natl Acad Sci USA* 105(8): 3128–3133.
3. Ahlemeyer B, Baumgart-Vogt E (2005) Optimized protocols for the simultaneous preparation of primary neuronal cultures of the neocortex, hippocampus and cerebellum from individual newborn (P0.5) C57Bl/6J mice. *J Neurosci Methods* 149(2): 110–120.
4. Liu XH, Lü GW, Cui ZJ (2002) Calcium oscillations in freshly isolated neonatal rat cortical neurons. *Acta Pharmacol Sin* 23(7):577–581.
5. Zhang SL, et al. (2001) Effect of dl-praeruptorin A on ATP sensitive potassium channels in human cortical neurons. *Acta Pharmacol Sin* 22(9):813–816.
6. Zhang W, Jin HW, Wang XL (2004) Effects of presenilins and beta-amyloid precursor protein on delayed rectifier potassium channels in cultured rat hippocampal neurons. *Acta Pharmacol Sin* 25(2):181–185.
7. Logothetis DE, Petrou VI, Adney SK, Mahajan R (2010) Channelopathies linked to plasma membrane phosphoinositides. *Pflugers Arch* 460(2):321–341.

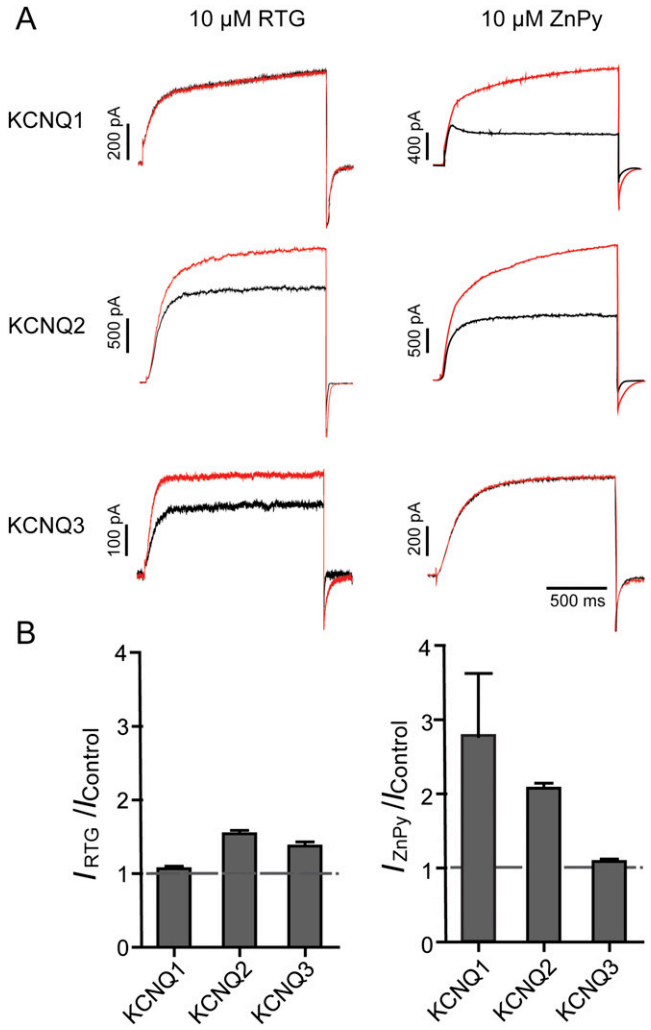

Fig. S1. Retigabine and zinc pyrithione (ZnPy) display differential selectivity for KCNQ subtypes. (*A*) Whole cell currents of CHO cells transfected individually with KCNQ1, KCNQ2, or KCNQ3 were recorded in the absence (black) and presence (red) of 10 μM retigabine (*Left*) or ZnPy (*Right*). Holding potential was −100 mV (retigabine), or −80 mV (ZnPy), respectively. KCNQ currents were elicited by depolarization to −10 mV (retigabine) or +50 mV (ZnPy), respectively. (*B*) Histogram shows retigabine and ZnPy effects for the indicated homomeric KCNQ channels. Normalized current amplitude is shown. Dashed lines indicate mean value of 1 (i.e., no change to control; $n \geq 3$).

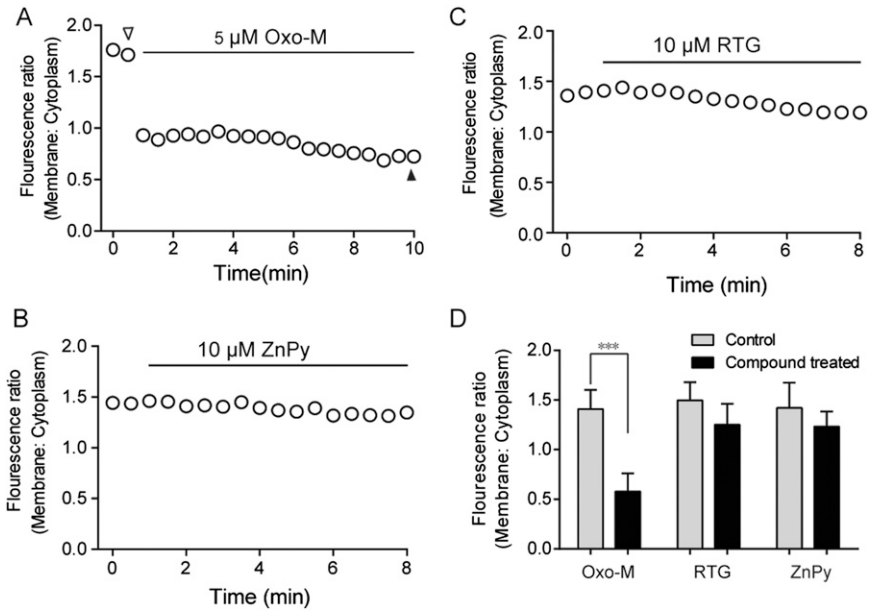

Fig. S2. Activators have no effect on fluorescence ratio between membrane and cytoplasm. CHO cells cotransfected with KCNQ2 and M1 (GFP-PLC-PH) as a marker to monitor translocation of phosphatidylinositol 4,5-bisphosphate (PIP_2) using fluorescence imaging. Time courses of representative fluorescence ratio between membrane and cytoplasm are shown for cells treated with oxotremorine M (Oxo-M) (*A*), retigabine (*B*), or ZnPy (*C*). (*D*) Histogram shows the fluorescence ratio before (open triangle) and at end of drug (filled triangle) application as indicated ($n = 3$).

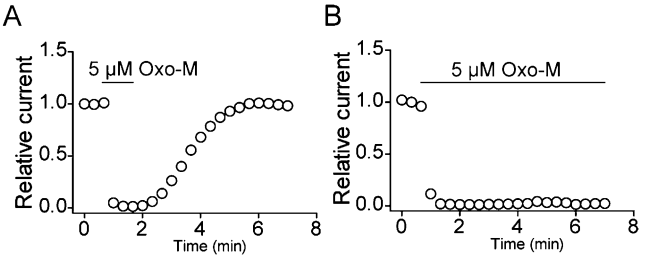

Fig. S3. Suppression of KCNQ2 current by activating M1 receptors. Time course of KCNQ current in CHO cells transfected with KCNQ2 and M1 receptors. (*A*) Treatment with 5 μM Oxo-M suppresses current rapidly and current recovers 150 s after removal of Oxo-M. Depending on the holding time course and cell conditions, the recovery may be partial or require a longer time course. (*B*) Perfusion with 5 μM Oxo-M suppressed KCNQ2 for more than 10 min.

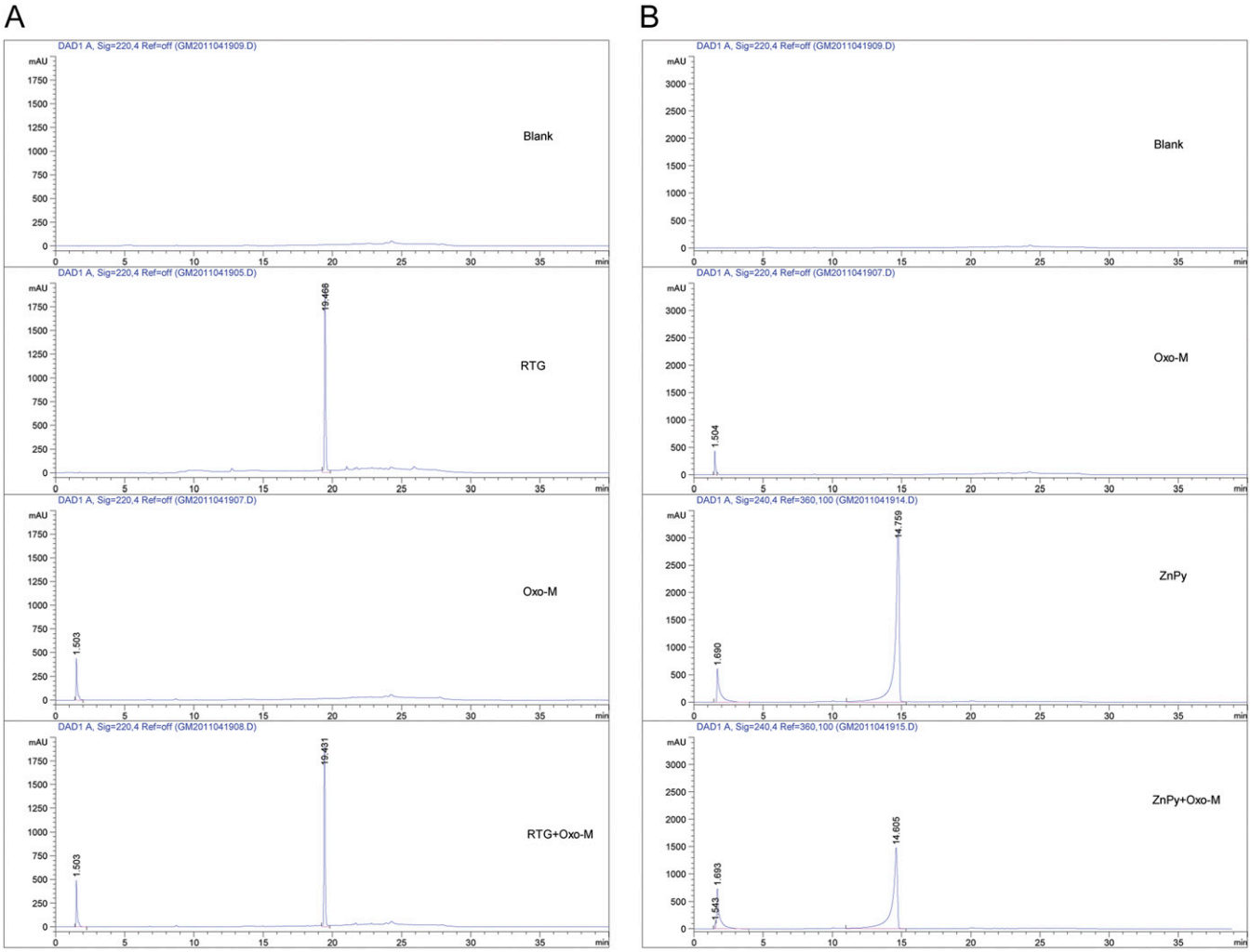

Fig. S4. HPLC analysis excludes the reaction between Oxo-M and activators. HPLC was performed in blank, Oxo-M alone, retigabine or ZnPy alone, or Oxo-M and retigabine/ZnPy mixtures. The corresponding chromatogram and peak of the tested compounds are indicated. Panel *A* is for retigabine series. Panel *B* is for zinc pyrithione series.

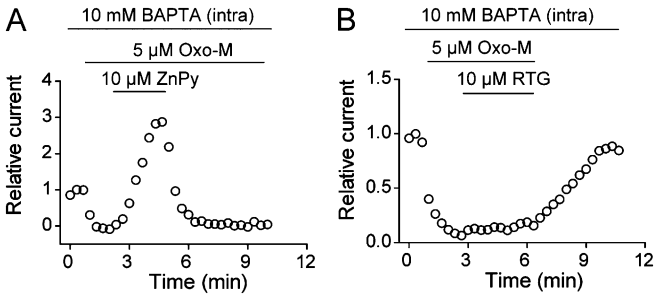

Fig. S5. Effects of ZnPy (*A*) and retigabine (*B*) in the presence of Oxo-M. Perforated patch recording was performed using CHO cells cotransfected with KCNQ2 and human muscarinic type 1 (M1) receptor. KCNQ current was monitored. M1 receptor was activated by 5 μM Oxo-M. Recordings were performed in the presence of 10 mM 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid included in the intracellular pipette solution.

Table S1. Effects of ZnPy on biophysical parameters of KCNQ3^{A278T} after activation of M1 receptor

	$V_{1/2}$, mV	$\tau_{activation}$, ms	$\tau_{deactivation}$, ms
Control	−27.6 ± 0.8	55.5 ± 3.4	29.3 ± 5.6
Five micromoles Oxo-M	−25.4 ± 2.8	59.0 ± 7.8	NA
Five micromoles Oxo-M/10 μM ZnPy	−34.7 ± 1.3	116.7 ± 3.6	45.6 ± 2.0

All values are mean ± SEM ($n \geq 4$). NA, not applicable.