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Novel Neuroprotective K^+ Channel Inhibitor Identified by High-Throughput Screening in Yeast

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ABSTRACT

Discovery of K^+ channel modulators is limited by low-throughput capacity of existing K^+ channel assays. To enable high-throughput screening for novel pharmacological modulators of K^+ channels, we developed an assay based on growth of yeast that functionally expresses mammalian Kir2.1 channels. Screening of 10,000 small molecules from a combinatorial chemical library yielded 42 potential Kir2.1 inhibitors. One compound, 3-bicyclo[2.2.1]hept-2-yl-benzene-1,2-diol, was confirmed to inhibit K^+ channels in patch-clamp measurements in mammalian cells with EC_{50} values of 60 and 1 μM for Kir2.1 and Kv2.1 channels, respectively. Inhibition of Kv2.1 channels decreased in the presence of the external pore blocker tetraethylammonium (TEA) and depended on a residue required for extracellular TEA action, suggesting that the identified compound targets the external mouth of the channel. Furthermore, at the nontoxic concentration of 3 μM, the identified compound completely abolished in vitro neuronal apoptosis mediated by Kv2.1 channels. Therefore, yeast-based screening has identified a novel uncharged neuroprotective mammalian K^+ channel inhibitor.

Potassium (K^+) channels play a pivotal role in electrical activity of all excitable tissues. Abnormalities in K^+ currents result in cardiovascular, neurological, renal, and endocrine pathology (Wickenden, 2002). The recent discovery of apoptosis mediation by K^+ currents (Yu et al., 2001) points to the role of K^+ homeostasis in cellular proliferation and degeneration. Pharmacological modulation of specific K^+ currents is recognized as a major strategy in treatment of a broad range of disorders (Wickenden, 2002). However, most existing small-molecule modulators affect K^+ channels at high micromolar to millimolar concentrations and lack sufficient target specificity (Wickenden, 2002). Given the great diversity of K^+ channels, development of more potent and specific K^+ channel drugs is a high priority of molecular pharmacology.

Despite the need for new K^+ channel modulators, the low-throughput capacity of traditional ion channel assays hinders the discovery of effective compounds (Worley et al., 2002; Willumsen et al., 2003). As an approach for high-throughput screening for K^+ channel modulators, we have used yeast that functionally express mammalian K^+ channels. Yeast require K^+ and so cannot grow in low [K^+] medium when their endogenous K^+ transporters trk1 and trk2 are disrupted (Gaber et al., 1988; Ko et al., 1990). However, growth of Δtrk1trk2 knockout yeast in low [K^+] medium can be rescued (i.e., genetically complemented) by mammalian Kir2.1 channel expression (Tang et al., 1995). Because this growth in low [K^+] medium depends on the activity of the mammalian channel, it might be possible to screen for Kir2.1 channel modulators by monitoring yeast growth.

Here, with such a system, we identified a novel K^+ channel inhibitor in an initial screen of 10,000 compounds. The identified compound is an uncharged small molecule that is shown to act at the external mouth of the channel pore. Strikingly, this first hit acts in the low micromolar range to prevent Kv2.1 channel-dependent neuronal apoptosis in vitro.

Materials and Methods

Yeast Culture. *Saccharomyces cerevisiae* host strain SGY1528 originates from Tang et al. (1995) and has the following genotype: *MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 trk1::HIS3 trk::TRP1*. Disruption of the native potassium transporters TRK1 and TRK2 renders the SGY1528 strain incapable of growing in medium with 2 mM KCl. K^+-free media contained 2% glucose, 0.16% yeast nitrogen base without amino acids, sodium, and ammonia, 8 mM H_3PO_4, 2 mM $MgSO_4$, 0.2 nM $CaCl_2$, 0.1% vitamin mix, 0.1% trace elements mix, 0.2% adenine, and deionized water (adapted from Tang et al., 1995). Vitamin mix contained 0.0002% of each of the following: biotin, calcium pantothenate, niacin, folic acid,

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ABBREVIATIONS: HEK, human embryonic kidney; DTDP, 2,2′-dithiodipyridine; compound 48F10, 3-bicyclo{2.2.1}hept-2-yl-benzene-1,2-diol; LDH, lactate dehydrogenase; TEA, tetraethylammonium; wt, wild type

p-aminobenzoic acid, pyridoxine-HCl, riboflavin, and thiamine-HCl. Trace element mix contained (in a 1 to 10 ng/liter range) boric acid, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulfate, and potassium iodide. Media were adjusted to pH 6.0 with arginine base and sterilized by filtering. KCl was added to reach the desired concentration. Yeast media ingredients were from Qbiogene Inc. (Carlsbad, CA). Yeast transformation was performed using the alkali cation method (Qbiogene Inc.). For growth assay performed in 96-well plates, logarithmic cultures with OD_{560} = 0.05 were dispensed in 50-μl aliquots per well and incubated at 30°C in a humidified incubator for 48 h. Yeast cultures were manually seeded in 96-well plates using multichannel pipettors. Spaces between wells were filled with water to prevent culture evaporation. Optical density of yeast cultures was measured by the Victor plate reader (PerkinElmer Life and Analytical Sciences, Boston, MA) at λ = 560 nm with a speed of 1 well/s (to prevent sedimentation artifacts). Plates were agitated before measurements.

DNA Manipulation. Genes encoding Kir2.1, Kir3.2 T188I, and KAT1 channels were cloned into pYMet vector, a derivative of yeast vector pYES (Invitrogen, Carlsbad, CA) in which GAL1 promoter is substituted for MET25 promoter (Minor et al., 1999). The MET25 promoter is repressible by 1 mM methionine. Kir3.2 mutant T188I, which complements potassium transport deficiency in yeast, originates from Yi et al. (2001). Plant K^+ channel KAT1 (Sentenac et al., 1992) was a gift from Dr. Sentenac (Centre National de la Recherche Scientifique, Montpellier, France). For expression in mammalian cells, genes encoding Kir2.1, Kv2.1, and Kv1.5 channels were placed under control of pCMV promoter in pCDNA3 or pRc/cytomegalovirus vectors (Invitrogen). Mutagenesis was performed using overlapping (four-primer) polymerase chain reaction technique.

Chemical Library. The PrimeSet chemical library (ChemBridge Corporation, San Diego, CA) consists of 10,000 organic molecules dissolved in dimethyl sulfoxide at 10 mM in individual wells of 96-well plates. Compounds were transferred from master plates to test plates using robot Biomek 2000 (Beckman Coulter, Fullerton, CA). Molecular masses of the library compounds are normally distributed between 250 and 500 Da.

Electrophysiology. Kir2.1 channels were expressed in HEK293 cells lacking native inwardly rectifying currents whereas Kv2.1 and Kv1.5 channels were expressed in Chinese hamster ovary cells. Plasmids expressing K^+ channels were cotransfected with plasmid expressing green fluorescent protein using the LipofectAMINE reagent (Invitrogen). Two or 3 days after transfection, cells exhibiting green fluorescence were used for whole-cell current recordings. The pipette solution for recording of Kir2.1 activity had the following composition: 140 mM KCl, 10 mM K-HEPES, 2 mM $MgCl_2$, and 1 mM K-EGTA, pH 7.2. For Kv experiments, 1 mM ATP was added. Bath solution contained 140 mM NaCl, 5.4 mM KCl, 10 mM HEPES, 10 mM glucose, 2 mM $CaCl_2$, and 0.8 mM $MgCl_2$, pH 7.4.

Cortical Culture and Apoptosis Assays. Cortical cultures were prepared from embryonic day 16 Sprague-Dawley rats as described previously (Hartnett et al., 1997). Toxicity assays were performed on 4-week-old cultures (25–29 days in vitro). To induce apoptosis, cells were exposed to 10 μM 2,2′-dithiodipyridine (DTDP) (Sigma-Aldrich, St. Louis, MO) in minimum Eagle's medium for 10 min at 37°C in 5% CO_2 (Aizenman et al., 2000; McLaughlin et al., 2001). In test samples, compound 48F10 was added to the incubation medium for a final concentration of 1 to 3 μM. Neuronal viability was visually determined 18 to 24 h after DTDP exposure by phase contrast microscopy using a lactate dehydrogenase (LDH)-based in vitro toxicology assay kit (Sigma-Aldrich). Media samples were analyzed spectrophotometrically to obtain a measure of cytoplasmic LDH release from dead and dying neurons.

Results

Identification of Potential Mammalian K^+ Channel Inhibitors by Screening in Yeast. We used a yeast strain that was engineered so that its growth depends on functional expression of mammalian Kir2.1 channels and developed a procedure for individually applying thousands of chemical compounds to yeast cultures to detect growth changes. Before proceeding to library screening, we proved that the assay is capable of detecting known Kir2.1 channel inhibitors when yeast are grown in 96-well plates. The host strain SGY1528 was transformed with the pYMetKir2.1 plasmid expressing Kir2.1 under control of the methionine-repressible promoter MET25. Expression of Kir2.1 channel enables the transformant to grow in selective medium with 0.5 to 2 mM K^+. Figure 1A shows that inhibition of Kir2.1 channel by Cs^+ can be detected in the assay format (boxed wells). Specifically, 10 mM Cs^+ abolished growth of SGY1528/Kir2.1 in 96-well plates in low but not high [K^+] media. Neither rubidium nor tetraethylammonium (TEA), two ions that block several K^+ channels, but not Kir2.1 channels, inhibited growth. Blocking expression of Kir2.1 with methionine also inhibited growth in low but not high [K^+] media.

Next, we used this assay to screen a library of 10,000 organic molecules. Aliquots of compounds were robotically dispensed from 96-well master plates to test plates. Wells containing 20 μM concentrations of each compound were seeded with 50 μl of early logarithmic yeast culture in either high [K^+] medium to test for intrinsic toxicity or low [K^+] medium to test for Kir2.1 channel inhibition. Each plate included control wells without compound and wells with Kir2.1 inhibitors, Cs^+, and methionine. After 48 h of incubation, culture densities were determined by absorbance with a plate reader. Wells in which yeast growth was changed in low [K^+] plates but unaffected in high [K^+] plates were sought as primary hits. We did not find any compounds that specifically enhanced Kir2.1-dependent growth that could be channel activators. Of the 10,000 compounds tested, 168 inhibited yeast growth at low, but not high, [K^+] media that might be K^+ channel inhibitors with low toxicity. Thirty-six compounds completely abolished yeast growth at high [K^+] and were thus considered toxic. A representative primary screening plate with the primary hit compound 48F10 is shown in Fig. 1A. Compound 48F10 was identified as 3-bicyclo [2.2.1]hept-2-yl-benzene-1,2-diol (Fig. 1B). Because the primary screening was qualitative, all primary hit compounds were retested in multiple growth measurements, as is shown in the example of 48F10 (Fig. 2C). Forty-two compounds with inhibition higher than 50% and toxicity below 20% were finally selected. To narrow this group, we tested compound specificity toward mammalian channels Kir2.1 and Kir3.2 versus plant channel KAT1 and the yeast K^+ transporters. Isogenic strains expressing Kir2.1, Kir3.2, KAT1, or wild-type strain with endogenous TRK intact were grown in low and high [K^+] media in the presence and absence of 50 μM test compounds. Because K^+ transport in yeast expressing different K^+ channels was rescued to different degrees, [K^+] in the selective media was adjusted to provide a comparable growth rate in all tested cultures. Figure 1D shows that 48F10 inhibited growth that depends on mammalian channels Kir2.1 and Kir3.2 but not the plant channel or yeast transporters. This was one of six compounds tested that exhibited this type of specificity. Compound 48F10 was selected for further analysis because its lack of charge and amines is not typical for K^+ channel blockers.

Inhibition of K^+ Channels in Mammalian Cells. Electrophysiological measurements established that 48F10 inhibits Kir2.1 current in mammalian cells. Figure 2A shows that 48F10 inhibits whole-cell Kir2.1 current evoked by voltage steps in HEK293 cells. Current was restored within 10 s after washout (i.e., as fast as possible with our perfusion system), indicating reversible inhibition (data not shown). As seen in Fig. 2B, inhibition of inwardly rectifying current by 48F10 occurred at all voltages where the channel was open. To measure outward currents at positive voltages, we introduced mutations D172N and E224G that attenuate channel block with magnesium and polyamines (Yang et al., 1995). No obvious voltage dependence was evident with the weakly rectifying Kir2.1 mutant (Fig. 2B, bottom). The dose-response curve for 48F10 indicates a half-blocking concentration (EC_{50}) of 60 μM and a Hill coefficient of 1 (Fig. 2C). These patch-clamp measurements demonstrate the validity of screening for mammalian K^+ channel modulators based on yeast growth.

In addition to its effects on Kir2.1 current, 48F10 was observed to inhibit voltage-gated Kv2.1 delayed rectifier current with an EC_{50} of 1 μM (Fig. 3, A and B). Inhibition did not change the voltage dependence of channel gating (Fig. 3C). In contrast, the Kv1.5 channel showed little sensitivity to this compound (Fig. 4A). Thus, among the three channels tested in mammalian cells, 48F10 showed at least a 1000-fold range in apparent affinity.

Mechanism of Action of Compound 48F10. Two independent approaches demonstrate that 48F10 acts at a site that overlaps with the site of external TEA block (Fedida et al., 1999). First, we examined whether a residue that is critical to external TEA sensitivity affects 48F10 inhibition. Human Kv1.5 channel is resistant to external TEA but becomes sensitive with the mutation R487Y at the outer mouth of the pore (Fedida et al., 1999). We introduced the analogous mutation R476Y in the rat Kv1.5 channel to induce sensitivity to external TEA (data not shown). Figure 4, A and B, shows that the mutation R476Y also renders Kv1.5 sensitive to 48F10 (i.e., EC_{50} is 6 μM). Second, we examined whether the apparent affinity of 48F10 for Kv2.1 is affected by TEA. If these two agents act independently, then a given dose of 48F10 should block the same fraction of current regardless of the presence or absence of extracellular TEA. However, 48F10 was much less effective at inhibiting the residual Kv2.1 current in the presence of 20 mM TEA, suggesting that these two compounds act at overlapping sites (Fig. 4C). Taken together, these results demonstrate direct action of 48F10 at the external mouth of Kv channels.

Neuroprotection by Compound 48F10. K^+ efflux via Kv2.1 channels is essential for caspase-dependent apoptosis in neurons (Yu et al., 1997; Pal et al., 2003). In fact, blocking delayed rectifier current with 5 mM TEA prevents neuronal death (Yu et al., 1997; Wang et al., 2000). Therefore, we tested whether 48F10, a much more potent inhibitor of Kv2.1 channels, could attenuate neuronal apoptosis. Mixed cultures of cortical neurons and glia were exposed to 10 μM DTDP, a membrane-permeant oxidizing agent that induces Kv2.1-dependent apoptosis in neurons but not glia (Aizenman et al., 2000; McLaughlin et al., 2001). As expected, a 10-min exposure to DTDP caused significant neuronal death within a day, as indicated by a decreased number of large phase-bright neuronal cell bodies and LDH release from dead

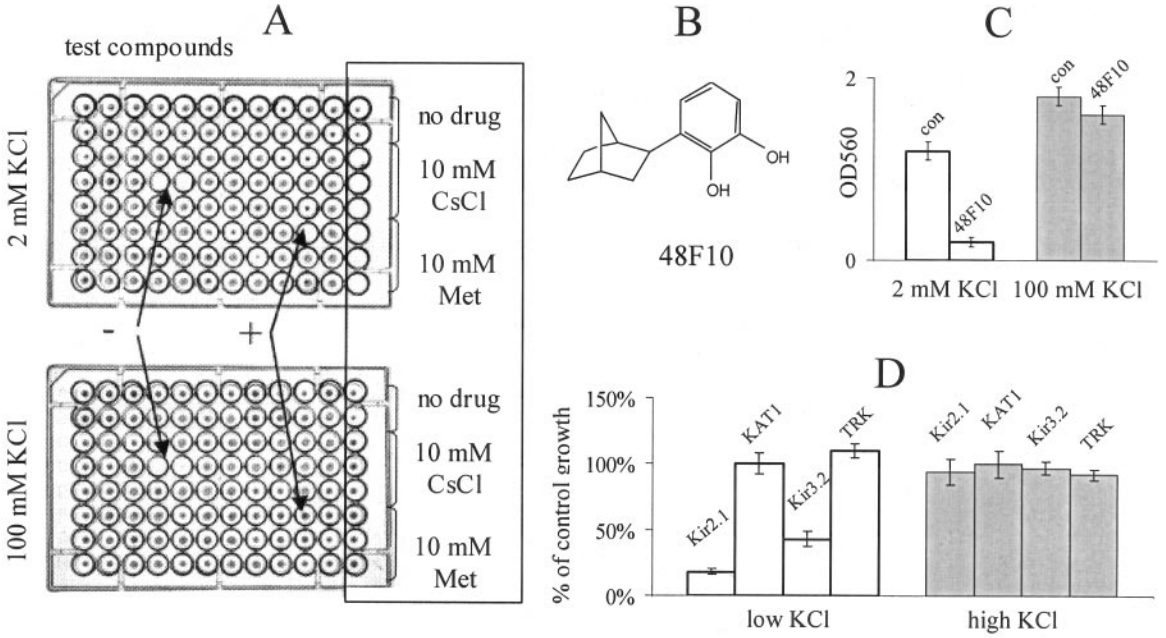

Fig. 1. Screening in yeast identifies compound 48F10 as a potential Kir2.1 channel inhibitor. A, representative primary screening plates. Boxed column contains wells with control inhibitors of Kir2.1 channel (CsCl and methionine) and wells without inhibitor. Toxic compounds that inhibit yeast growth in media with low and high [K^+] are indicated with negative (−) arrows. Primary positive compound 48F10 that inhibits growth at low but not high [K^+] is indicated with positive (+) arrows. B, structure of compound 48F10, 3-bicyclo [2.2.1]hept-2-yl-benzene-1,2 diol. C, measurements of yeast growth in the presence and absence of 50 μM 48F10. Bars represent mean optical density of yeast cultures in 10 wells ± S.E.M. after 48 h of incubation. D, growth of yeast expressing different K^+ channels in the presence of 48F10. Two-milliliter cultures were grown on a rotary shaker in high [K^+] or low [K^+] media with 50 μM 48F10 and without 48F10 (control). Low [K^+] media contained 1 mM (Kir2.1), 0.5 mM (Kir3.2), or 0.25 mM (KAT1 and TRK) KCl. Optical density of test and control cultures was measured before and after 48 h of incubation. Bars show mean percentage of control growth ± S.E.M. (n = 3–5 independent cultures).

cells (Fig. 5). Furthermore, overnight exposure of cultures to 1 to 3 μM 48F10 did not affect neuronal survival, demonstrating that the channel inhibitor is not toxic. Finally, we found that application of 1 to 3 μM 48F10 after DTDP treatment prevented death of cortical neurons (Fig. 5). Thus, this compound is ~5000-fold more neuroprotective than TEA.

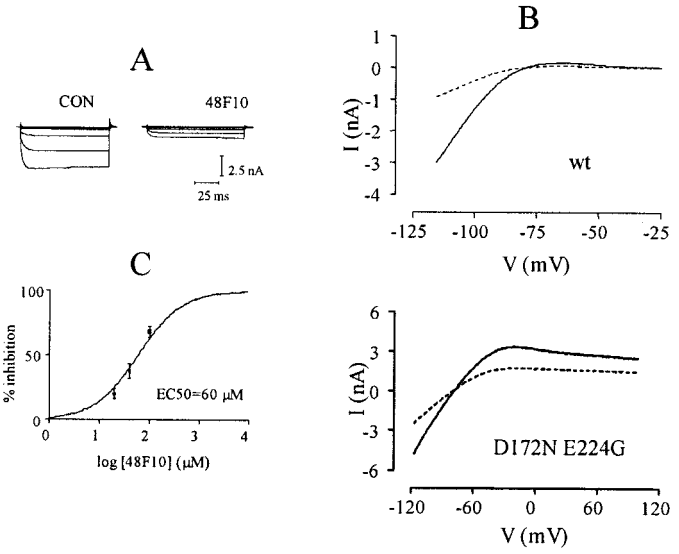

Fig. 2. Compound 48F10 inhibits Kir2.1 current. A, representative macroscopic currents recorded from HEK293 cells expressing Kir2.1 channel in the presence and absence of 100 μM 48F10. Currents were elicited by voltage steps from −120 to 0 mV in 10-mV increments from holding potential −70 mV. B, currents versus voltage in the presence (dashed line) and absence (solid line) of 100 μM 48F10 generated by voltage ramps. Currents elicited by negative voltages were recorded from cells expressing wt Kir2.1 (B, top). Currents elicited by positive voltages were recorded from D172N/E224G mutant (B, bottom). C, dose-response relationship of 48F10 block of Kir2.1 current. The degree of block was measured at −120 mV. The smooth line is a fit of experimental data with a Hill coefficient of 1 (n = 4).

Discussion

Yeast-Based Screening for Mammalian K+ Channel Modulators. In this study, we developed a novel assay for discovery of K^+ channel modulators by screening chemical libraries in yeast. The use of yeast expressing a mammalian K^+ channel for K^+ channel drug discovery has been suggested previously (Hahnenberger and Kurtz, 1997); however, based on measurement of medium acidification, that approach was successful only for confirming a few model inhibitors, not for discovery of new drugs (Hahnenberger et al., 1996). Our assay monitors optical density of yeast culture growth as a reporter of activity of mammalian channel Kir2.1. We validated this approach by identifying a genuine K^+ channel inhibitor among 10,000 compounds.

Although screening chemical libraries for modulators of enzymatic activities is a usual practice, effective high-throughput screening for ion channel modulators has been limited thus far to the cystic fibrosis conductance regulator (Ma et al., 2002). This assay, with its 5000- compound-per-day capacity, requires customized automated instrumentation and was designed specifically for the cystic fibrosis conductance regulator. We sought to develop an assay for K^+ channels that potentially allows screening of $>10^5$ compounds. Ten thousand compounds were mixed with 10,000 aliquots of yeast culture in 125 test microplates and 125 control microplates. Preparation of these primary plates consumed 10 min per plate, including robotic compound dispensation and manual yeast seeding. After 48 h of incubation, yeast growth was measured by a plate reader with multiplate

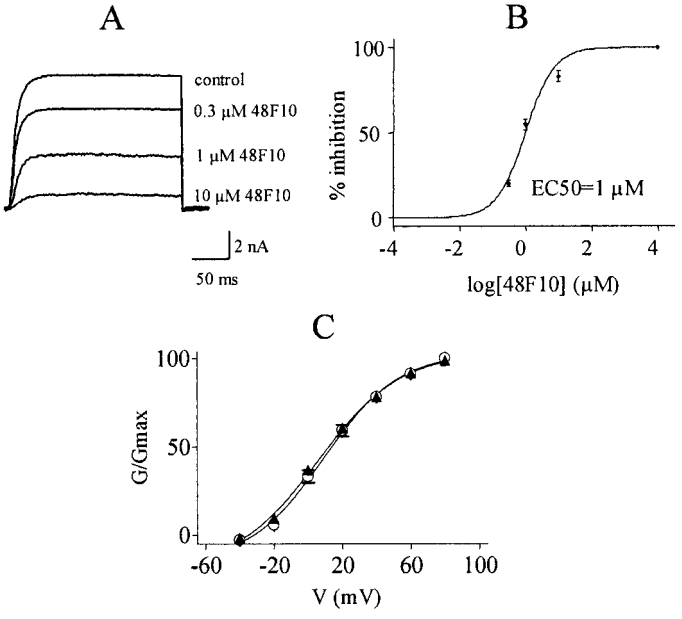

Fig. 3. Compound 48F10 inhibits Kv2.1 current. A, representative current traces recorded from Chinese hamster ovary cells expressing Kv2.1 channel in the presence and absence of 48F10 elicited by a voltage step to +40 mV from a holding potential of −60 mV. B, dose-response relationship of 48F10 block of steady-state Kv2.1 current. C, conductance versus membrane potential plot. Boltzmann fits yield half-maximal voltages of 11.7 mV for control and 13.2 mV in 48F10. Slope factors were 17.4 for control and 16.9 for 48F10 (n = 7).

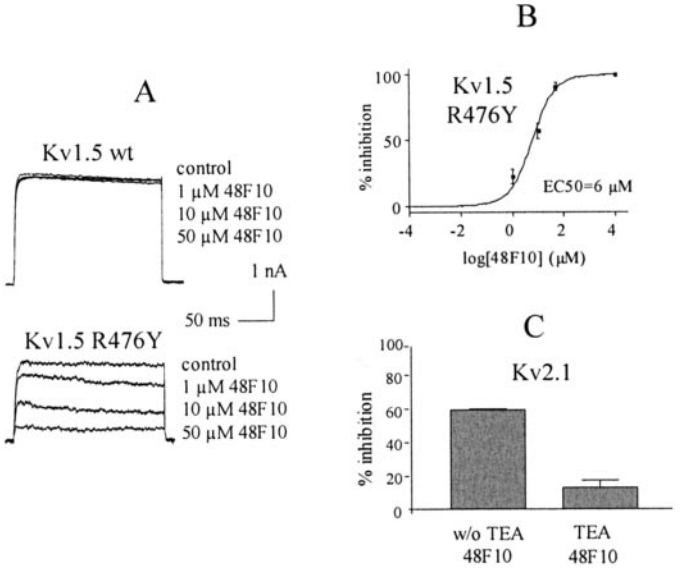

Fig. 4. Compound 48F10 acts at the external mouth of Kv channels. A, mutation R476Y in the external mouth of Kv1.5 channel renders Kv1.5 channel sensitive to 48F10. Representative current traces elicited by voltage steps to +40 mV from −60 mV from cells expressing wild-type or R476Y Kv1.5 channels in the presence and absence of 48F10.B, dose-response relationship of 48F10 block of R476Y Kv1.5 channel. Steady-state current was measured at +40 mV. C, blocking percentage of Kv2.1 current by 1 μM 48F10 in the presence and absence of 20 mM TEA. In the latter case, Kv2.1 current was inhibited by ~80% by 20 mM TEA before application of 1 μM 48F10 (n = 14 for control and 7 for 48F10). Kv2.1 current was completely restored after TEA and 48F10 washout.

capacity at a speed of one plate every 2 min. Overall processing time, not including incubation, was 50 h for 10,000 compounds, or 20 s per compound. Primary screening required as little as 5 to 20 ng of test compound. The assay does not involve any sophisticated equipment. The yield of positive compounds in the primary round of screening in yeast was 2% of total. Because the size of resulting sublibrary did not allow us to proceed to electrophysiology in low-throughput conditions, we added a quantitative second round of yeast selection with a threshold of inhibition >50% and toxicity <20%. The final yield of the quantitative step was 0.4% of original library. Thus, even performed on a limited scale, yeast-based assay demonstrated its high-throughput capacity.

In addition to channel modulation, compound toxicity and specificity were built into the screening assay in yeast. Compounds were tested in vivo under conditions in which channel function was not required (i.e., in the presence of high [KCl]) to exclude toxic agents. To select inhibitors specific to mammalian K^+ channels, we included a test with a phylogenetically distant plant K^+ channel.

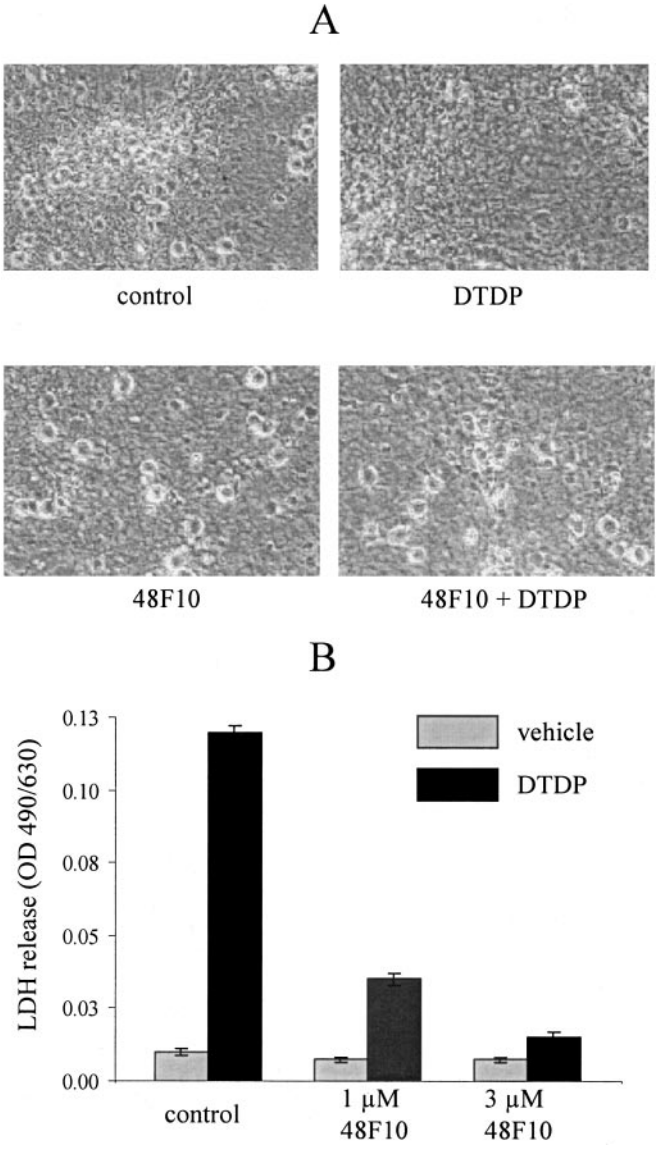

Fig. 5. Compound 48F10 prevents neuronal death in cortical cultures. A, phase-contrast micrographs of cultured cortical neurons after a 10-min exposure to 10 μM DTDP in the presence and absence of 3 μM 48F10. B, LDH release from cortical cultures 24 h after a 10-min exposure to 10 μM DTDP. Cultures were maintained in the presence and absence of 48F10. Bars represent mean ± S.E.M. LDH-specific absorbance in three independent cultures.

Although compound 48F10 was identified using Kir2.1 as a target, it proved to have a 60-fold higher apparent affinity for the Kv2.1 delayed rectifier channel. Interestingly, 48F10 did not potently inhibit another voltage-gated delayed rectifier, Kv1.5. Thus, the screen using Kir2.1 identified a compound that displays a wide range of potencies among different channels. This demonstrates that basing the yeast assay on one target K^+ channel has the potential of detecting more potent modulators of other K^+ channels.

Properties of the Identified K^+ Channel Inhibitor. Compound 48F10 attracted our attention because it lacks structural similarity to well understood K^+ channel pore blockers and could therefore act by a novel mechanism. For example, the structure of 48F10 and its lack of ionizable atoms are distinct from the inner pore blockers magnesium and linear polyamines (Lopatin et al., 1995; Xie et al., 2003). In fact, a double mutation (D172N/E224G) that attenuates block of Kir2.1 current by magnesium and polyamines (Yang et al., 1995) does not affect inhibition of Kir2.1 by 48F10 (Fig. 2B). Thus, this inhibitor does not mimic or facilitate block by Kir2.1 native gating particles. Exclusion of a Kir-specific gating effect is also supported by the lack of a change in the voltage dependence of Kv2.1 channel gating. These results concerning the internal pore and gating mechanisms led us to consider an alternative site of action.

Two independent findings suggest that 48F10 acts at the external mouth of the channel. First, we examined 48F10 blocking behavior in the presence of TEA, a known external conduction blocker (Fedida et al., 1999). This experiment was performed on Kv2.1 channels that are sensitive to external TEA. The lesser degree of 48F10 inhibition of Kv2.1 currents in the presence of TEA, although measured at one concentration of TEA and 48F10, (Fig. 4B) suggests that TEA binding to external mouth of the channel prevents 48F10 action. Mechanistically, this is most easily explained by overlapping binding sites in external mouth of the pore for TEA and 48F10. Alternatively, the apparent competition between TEA and 48F10 could be caused by allosteric distortion of remote 48F10 binding site after TEA binding to the outer pore. However, in support of the first mechanism, we demonstrated that mutation of a residue in external mouth of the pore that influences TEA action changes channel affinity for 48F10. Specifically, substitution of arginine with tyrosine at position 476 renders the Kv1.5 channel sensitive to 48F10 and TEA (Fig. 4A). Taken together, these results locate the site of action of 48F10 at the external mouth of Kv2.1 and Kv1.5 channels, a region usually occupied by charged ions and water.

How does the 48F10 molecule interact with K^+ channels? Structurally, compound 48F10 includes two moieties: catechol (benzenediol) and norbornane (3-bicyclo[2.2.1]heptyl) (Fig. 1B). Nothing is known about modulation of K^+ channels specifically by norbornane and similar groups. Catechol in concentrations above 0.1 mM has been used widely to inhibit native K^+ currents (Ito and Maeno, 1986; Erdelyi and Such, 1988; Kuenzi and Dale, 1998; Bretschneider et al., 1999; Hess and El Manira, 2001); however, the mechanism of its

action remains unknown. Our data on catechol inhibition of cloned Kv channels indicate that the site of catechol action is different from that of 48F10, suggesting that 48F10 may constitute a novel pharmacophore for K^+ channels (manuscript in preparation).

Recently, a critical role for Kv2.1 channel in neuronal apoptosis was demonstrated using dominant-negative subunits (Pal et al., 2003). Because ~10 mM TEA is required for protection from neuronal death (Yu et al., 1997; Wang et al., 2000), we tested whether 48F10, which inhibits Kv2.1 with an EC_{50} of 1 μM, attenuates neuronal apoptosis. We found that 1 to 3 μM 48F10 was not toxic for two types of cells, yeast (Fig. 1, C and D) and neurons (Fig. 5A) and prevented DTDP-induced neuronal death in cortical cultures (Fig. 5). Thus, 48F10 is ~5000-fold more neuroprotective than the traditional delayed rectifier blocker TEA. 48F10 is distinct from TEA in that it is uncharged and so could pass through the blood-brain barrier. Therefore, compounds related structurally to 48F10 may prevent neuronal apoptosis in vivo.

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