Impermeability of the GIRK2 weaver channel to divalent cations

PING HOU, ANKE DI, PING HUANG, CHARLOTTE B. HANSEN, AND DEBORAH J. NELSON
Department of Neurobiology, Pharmacology, and Physiology,
The University of Chicago, Chicago, Illinois 60637

Hou, Ping, Anke Di, Ping Huang, Charlotte B. Hansen, and Deborah J. Nelson. Impermeability of the GIRK2 weaver channel to divalent cations. Am J Physiol Cell Physiol 278: C1038–C1046, 2000.—A single amino acid mutation (G156S) in the putative pore-forming region of the G protein-sensitive, inwardly rectifying K+ channel subunit, GIRK2, renders the conductance constitutively active and nonselective for monovalent cations. The mutant channel subunit (GIRK2wv) causes the pleiotropic weaver disease in mice, which is characterized by the selective vulnerability of cerebellar granule cells and Purkinje cells, as well as dopaminergic neurons in the mesencephalon, to cell death. It has been proposed that divalent cation permeability through constitutively active GIRK2wv channels contributes to a rise in internal calcium in the GIRK2wv-expressing neurons, eventually leading to cell death. We carried out comparative studies of recombinant GIRK2wv channels expressed in Xenopus oocytes and COS-7 cells to determine the magnitude and relative permeability of the GIRK2wv conductance to Ca2+. Data from these studies demonstrate that the properties of the expressed channel differ in the two systems and that when recombinant GIRK2wv is expressed in mammalian cells it is impermeable to Ca2+.

MATERIALS AND METHODS
cDNA clone. GIRK1 was cloned from a RIN cell library and had a predicted amino acid sequence identical to the cardiac clone originally described (8). GIRK2 and GIRK2wv were gifts from Dr. P. Kofuji (California Institute of Technology, CA). The m2 muscarinic receptor was purchased from Clontech (Clontech, CA) in the pGEM3Z vector. All GIRK constructs were subcloned into either the pMXT vector, obtained from Dr. P. Kofuji (California Institute of Technology, CA), for oocyte expression or pEGFPN3 (Clontech, CA). The m2 muscarinic receptor was purchased from Clontech (Clontech, CA) for mammalian expression. The m2 receptor was linearized with Hind III, and cRNA was transcribed using the T7 polymerase mMessage mMachine kit (Ambion). All GIRK constructs were linearized with Sal I, and cRNA was transcribed using T3 polymerase mMessage mMachine kit (Ambion). The cRNA concentration was determined by ultraviolet light absorption at 260 nm (A260) and confirmed by intensity on ethidium bromide stained agarose gels. For mammalian cell expression, GIRK1, GIRK2, and GIRK2wv were fused to enhanced green fluorescent protein (EGFP) at the carboxy terminus in the pEGFPN3 vector (Clontech).

Oocyte electrophysiology. Oocytes were injected with 2 ng of m2 muscarinic receptor cRNA and 5 ng of each GIRK subunit cRNA along with 12.3 ng GIRK5 (KA1) antisense cRNA. Injected oocytes were maintained in OR-2+ solution containing (in mM) 96 NaCl, 2.5 KCl, 1 CaCl2, 2 MgCl2, 5 HEPES, 2.5 sodium pyruvate, and 50 µg/ml gentamicin. Nominally Ca2+-free solutions were used to incubate and maintain oocytes expressing GIRK2wv.
Two-microelectrode voltage-clamp recordings were performed 3 days postinjection using a TURBO TEC-10C amplifier (NPI, Tamm, Germany). Data were acquired using Pulse software (HEKA, Lambrecht, Germany), an ITC-16 interface (Instrutech, Great Neck, NY), and an IBM-compatible PC. Microelectrodes were filled with 3 M KCl and had resistances of 0.5–2 MΩ. During electrophysiological recordings, oocytes were continuously superfused with a bath solution of 90 mM NaCl or KCl, 1 mM MgCl2, and 5 mM HEPES (pH 7.6 with NaOH/KOH). Na+ was isosmotically replaced with N-methyl-
-glucamine (NMDG) in solutions used to investigate divalent permeability. The divalent content of these solutions was varied at constant osmolarity and contained (in mM) 5, 20, or 70 CaCl2 with 35 NMDG-Cl, 1 MgCl2, and 5 mM HEPES (pH 7.6 with NaOH/KCl). G protein-dependent currents were induced with the addition of 5 μM carbachol (Sigma, St. Louis, MO) to the bathing solution. In all experiments, the holding potential was −80 mV; test potentials were delivered once every second and stepped between −150 and 50 mV in 20-mV increments. Data collection and analysis were performed using Pulse/Pulse Fit (HEKA), and data plotted using the integrated graphics package IGOR (WaveMetrics, Lake Oswego, OR).

RESULTS

Comparison of GIRK1/2 and GIRK2wv channels expressed in Xenopus oocytes and COS-7 cells. The goal of this study was to quantitate the magnitude of the Ca2+ permeability of recombinant GIRK2wv channels compared with heteromultimeric GIRK1 + GIRK2 (GIRK1/2) channels. We compared expression of the wild-type and mutant channels in Xenopus oocytes to that in mammalian COS-7 cells.

Recombinant GIRK subunits coassemble with endogenous Xenopus GIRK5 subunits to form functional channels (5). Antisense cDNA against GIRK5 (KHA1) has been previously reported to knock out endogenous GIRK5 expression in oocytes (5, 15). Therefore, antisense cDNA against GIRK5 was co-injected in all our studies to prevent endogenous GIRK5 expression and coassembly.

Figure 1, A and B, compares expression of the heteromultimeric GIRK1/2 and GIRK2wv channels in a representative Xenopus oocyte and mammalian cell. Both expression systems gave rise to carbachol-induced currents that were inwardly rectifying and Ba2+ sensitive. When expressed in oocytes, GIRK1/2 was associated with a large basal (carbachol-independent) current in high-K+ solutions. The corresponding basal current was absent in the COS-7 cells. Average peak current amplitude of the carbachol-sensitive K+ current was −3.5 ± 0.3 nA (n = 41) at −150 mV in Xenopus oocytes and −1.2 ± 0.3 nA at −160 mV (n = 5) in COS-7 cells.

As has been previously observed in a number of other laboratories, the weaver mutation in GIRK2 induces the expression of recombinant channels, which are highly permeable to Na+ and independent of G protein-induced gating, as can be seen in Fig. 2, A and B. Large basal currents in both high-Na+ and high-K+ solutions were observed in oocytes as well as COS-7 cells. The magnitude of the G protein-independent Na+ current
Methods. Cells chosen for electrophysiological recording were identified by their green fluorescence. A: 2-microelectrode current recordings from an oocyte injected with cRNA for m2 muscarinic receptors, GIRK1 and GIRK2, and the cDNA along with m2 receptor cDNA as described in MATERIALS AND METHODS. Cells chosen for electrophysiological recording were identified by their green fluorescence. B: whole cell patch voltage-clamp recordings from a single COS-7 cell transiently transfected with the GIRK1-EGFP and GIRK2 cDNA as described in MATERIALS AND METHODS. Cells chosen for electrophysiological recording were identified by their green fluorescence.}

Fig. 1. Coexpression of GIRK1 with GIRK2 in Xenopus oocytes leads to larger G protein-independent currents than coexpression in mammalian cells. A: 2-microelectrode current recordings from an oocyte injected with cRNA for m2 muscarinic receptors, GIRK1 and GIRK2. A, left: 3 families of superimposed current traces elicited by 1-s voltage steps to potentials between −150 and 50 mV from a holding potential of −80 mV. Dotted lines, zero current level. First set of currents was recorded from an oocyte in a solution in which all the NaCl was replaced with KCl. Second set of currents represents residual current remaining after adding 500 µM Ba²⁺ to high-K⁺ carbachol-containing solution. All 3 families of current were obtained from the same oocyte. Corresponding current-voltage (I-V) relations are plotted to the right of current traces. Current magnitude was determined at the point in the current traces where the currents were maximum. B: whole cell patch voltage-clamp recordings from a single COS-7 cell transiently transfected with the GIRK1-EGFP and GIRK2 cDNA as described in MATERIALS AND METHODS. Cells chosen for electrophysiological recording were identified by their green fluorescence. B, left: 3 families of superimposed current traces elicited by 200-ms voltage steps to potentials between −160 and 50 mV from a holding potential of −80 mV. As in A, the dotted lines indicate zero current level. First set of currents was recorded from an oocyte in a solution in which all the external NaCl was isosmotically replaced with KCl. Second set of currents represents difference currents obtained by subtracting currents in high-K⁺ solution from those obtained in the identical solution containing in addition 5 µM carbachol. Third set of currents was obtained when 200 µM Ba²⁺ was added to high-K⁺ carbachol-containing solution. Associated I-V relations are plotted to the right of current traces. Note that, compared with the current GIRK1/2 currents recorded in the oocyte in high-K⁺ solutions, currents recorded from the mammalian cell preparations failed to show a significant expression of G protein-independent (basal) current activation.

Fig. 2. Comparison of GIRK2w expression in Xenopus oocytes and mammalian cells. A: currents were recorded from oocytes as in Fig. 1A. Three families of current traces shown at left were recorded in solutions containing high K⁺, high Na⁺, high Na⁺ containing 300 µM QX314, and a solution in which all the monovalent cations had been replaced with N-methyl-D-glucamine (NMDG). Associated I-V relations are plotted to the right. B: currents recorded from a single transfected mammalian cell as in Fig. 1B. External solutions are given above each family of current traces. Associated I-V relations are plotted to the right.
pressed in the oocytes was 74 ± 4% (n = 5) at −150 mV also in high-Na+ external solutions. The percentage of QX-314-induced current inhibition in the oocytes was calculated following leak subtraction. Leak current was determined in solutions in which all the permeant cations were isosmotically replaced with the large impermeant cation NMDG. It should be noted that QX-314 failed to inhibit monovalent cation current in a small percentage of oocytes in which the leak-subtracted GIRK2 wv current-voltage (I-V) relationship was linear (data not shown).

Divalent permeability of uninjected oocytes. To quantitate the magnitude of the GIRK2 wv-induced Ca2+ influx pathway, it was necessary to identify basal divalent permeability through endogenous, voltage-gated Ca2+ channels in uninjected oocytes exposed to solutions containing elevated divalent concentrations. Characterization of endogenous voltage-activated Ca2+ channels in oocytes has been previously established (2, 9, 12). A comparative summary of the magnitude of both inward and outward current at −150 and 50 mV, in high and low Ca2+ solutions is plotted in Fig. 3 as a function of internal 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) buffering. Charnet and colleagues (1) have previously reported the use of BAPTA injections to buffer the influx of Ca2+ in oocytes, thereby preventing activation of the endogenous Ca2+-activated anion conductance.

As can be seen in Fig. 3A, oocytes exposed to extracellular solutions containing 70 mM Ca2+ showed significant outward current at 50 mV [2,400 ± 380 nA (n = 8)] compared with currents in 5 mM Ca2+-containing solutions [450 ± 57 nA (n = 8)]. The enhancement of outward current in high Ca2+ at the depolarized potential was prevented if the oocytes were injected with 50 nl of 100 mM BAPTA before current recording. Inward currents showed no significant change in amplitude on raising external Ca2+ and were unaffected by internal BAPTA buffering. Outward currents at 50 mV in the presence of internal BAPTA buffering were 330 ± 28 and 250 ± 26 nA (n = 6) in 5 and 70 mM Ca2+, respectively (Fig. 3B). A relative comparison of current amplitude at −150 and 50 mV for the high- and low-Ca2+-containing solutions in the presence and absence of internal BAPTA buffering is given in Fig. 3C.

Direct measurement of current carried by Ca2+ in oocytes and mammalian cells expressing GIRK1/2 and GIRK2 wv. To determine the magnitude of divalent current carried by GIRK2 wv channels compared with GIRK1/2 channels, experiments on both oocytes and COS-7 cells were performed in solutions in which Ca2+ was the only permeant cation in the extracellular solution. Oocytes were injected with 100 mM BAPTA prior to current recording in high divalent solutions to block activation of the contaminating Ca2+-activated Cl− current. Figure 4 illustrates data obtained from both oocytes and COS-7 cells expressing GIRK1/2. Both basal and carbachol-induced K+ currents were recorded to ensure that cells were expressing G protein-activated GIRK channels. Sequential exposure of COS-7 cells to 5 and 70 mM Ca2+-containing solutions failed to result in current activation. Current amplitudes at −150 mV for oocytes showed no significant change on switching from low- to high external Ca2+.

A comparison of current data obtained from both oocytes and COS-7 cells expressing either GIRK1/2 or GIRK2 wv channels in external solutions containing 5 mM Ca2+ is given in Fig. 5, A and B. Current in solutions containing high Na+ was recorded to confirm that cells were expressing GIRK2 wv channels. Exposure of COS-7 cells to 5 mM Ca2+-containing solutions did not elicit current activation. However, Ca2+ currents were observed in 5 mM Ca2+ solutions in oocytes expressing GIRK2 wv channels. A comparison of cur-

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Fig. 3. Summary of inward and outward current amplitudes in uninjected oocytes as a function of increasing external divalent concentrations in the presence and absence of internal BAPTA buffering. All experiments were obtained from uninjected oocytes using voltage protocols described in Fig. 1. Oocytes were injected with high concentrations of BAPTA as described in MATERIALS AND METHODS. A: representative currents and I-V relationship from a single oocyte in solutions containing 5 and 70 mM Ca2+ as the only permeant species. B: currents from an oocyte injected with BAPTA in the presence of the low and high Ca2+-containing solutions. C: comparison of current amplitude for two Ca2+-containing solutions in the presence and absence of internal Ca2+ buffering. Peak current amplitude was determined at 50 and −150 mV. In all experiments, Ca2+ was the only permeant cation in the external solution. Isosmolarity was obtained by addition of sucrose to the low-Ca2+ solution.
containing solutions, demonstrating that the shifts in reversal potential in the GIRK2wv-expressing oocytes were not attributable to a background current. Ca\(^{2+}\) current in the GIRK2wv-expressing oocytes in the presence of QX314 was 1,032 ± 277 nA (n = 7) in 20 mM Ca\(^{2+}\) and did not change from current recorded in the absence of QX314, indicating that either the GIRK2wv channel when expressed in oocytes is not sensitive to QX314 in the presence of high external divalent concentrations or that the divalent permeable pathway is not due to GIRK2wv expression. Niflumic acid, a potent Ca\(^{2+}\)-activated Cl\(^{-}\) channel blocker in oocytes with a dissociation constant (K\(_d\)) of 17 µM (20) did not inhibit outward or inward current at a concentration of 1 mM (data not shown), indicating that the QX314-insensitive divalent current was not due to the endogenous Ca\(^{2+}\)-activated Cl\(^{-}\) channels.

We were unable to observe either an increase in current or shift in reversal potential on increasing external Ca\(^{2+}\) in COS-7 cells expressing GIRK2wv channels. The reversal potential in the mammalian cell experiments was \(-28.6 ± 5.2\) mV (n = 5) in 5 mM Ca\(^{2+}\) and \(-23.0 ± 5.6\) mV (n = 5) in 70 mM Ca\(^{2+}\)-containing solutions.

Intracellular calcium measurements. To further investigate whether constitutively active GIRK2wv channels expressed in mammalian cells could give rise to significant changes in levels of intracellular calcium (Ca\(^{2+}\)), as has been reported for neurons cultured from weaver mice (4, 21), we carried out digital fluorescent imaging experiments in COS-7 cells transfected with GIRK1/2 or GIRK2wv where GIRK1 and GIRK2wv were tagged with EGFP. Fura 2 was used to detect resting Ca\(^{2+}\) in nontransfected COS-7 cells, COS-7 cells cotransfected with GIRK1-EGFP, and GIRK2wv-EGFP. Cells were loaded with fura 2-AM for 1 h before digital fluorescent imaging experiments. The resting 340/380 ratios (R) among the three groups were indistinguishable. These data are summarized in Fig. 7. The dynamic range of the cellular response to changes in Ca\(^{2+}\) was determined in experiments in which cells were sequentially exposed to a solution containing 10 µM ionomycin in the presence of 2 mM Ca\(^{2+}\), followed by a solution change to one in which the free Ca\(^{2+}\) concentration was buffered to zero in the presence of 1 mM EGTA as seen in Fig. 7A. The mean resting 340/380 nm fluorescence intensity ratio (R\(_{340/380}\)) values were 0.6 ± 0.02 (n = 43) for nontransfected cells, 0.55 ± 0.09 (n = 32) for GIRK1/GIRK2-expressing cells, and 0.57 ± 0.03 (n = 34) for the GIRK2wv-expressing cells (Fig. 7B). We were unable to detect a change in the R\(_{340/380}\) values on changing from low (2 mM) to high (70 mM) external Ca\(^{2+}\) in either the GIRK1/2- or GIRK2wv-transfected cells. The average of the change in the R\(_{340/380}\) in individual cells on increasing extracellular Ca\(^{2+}\) from 2 to 70 mM was 0.28 ± 0.02 (n = 5) for GIRK1/2 and 0.21 ± 0.03 (n = 9) for GIRK2wv as summarized in Fig. 7C.

**DISCUSSION**

Expression and activation of GIRK2wv in oocytes has been associated with a large increase in the endogenous
Ca\(^{2+}\)-activated Cl\(^{-}\) current, indicative of a large divalent influx in Ca\(^{2+}\)-containing solutions (14). This observation, along with the increased vulnerability of oocytes to cell death in Ca\(^{2+}\)-containing solutions (18), has suggested that the GIRK2\(^{wv}\) channel might allow for a significant Ca\(^{2+}\) “leak.” These observations prompted our investigation of the magnitude of the GIRK2\(^{wv}\) divalent permeability in heterologous expression systems.

In this study, we have compared expression of GIRK1/2 and GIRK2\(^{wv}\) channels in both oocytes and mammalian cells. We compared the divalent permeability of the wild-type to the mutant GIRK2\(^{wv}\) channels and found that oocytes expressing the mutant channels

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**Fig. 5.** GIRK2\(^{wv}\) expression in oocytes but not mammalian cells gives rise to a significant inward current in 5 mM Ca\(^{2+}\)-containing solutions over that seen in GIRK1/2-expressing cells. A: experiments were carried out in divalent solutions using voltage protocols as in Fig. 1. Current traces are from representative oocytes expressing GIRK1/2 and GIRK2\(^{wv}\). Amplitude of the inward current at \(-150\) mV for uninjected, GIRK1/2, and GIRK2\(^{wv}\)-expressing oocytes is compared on right. Divalent inward current was significantly greater in the GIRK2\(^{wv}\)-expressing oocytes over that observed for the GIRK1/2-expressing oocytes (\(P < 0.001\)) as well as for the uninjected oocytes (\(P < 0.0001\)). Level of significance between mean currents in 3 populations of oocytes was determined using General Linear Models (SAS, Carey, NC) with a Tukey correction for analysis of unequal cell sizes. B: currents from representative COS-7 cells transiently transfected with GIRK1-EGFP + GIRK2 and GIRK2\(^{wv}\)-EGFP fusion protein. There was no significant increase in inward current in 2 mM Ca\(^{2+}\)-containing solutions for the GIRK2\(^{wv}\)-expressing cells over that seen for the GIRK1/2-expressing cells as summarized in the bar graph to the right of the current traces.

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**Fig. 6.** Comparison of representative currents recorded from oocytes and mammalian cells expressing GIRK1/2 and GIRK2\(^{wv}\) in 70 mM Ca\(^{2+}\)-containing solutions. A: representative families of current traces from 2 different oocytes expressing either GIRK1/2 or GIRK2\(^{wv}\) in external solutions containing 70 mM external Ca\(^{2+}\). Summary of maximum inward current at \(-150\) mV is given to right of the current traces. Inward current in the GIRK2\(^{wv}\)-expressing oocytes was significantly increased (\(P < 0.001\)) over that observed for the GIRK1/2-expressing oocytes. Statistical analysis was performed as in Fig. 5, comparing mean inward current data between uninjected, GIRK1 + GIRK2, and GIRK2\(^{wv}\)-expressing oocytes. Results of statistical analysis demonstrated that in the oocytes the difference between the mean current amplitude was significantly different as a function of channel type. There was no statistical difference in mean current between channel types as a function of external Ca\(^{2+}\) concentration. B: families of current traces from 2 representative COS-7 cells expressing GIRK1-EGFP + GIRK2 and GIRK2\(^{wv}\)-EGFP. Summary of maximum inward current at \(-160\) mV is given to right of current traces. Note there was no significant difference in current amplitude in the high external Ca\(^{2+}\) solutions between the cells expressing mutant and wild-type channels.
Fig. 7. Comparison of resting Ca^{2+} levels in GIRK1 + GIRK2 vs. GIRK2wv-expressing COS-7 cells. Relative levels of intracellular Ca^{2+} were determined as the 340/380 nm fluorescence intensity ratio (R_{340/380}) in fura 2 loaded COS-7 cells expressing either GIRK1 + GIRK2 or GIRK2wv using digital imaging techniques. A: resting levels of R_{340/380} in 13 cells before and after exposure to external solutions containing 10 µM ionomycin. Ionomycin-containing solution was added at arrow. When the R_{340/380} appeared to reach a maximum value, the external solution was changed to one containing 2 mM Ca^{2+} and 1 mM EGTA. B: plot of the average resting R_{340/380} in nontransfected cells, cells expressing GIRK1 + GIRK2, and cells expressing GIRK2wv in an external solution containing 2 mM Ca^{2+}. Average maximal response to ionomycin in the GIRK2wv cells is shown in bar at right. C: comparison of the difference in resting R_{340/380} for cells in external solutions containing 70 mM Ca^{2+} minus that obtained in 2 mM Ca^{2+}. Cells were exposed to the Ca^{2+}-containing solution and the differences in the response in the intracellular fura 2 fluorescence. Consistent with the previous anecdotal observation made by Navarro and co-workers (13), we were unable to observe an increase in inward current in Chinese hamster ovary (data not shown) as well as COS cells transfected with the GIRK2wv gene in 70 mM Ca^{2+} containing external solutions.

It is tempting to generalize that the weaver mutation in the signature sequence of all K^+ selective channels would produce a similar loss in K^+ selectivity in the outwardly as well as the inwardly rectifying K^+ conductances. Interestingly, this same mutation has been found in a member of the six-transmembrane family of K^+ channels, KCNQ4, localizes its expression to cochlear outer hair cells and maps to the DFNA2 locus for a form of nonsyndromic dominant deafness (7). A mutation in this gene in the DNFA2 pedigree exchanges the G for an S (G285S) in the GYG sequence in the pore of that channel, identical to the mutation in GIRK2wv. The G285S mutation in KCNQ4 exerts a strong dominant negative effect on wild-type KCNQ4, and its loss leads to slow cellular degeneration (7), although the precise
pathogenesis is unknown. KCNQ4 codes for a six-transmembrane domain \(K^+\) channel subunit protein that is assumed to form a functional heterotetramer with other members of the KCNQ family. Unlike GIRK2wv, which has an equivalent mutation in the signature sequence, the mutation G285S in KCNQ4 does not appear to form functional homomultimers as does GIRK2wv. Coexpression studies with the mutant KCNQ4 G285S and other members of the KCNQ family carried out to date show that coexpression of the mutant subunit reduces current expression by \(-90\%\). The remaining current is \(K^+\) selective over \(Na^+\) or \(Ca^{2+}\) (7), unlike the selectivity profile of the mutant GIRK2wv. Thus similar pore mutations in the outward and inwardly rectifying \(K^+\) channel families would appear to have significantly different functional phenotypes with respect to changes in channel selectivity and ability to form functional homo- and heteromultimers (6, 16). The two transmembrane domain GIRK subunits appear to tolerate changes in pore-forming residues allowing for the formation of hetero- as well as homomultimeric channels. The six-transmembrane domain \(K^+\) channels appear to require a more rigid scaffolding intolerant of similar changes in pore-forming residues.

In addition to the observed differences in selectivity between GIRK1/GIRK2 heteromultimers and GIRK2wv homomultimers, we observed a consistent difference in the kinetics of current activation for the two channels at the most hyperpolarized potentials when expressed in Xenopus oocytes. Current activation for GIRK2wv expressed in oocytes was instantaneous, whereas the kinetics of activation for GIRK1/GIRK2 were much slower. Similar differences in the time course of current activation on hyperpolarization between recombinant GIRK1/GIRK2 and GIRK2wv channels have been observed by Slesinger et al. (16). Differences in time course of current activation between the wild-type GIRK1/GIRK2 channels and the mutant GIRK2wv channels were not observed when the recombinant channels were expressed in the mammalian cell background. These differences in activation kinetics, which we observed exclusively in the oocyte expression experiments, are consistent with the weak inward rectification of the currents also observed for the mutant GIRK2wv channel in the oocyte system in our studies as well as those of Kofuji et al. (6). Rectification in the inwardly rectifying \(K^+\) channels is a result of \(Mg^{2+}\) and/or polyamine binding to an intracellular site, thereby blocking monovalent cation permeation in the outward direction (3, 11, 17, 19). It may be that the reduced rectification seen for GIRK2wv when expressed in oocytes may be due to weak binding and/or permeation of a class of cytoplasmic polyamines not present in the mammalian cells.

In summary, results for our investigation indicate that the modest \(Ca^{2+}\) influx through GIRK2wv homomeric channels expressed in oocytes differs from that observed for channels expressed in mammalian cells and may represent the formation of a functional channel arising from coassembly with an unidentified endogenous subunit of the oocyte. Coassembly with the endogenous Xenopus oocyte subunit GIRK5 is unlikely, in that our experiments were conducted using antisense against GIRK5, which would have prevented its expression. Recombinant GIRK2wv channel expression in mammalian cells was not associated with either an observable \(Ca^{2+}\) permeation through the conductance nor an increase in intracellular \(Ca^{2+}\) over that observed in nontransfected cells. Our data suggest that the elevation in \(Ca^{2+}\) associated with neuronal cell death in murine cells expressing the gene may not be due to divalent permeation through the GIRK2wv homomeric channels but may be due instead to toxicity induced through chronic depolarization, allowing for \(Ca^{2+}\) influx through voltage-dependent \(Ca^{2+}\)-permeable pathways.

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