The Inwardly Rectifying K⁺ Channel Subunit GIRK1 Rescues the GIRK2 weaver Phenotype

Ping Hou, Shuizhong Yan, Weijen Tang, and Deborah J. Nelson

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The weaver (wv) gene has been identified as a glycinase to serine substitution at residue 156 in the H5 region of inwardly rectifying K⁺ channel, GIRK2. The mutation is permissive for the expression of homotetrameric channels that are nonselective for cations and G-protein-independent. Coexpression of GIRK2wv with GIRK1, GIRK2, or GIRK3 in Xenopus oocytes along with expression of subunit combinations linked as dimers and tetraters was used to investigate the effects of the pore mutation on channel selectivity and gating as a function of relative subunit position and number within a heterotetrameric complex. GIRK1 formed functional, K⁺-selective channels with GIRK2 and GIRK3. Coexpression of GIRK2wv with GIRK1 gave rise to a component of K⁺-selective, G-protein-dependent current. Currents resulting from coexpression of GIRK2wv with GIRK2 or GIRK3 were weaver-like. Current from dimers of GIRK1-GIRK2wv, GIRK2-GIRK2wv, and GIRK3-GIRK2wv was phenotypically similar to that obtained from coexpression of monomers. Linked tetramers containing GIRK1 and GIRK2wv in an alternating array gave rise to wild-type, K⁺-selective currents. When two mutant subunits were arranged adjacent in a tetramer, currents were weaver-like. These results support the hypothesis that in specific channel stoichiometries, GIRK1 rescues the weaver phenotype and suggests a basis for the selective neuronal vulnerability that is observed in the weaver mouse.

Key words: K⁺ channels; weaver mice; G-proteins; Xenopus oocytes; voltage clamp; neurons

Received May 19, 1999; revised July 16, 1999; accepted July 22, 1999.

This work was supported by National Institutes of Health Grants R01 GM36823 and R01 GM 54266. We thank Drs. Aaron P. Fox, Henry A. Lester, Anke Di, and Dorothy A. Hanck for many helpful discussions as well as Clark Lin and Boris Krupe for technical assistance.

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The Journal of Neuroscience, October 1, 1999, 19(19):8327–8336

MATERIALS AND METHODS

DNA clone. GIRK1 was cloned from a rat insulinoma tumor cell (RIN) library and has a predicted amino acid sequence identical to the cardiac clone originally described (Kubo et al., 1993b); GIRK2 and GIRK2wv, GIRK3, and GIRK5, were generous gifts from Drs. P. Kofuji (California Institute of Technology, Pasadena, CA), A. Karschin (Max-Planck-
Table 1. Homomeric expression of GIRK1, GIRK2, and GIRK3 is inhibited in the presence of antisense to GIRK5 and synergistically enhanced when coexpressed with cloned GIRK5

<table>
<thead>
<tr>
<th>Coexpression experiment</th>
<th>GIRK1</th>
<th>GIRK2</th>
<th>GIRK3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>$I_{\text{carb}}$</td>
<td>Basal</td>
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<td>Control</td>
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<td>0.53 ± 0.09 (8)</td>
<td>0.66 ± 0.07 (10)</td>
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<tr>
<td>GIRK5 antisense</td>
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<td>0.10 ± 0.02 (6)</td>
<td>0.46 ± 0.03 (5)</td>
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<td>GIRK5</td>
<td>1.95 ± 0.26 (8)</td>
<td>5.64 ± 0.79 (8)</td>
<td>1.07 ± 0.14 (11)</td>
</tr>
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</table>

All oocytes were coinjected with the muscarinic receptor m2 as described in Materials and Methods. Peak currents were measured at −150 mV and averaged. The G-protein-dependent current was determined as the difference between the current determined in the presence of 5 μM carbachol added to the bath solution minus the current determined in the absence of carbachol stimulation. The number of oocytes for each experimental condition is given in parentheses. Currents were recorded in solutions in which all the Na⁺ was osmotically replaced with K⁺.

OR-2⁺ solution, which has been shown to enhance cellular survival by preventing possible swelling and Ca²⁺ overload (Silverman et al., 1996a). In those experiments investigating the effects of free Gop on basal current activity, oocytes were maintained in a high glucose (5 mM)-containing solution. Oocytes were injected with 50 nl containing constant amounts of each single subunit cRNA (−5 ng), m2 receptor (−2.5 ng) together with 12.5 ng of fully phosphothiolated GIRK5 antisense oligonucleotide KHA1 (5'–CTGAGAATTCGCTTCATCTG–3') prepared at HHMI.

Electrophysiology. Two-electrode voltage recordings were performed 2–3 d after injection at room temperature using a TURBO TEC-10C amplifier (NPI, Tamm, Germany), 1Tc-16 interface (Instrutech, Great Neck, NY), and IBM-compatible PC. Microelectrodes were filled with 3 M KCl and had resistances of 0.5–2 MΩ. 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. G-protein-dependent currents were induced with the addition of 5 μM carbachol (Sigma, St. Louis, MO)

Figure 1. G-protein-dependent and independent K⁺ current activation in oocytes injected with cRNA for GIRK1, 2, and 3 as well as combinations of GIRK1, GIRK2, or GIRK3 with GIRK5 or antisense for GIRK5. Currents were recorded using a two-microelectrode voltage clamp from oocytes injected with cRNA for the m2 muscarinic receptor, GIRK1, 2, 3, and 5, and GIRK5 antisense oligonucleotide KHA1, as described in Materials and Methods. Currents were recorded from a holding potential of −80 mV in response to step voltages between −150 and 50 mV in 20 mV increments, the interval between steps was 1 sec. The basal current was determined in a solution in which all the Na⁺ was isosmotically replaced with K⁺; G-protein current activation was determined in response to 5 μM carbachol added to the extracellular solution. Bars represent a summary of current data at −150 mV for all subunit combinations recorded 72 hr after injection for both basal K⁺ and total current (basal plus carbachol-induced in high K⁺ solutions). Bars represent mean ± SEM measure at −150 mV.
to the bath 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 intervals. Data collection and analysis were performed using Pulse/Pulse Fit (Heka, Lambrecht, Germany), and data were plotted using the integrated graphics package Igor (WaveMetrics, Lake Oswego, OR). Data are presented as mean ± SEM with the number of oocytes in parentheses. All experiments were conducted at room temperature.

**G<sub>lu</sub> protein purification.** Expression of the N-terminal hexohistidinetagged short form of G<sub>lu</sub> was performed in *Escherichia coli* BL21(DE3) that carried pUBS520 and H6-pQE60-G<sub>lu</sub> grown to cell density of OD<sub>600</sub> = 0.4 at 30°C and induced by adding IPTG and chloramphenicol to a final concentration of 30 and 1 μM, respectively. The expression of DNA from pUBS520 enhanced G<sub>lu</sub> expression threefold to fourfold. After a 19 hr induction period, *E. coli* were harvested and lysed; G<sub>lu</sub> was purified using the nickel-nitritolriacetic acid (Ni-NTA) and FPLC Q-Sepharose column as described (Lee et al., 1994). Coomassie blue staining of protein purification.

**RESULTS**

**Coexpression of monomeric GIRK subunits in *Xenopus* oocytes**

Expression of recombinant GIRK channels was studied in *Xenopus* oocytes after injection of GIRK1, GIRK2, GIRK3, and GIRK5 subunit cRNA or combinations of two isoforms along with m2, muscarinic receptor cRNA. In all experiments, G-protein-independent (basal) as well as G-protein-dependent (carbachol-induced) currents were recorded in solutions containing 90 mM K<sup>+</sup>. Currents were recorded at 36–72 hr after injection. Experiments were replicated in at least three batches of oocytes.

Recombinant GIRK subunits coassemble with endogenous *Xenopus* GIRK5 subunits to form functional channels (Hedin et al., 1996). Antisense against GIRK5 (KHA1) has been previously reported to knock out endogenous GIRK5 expression in oocytes (Silverman et al., 1996b). We conducted experiments to compare levels of homomeric GIRK subunit expression in the presence and absence of coinjected antisense against GIRK5. In addition, we examined the synergistic enhancement of homomeric GIRK subunit expression in the presence of coexpressed GIRK5. Data comparing carbachol-induced current amplitude to basal current amplitude in high K<sup>+</sup> solutions for GIRK1, GIRK2, and GIRK3 are summarized in Table 1. Current expression is synergistically enhanced for GIRK1 and GIRK2 isoforms in the presence of coexpressed GIRK5. Summary of current data at −150 mV for all subunit combinations determined 72 hr after injection for both basal K<sup>+</sup> (solid bars) and total current (white bars). Bars represent mean ± SEM based on recordings from 10–40 oocytes taken from at least three batches.

**Selectivity of heteromultimeric GIRK2wv channel is controlled by GIRK1 subunit association**

To determine whether GIRK2wv forms functional heterooligomers, we performed coexpression experiments with wild-type GIRK1, 2, or 3. GIRK2wv was coinjected at a ratio of 1:1 with wild-type cRNA. The selectivity of both the basal as well as the carbachol-induced current is compared for each of the coexpression studies in Figure 3. When GIRK1 was coexpressed with GIRK2wv, currents resembled those obtained after coexpression of GIRK1 with GIRK2 in that (1) the G-protein-independent
current was $K^+$-selective and (2) there was a significant amount of $K^+$-selective carbachol-induced current. When GIRK2 was coexpressed with GIRK2wv, both basal- and carbachol-induced $K^+$ currents were significantly reduced as compared to GIRK1 + GIRK2wv expression. GIRK3 coexpression with GIRK2wv resembled GIRK2wv homomeric currents in that the G-protein-independent component was nonselective, and the carbachol-sensitive component was absent. Data for the GIRK2wv coexpression studies are compared and summarized in Figure 3C.

The presence of GIRK1, not the number of wv subunits, determines channel phenotype

The family of GIRK channels appears to form functional tetramers (Dascal, 1997; Jan and Jan, 1997b; Corey et al., 1998). Therefore, functional channels in coexpression experiments may exist in several possible channel stoichiometries. To constrain the possible functional combinations, we constructed and expressed heterodimers containing a GIRK2wv subunit. The selectivity of the heterodimer recombinant currents is shown in Figure 4. A comparison of the basal as well as the carbachol-induced current for the dimeric constructs was compared to that obtained for expression of GIRK2wv monomers. We found that the GIRK1-wv dimer showed a phenotypic profile that resembled the wild-type GIRK1–2 dimer currents with respect to (1) an insignificant basal $Na^+$ permeability and (2) an equivalent basal- and carbachol-induced $K^+$ current. However, more importantly, both GIRK2-wv and GIRK3-wv dimer constructs gave rise to a basal or G-protein independent $Na^+$ current and no carbachol-induced $K^+$ current, a phenotypic profile that was identical to the GIRK2wv monomeric currents. Each of the dimeric constructs presumably gave rise to tetrameric channels containing two GIRK2wv subunits with two possible stoichiometries where identical subunits were positioned either across from or adjacent to each other. Current phenotypes determined by selectivity as well as G-protein dependence differed according to the wild-type subunit linked to GIRK2wv subunit as summarized in Figure 4C.

GIRK2wv subunit stoichiometry and positional effects

The selectivity and G-protein dependence of the currents obtained from the dimer expression experiments strongly resembled data obtained in the coexpression studies. This may reflect that subunit coassembly may not be random but involve a preferred arrangement around the pore. To date, studies on the stoichiometry of GIRK channels have relied on the formation of multimeric concatemers. This approach has been successfully used to constrain the stoichiometry and relative position of both voltage-gated and inwardly rectifying $K^+$ channel subunits (Liman et al., 1992; Yang et al., 1995; Silverman et al., 1996b; Tucker et al., 1996). Following this approach, we linked GIRK1 and GIRK2wv subunits into tetrameric constructs. The positional effect of the GIRK2wv subunit was investigated using tetramers that contained two mutant subunits in one of two alternate patterns. Identical subunits were either adjacent (1–1-wv-wv) or linked as an alternating array (1-wv-1-wv). Data obtained from the expres-
The basal current for the 1-wv-1-wv tetramer was K\textsuperscript{+}-selective and resembled that obtained for GIRK1-GIRK2\textsuperscript{wv} coexpression and GIRK1-wv dimer expression. In contrast, the 1–1-wv-wv basal currents were nonselective and were similar to monomeric GIRK2\textsuperscript{wv} currents (Fig. 5A). The relative amplitude of the carbachol-induced currents in Na\textsuperscript{+} versus K\textsuperscript{+} containing solutions for all the aforementioned constructs is compared in Figure 5B. The expression of 1-wv-1-wv resulted in a significant G-protein-dependent K\textsuperscript{+}-selective current (−1.3 ± 0.2 μA at −150 mV; n = 16) when compared to that obtained for the 1–1-wv-wv tetramer (−0.6 ± 0.12 μA at −150 mV; n = 12) as seen in Figure 5B. Overall, the expression levels of 1–1-wv-wv were comparable to that obtained for the GIRK2\textsuperscript{wv} homomultimeric current.

The pharmacological sensitivity to block by 500 μM external Ba\textsuperscript{2+} for both concatameric as well as monomeric constructs is compared in Figure 5, C and D. Ba\textsuperscript{2+} sensitivity is expressed as the fraction of the total current in high K\textsuperscript{+} solutions (carbachol-sensitive plus insensitive K\textsuperscript{+}-selective current) inhibited after exposure to 500 μM Ba\textsuperscript{2+}. Currents obtained after coexpression of GIRK1 with GIRK2\textsuperscript{wv} and the 1-wv-1-wv tetramer maintained their Ba\textsuperscript{2+} sensitivity. The tetrameric 1–1-wv-wv currents were insensitive to 500 μM Ba\textsuperscript{2+}, similar to GIRK2\textsuperscript{wv} homomultimeric channels. A comparison of the Ba\textsuperscript{2+} sensitivity for wild-type heteromultimeric GIRK1/GIRK2 and homomultimeric GIRK2\textsuperscript{wv} channels is given in Figure 5D in which the Ba\textsuperscript{2+}-sensitive current is expressed as a fraction of the total current in high K\textsuperscript{+} solutions.

The possible channel stoichiometries in each of the GIRK2\textsuperscript{wv} expression experiments summarized in Figure 5 are depicted graphically in Figure 6. Note that dimeric GIRK1-GIRK2\textsuperscript{wv} expression could yield two theoretically possible tetrameric stoichiometries. Expression of the linked tetramers, which would give rise to both of the possible stoichiometries, gives currents that are separable on the basis of their G-protein sensitivity and basal Na\textsuperscript{+} conductance. Thus, the sum of the tetrameric currents does not give rise to a current that resembles the currents obtained by expression of the GIRK1-GIRK2\textsuperscript{wv} heterodimer. These results suggest that the tetramer with two adjacent GIRK2\textsuperscript{wv} subunits is not the preferred stoichiometry in the expression of heterodimers.
Comparative basal activation in high K⁺ for wild-type GIRK1/GIRK2 channels versus GIRK1-GIRK2wv dimers

The basal current in high K⁺ solutions was elevated for GIRK1 + GIRK2 heteromultimers (Figs. 2C, 3C), for GIRK1 + GIRK2wv (Fig. 3C), and for the dimeric construct GIRK1-GIRK2wv (Fig. 4). Such high levels of basal current activation does not occur in isolated neurons expressing GIRK channels (Surmeier et al., 1996; Slesinger et al., 1997; Rossi et al., 1998). It has been proposed that the high levels of basal activation seen with GIRK expression in the Xenopus oocyte expression system is a result of high levels of intracellular Na⁺ (Silverman et al., 1996a) or high levels of free Gαs (Vivaudou et al., 1997). In that the K⁺ over Na⁺ selectivity of the GIRK1/GIRK2wv heteromultimers served as an indicator of wild-type GIRK current, we performed experiments to determine if basal K⁺ current of channels containing the mutant subunit were differentially sensitive to free Gαs. Oocytes expressing either GIRK1 + GIRK2, the dimer GIRK1-GIRK2wv, or GIRK2wv were examined for current expression 36 hr after cRNA injection. Oocytes were maintained in solutions in high glucose (5 mM), low K⁺ (2.5 mM) solutions. Recordings were made in solutions in which all the Na⁺ was replaced with the large impermeant cation N-methyl-D-glucamine (NMDG) or 90 mM K⁺. Approximately 30 min before recording, half of the oocytes were injected with 50 nl of purified Gαs (40 μg/μl) to serve as a Gβγ sink. The amplitude of the basal- and carbachol-induced current amplitude was compared for all the constructs and is summarized in Figure 7. Na⁺-selective currents were also determined for the GIRK2wv homomultimeric currents. The GIRK2wv homomultimeric currents were unchanged in the presence of Gαs. The GIRK1 + GIRK2 currents as well GIRK1-GIRK2wv dimer currents responded to injection of the Gαsα by a significant decrease in the carbachol-induced current and a more modest decrease in the basal current. Thus, the two heteromultimeric channel constructs were indistinguishable based on their sensitivity of the basal K⁺ current to free circulating Gβγ.

DISCUSSION

In this report, we demonstrate that GIRK1 is capable of forming heteromultimeric channels with GIRK2wv in monomer coexpression studies as well as in linked concatemers. The presence of GIRK1 in a tetrameric GIRK1/GIRK2wv channel rescued the wild-type GIRK1/GIRK2 heteromultimeric phenotype, restoring K⁺ selectivity and G-protein-dependent current activation. Furthermore, the position of two GIRK2wv subunits within linked concatemers appears to determine current selectivity and G-protein dependence. GIRK2 and GIRK3 formed functional heteromultimeric channels with GIRK2wv; however, the heteromultimeric complexes retained GIRK2wv homomultimeric channel properties.
Our studies differ from those of Slesinger et al. (1996), who reported that expression of monomeric GIRK1 and GIRK2v wastewater rise to a significant decrease in the amplitude of the agonist-independent basal Na$^+$ as well as K$^+$ currents over that observed for oocytes expressing GIRK2v wastewater alone. In their studies, a GIRK2v wastewater construct was used in which the first nine amino acids were deleted. The truncation of the first nine amino acids in GIRK2v wastewater may have reduced the mutant subunit affinity for heteromultimer formation, thereby, giving rise to the difference in current expression between the two studies. In addition, our studies, unlike those of Slesinger et al. (1996), included antisense against the endogenous GIRK5. The presence of GIRK5 in their studies may have also contributed to heteromultimer formation with GIRK2v wastewater, thereby competing with GIRK1 as a companion subunit.

In our studies, GIRK1, GIRK2, and GIRK3 failed to form functional homomeric channels when expressed either as monomers (Fig. 1) or dimers (data not shown). The apparent discrepancy between our data and the data reported in previous investigations in which expression of homomultimeric GIRK1 and GIRK2 was obtained may be caused by significant amounts of coassembly with the endogenous Xenopus GIRK5 (Kofuji et al., 1995, 1996; Slesinger et al., 1996) or coexpression with G$p_y$, which has been reported to increase current levels 16-fold above activation through the m2 receptor alone (Velimirovic et al., 1996). The small but detectable (280 ± 30 nA) carbachol-sensitive current that we observed for GIRK2 coexpressed with GIRK5 antisense may represent GIRK2 homomultimeric current in our experiments.

GIRK1 appears to be necessary but not sufficient for channel formation. The other interacting subunits, GIRK2, GIRK3, and GIRK4, appear to lend subtle differences in conductance or open state probability to the functional channel depending on number or position within the heterotetrameric complex. Data in support of this hypothesis come from studies of GIRK1 and GIRK4 in which current expressed from linked concatamers was maximized when channels were comprised of alternating subunits within the tetramers (Silverman et al., 1996b; Tucker et al., 1996; Corey et al., 1998). The positional studies of GIRK1 and GIRK4 in linked concatamers (Silverman et al., 1996b; Tucker et al., 1996; Corey et al., 1998) suggested that the position of multiple subunits of GIRK2v wastewater within a heterotetramer might play a similar role in the determination of channel selectivity and $G$-protein dependence.

Unlike GIRK1, GIRK3 does not enhance either GIRK2 or GIRK2v wastewater current expression. In addition, GIRK3 does not seem to form functional heterotetramers with GIRK5 (Table 1). Therefore, GIRK3 seems to form heterotetramers exclusively with GIRK1.

**Positional effects of GIRK2v wastewater on channel selectivity and G-protein dependence within a tetramer**

Expression of the GIRK1 and GIRK2v wastewater tetrameric constructs yielded currents with biophysical signatures dependent on the position of the mutant subunits in the tetramer. The 1–1–1–1 wastewater tetramer currents were $G$-protein-dependent and K$^+$-selective. On the other hand, 1–1–1–1 wastewater tetramer currents were associated with a high basal Na$^+$ current and only a modest $G$-protein-dependent current in high K$^+$ solutions (Fig. 5A,B).

The most parsimonious explanation for the current data obtained from the tetrameric constructs relies on the assumption that when subunit DNA is fixed in a concatameric array, subunit proteins will align in the same manner in the membrane. Although highly likely, one could imagine an alternative scenario, whereby, the concatenated 1–1–1–1 wastewater sequence of cDNAs could give rise to subunits that arrange 1–1–1–1 wastewater with the long cytoplasmic segments connecting C to N termini twisted and interwoven. The abnormal arrangement of the C- and N-termini could provide an alternate explanation for the aberrant channel behavior observed with the 1–1–1–1 wastewater construct.

**Tetramers containing at least two nonwastewater subunits restore K$^+$ selectivity and G-protein sensitivity**

Previous coexpression studies of GIRK1 and GIRK2v wastewater monomers in oocytes have yielded conflicting results that could possibly be accounted for, in part, by subtle experimental differences in relative subunit concentrations. Kofuji et al. (1996) found that oocyte coexpression of GIRK1 with GIRK2v wastewater gave rise to currents that were similar in selectivity and G-protein sensitivity to GIRK2v wastewater homomultimeric currents. In other studies, coexpression of GIRK2v wastewater with GIRK1 at a ratio of coinjected cRNA of 1:1 led to a reduction in both basal- and carbachol-induced currents, compared to oocytes expressing GIRK1 + GIRK2.
Table 2. Anatomical correlation between GIRK subunit expression and cell survival in the weaver mouse

<table>
<thead>
<tr>
<th>Region</th>
<th>Age</th>
<th>GIRK1 protein</th>
<th>GIRK2 protein</th>
<th>Cell fate</th>
</tr>
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<td>+</td>
<td>Survival</td>
</tr>
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<td>Adult</td>
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<td>+</td>
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<td>++</td>
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<td>Survival</td>
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<td>Deep cerebellar nuclei</td>
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Staining intensities: +++, strong; ++, moderate; +, light; -, little to no background levels.

aLiao et al. (1996).
bSlesinger et al. (1996).
cSchein et al. (1998).

Correlation between cell survival and GIRK1 expression in the weaver mouse brain

It is our hypothesis that susceptibility to cell death among populations of neurons in the weaver mouse may result from differential isoform expression and, therefore, the availability of GIRK subunits to form functional channels. In that the homomultimeric GIRK2wv channel is the most pathological, our hypothesis would predict that cell death would be highest in those neurons that demonstrate the highest levels of GIRK2 expression. Substantia nigra (SN) is the primary target for cellular degeneration in the weaver mouse (Table 2), in which GIRK2 protein expression is highest and in which there is a considerable reduction in both GIRK2-positive cells as well as cell number with increasing age (Liao et al., 1996). Within the SN, the strongest GIRK2 expression was seen in the pars compacta, the region most vulnerable to cell death. The more laterally placed neurons in SN pars lateralis, which do not stain for GIRK2 protein expression, are for the most part spared in weaver homozygotes (Graybiel et al., 1990; Liao et al., 1996; Roffler-Tarlov et al., 1996; Wei et al., 1997; Schein et al., 1998). Thus, in the SN there is a direct correlation between the magnitude of GIRK2 expression and cell death.

The early cytarchitectural studies of the weaver mouse cerebellum conducted by Herrup and Trenkner (1987) revealed an apparent gradient in cell death with the selective loss of granule cells only in the medial cerebellum with a substantial number surviving in the lateral cerebellar cortex. Patterns of protein expression for the mutant GIRK2 protein in the weaver mouse have since provided an explanation for their initial observations. Schein et al. (1998) observed that Purkinje cells of the lateral cerebellum expressed little GIRK2 and were also spared. However, there was selective loss of Purkinje cells in the midline, which correlated with enhanced expression of GIRK2. Corroborating studies carried out by Schlesinger et al. (1996) on the highly vulnerable, developing weaver mouse cerebellar Purkinje cells also demonstrated expression of GIRK2 but not GIRK1.

The loss of granule cells in the external germinal layer, the internal granular layer, and the deep cerebellar nuclei of the cerebellum in the weaver mouse that express both GIRK1 and GIRK2 would, at first examination, appear to be an exception to our hypothesis, i.e., that elevated levels of GIRK1/GIRK2 expression might spare neurons through the formation of functional heteromultimeric channels. The apparent inconsistency could be accounted for on a number of levels. The weaver mutation could quantitatively exert variable toxicity in different neu-
neuronal populations depending on relative levels of protein expression with respect to wild-type isoforms e.g., GIRK1 (Liao et al., 1996; Roffler-Tarlov et al., 1996; Wei et al., 1997; Schein et al., 1998). The mutant toxicity could also be a function of the splice variant of GIRK2 which is expressed within a given region. To date, five splice variants of GIRK2 have been reported: GIRK2–1, GIRK2A (A1 and A2), GIRK2B, and GIRK2C (Iso-moto et al., 1996; Wei et al., 1998). Wei et al (1998) detected strong expression of GIRK2B and GIRK2C in the cerebellum and suggested that their respective proteins may play prominent roles in the mutation-induced pathology of the weaver mice. Isomoto et al. (1996) demonstrated that GIRK2B forms functional homomultimers. By analogy, GIRK2C may also form a functional channel. Based on their observations, we speculate that the weaver mutant forms of GIRK2B and 2C strongly expressed in the cerebellar granule cells may have a higher affinity for the formation of homomultimeric mutant channels than hetero- multimers with GIRK1, thereby giving rise to enhanced cell death.

In summary, our study addresses the issue of stoichiometric and functional relationships between GIRK channel subunits. Our results constitute strong evidence that GIRK1 forms heteromultimeric channels with GIRK2wv, restoring G-protein sensitivity and K\(^+\) selectivity and thereby suppressing the lethal weaver phenotype. Thus, different susceptibilities to cell death among different populations of neurons may result from differences in ratio of expression of GIRK subunit isoforms among the different neuronal populations.


