Molecular Determinants for Sodium-dependent Activation of G Protein-gated K⁺ Channels*

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G protein-gated inwardly rectifying K⁺ channels (GIRKs) are activated by a direct interaction with Gβγ subunits and also by raised internal [Na⁺]. Both processes require the presence of phosphatidylinositol bisphosphate (PIP₂). Here we show that the proximal C-terminal region of GIRK2 mediates the Na⁺-dependent activation of both the GIRK2 homomeric channels and the GIRK1/GIRK2 heteromeric channels. Within this region, GIRK2 has an aspartate at position 226, whereas GIRK1 has an asparagine at the equivalent position (217). A single point mutation, D226N, in GIRK2, abolished the Na⁺-dependent activation of both the homomeric and heteromeric channels. Neutralizing a nearby negative charge, E234S had no effect. The reverse mutation in GIRK1, N217D, was sufficient to restore Na⁺-dependent activation to the GIRK1N217D/GIRK2D226N heteromeric channels. The D226N mutation did not alter either the single channel properties or the ability of these channels to be activated via the m2-muscarinic receptor. PIP₂ dramatically increased the open probability of GIRK1/GIRK2 channels in the absence of Na⁺ or Gβγ but did not preclude further activation by Na⁺, suggesting that Na⁺ is not acting simply to promote PIP₂ binding to GIRKs. We conclude that aspartate 226 in GIRK2 plays a crucial role in Na⁺-dependent gating of GIRK1/GIRK2 channels.

The G protein-gated inwardly rectifying K⁺ channels (GIRKs) were the first channels to be shown to be gated by a direct interaction with the βγ subunits of GTP-binding proteins (1, 2, 3). This mechanism mediates the coupling of m2-muscarinic receptors with GIRK channels in the atria, and the generation of inhibitory postsynaptic currents by neurotransmitters acting on GABA$_B$, 5HT$_1A$ and A$_1$ receptors, in hippocampal neurons (4). More recently, other regulators of GIRK channels have been identified, which can activate these channels in the absence of G proteins. Recombinant GIRK1/GIRK4 channels and the native atrial channels were shown by Sui et al. (5) to be activated by a rise in cytosolic [Na⁺], with a threshold for activation of 3–10 mM and an EC$_{50}$ of ~40 mM. In isolated membrane patches, activation by Na⁺ required the hydrolysis of ATP. Subsequently, Sui et al. (6) showed that phosphatidylinositol 4,5-bisphosphate (PIP$_2$) mimics the ATP effects and that depletion or block of PIP$_2$ inhibits the activation of GIRK channels by both Gβγ and Na⁺. Huang et al. (7) reported similar findings and showed also that activation of GIRK channels, by applying PIP$_2$ in the presence of Na⁺, precluded further activation by Gβγ.

Activation by internal Na⁺ is not unique to atrial GIRK1/GIRK4 channels. GIRK1/GIRK2 heteromeric channels have also been shown to be activated by 20 mM Na⁺ in the presence of ATP (8), and a mutation in the pore region of GIRK2, which renders these channels permeable to both Na⁺ and K⁺, causes a large increase in their Gβγ-independent activity (9, 10, 11).

Some of the molecular aspects of G protein gating of GIRKs have already been elucidated. G proteins have been shown to interact with both the cytoplasmic N- and C-terminal regions of GIRK subunits. Huang et al. (12) made a series of GIRK1 C-terminal fusion proteins and reported that the region between amino acids 318 and 462 was involved in binding purified Gβγ. A recent study by Krapivinsky et al. (13) identified a single region within the C-terminal tail of GIRK4 that lies in close proximity to the second transmembrane segment (TM2) (amino acids 209–245) as being critical for Gβγ binding and channel activation. A point mutation within the proximal C terminus of GIRK4, substituting threonine for cysteine 216, drastically reduced the potency of Gβγ in activating not only GIRK4 homomeric channels but also GIRK1/GIRK4 heteromeric channels (13).

The aim of our study was to identify the region of GIRK1/GIRK2 channels that mediates their activation by internal Na⁺. Using a chimeric approach, combined with site directed mutagenesis of the GIRK subunits, we have identified the proximal C-terminal region of GIRK2 as playing a crucial role in the Na⁺-dependent activation of both GIRK2 homomeric and GIRK1/GIRK2 heteromeric channels. Within this region, GIRK2 has an aspartate at position 226, which is conserved in GIRK4 but is an asparagine in GIRK1. Substituting asparagine for aspartate 226 in GIRK2, abolished Na⁺-dependent activation of both the homomeric and GIRK1/GIRK2 heteromeric channels without affecting their ability to be activated via the m2-muscarinic receptors. The reverse mutation in the GIRK1 subunit (N217D) was sufficient to recover Na⁺-dependent activation of the heteromeric channel.

EXPERIMENTAL PROCEDURES

cDNA Clones—Standard PCR procedures were used to construct a strong Kozak consensus sequence GCCGCAACC immediately upstream of the ATG initiation codon in cDNA clones for GIRK1 and GIRK2. We used the longer of the GIRK2 splice variants, which contains 423 amino acids (14). These constructs were then subcloned into the EcoRI site of the pBG7.2 vector, which provides the 5’- and 3’-untranslated regions of the Xenopus β-globin gene. GIRK4 was also subcloned into the pBG7.2 vector, and the human muscarinic m2 re-

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FIG. 1. Activation of GIRK1/GIRK2 heteromeric channels by intracellular Na\(^+\) and PIP\(_{2}\). Multichannel recordings from oocytes coexpressing GIRK1 and GIRK2. A, channel activity recorded in the cell-attached mode ran down upon formation of an inside-out, isolated patch.
Female Xenopus laevis anesthetized with 0.3% (w/v) 3-a-mino benzoic acid (Sigma) and dissociated from connective tissue using 20 mM NaCl-free buffer (mM): 82.5 NaCl, 2.5 KCl, 1 MgCl₂, 5 HEPES, pH 7.6 with NaOH. Isolated oocytes were microinjected with 50 nl cRNAs dissolved in water. The in vitro transcription of cRNAs was as described previously in Stevens et al. (15). A total similar amount of channel subunit cRNA was injected into each oocyte. In some experiments, the human m2-muscarinic receptor cRNA was coinjected. Oocytes were incubated in ND96 at 18 °C.

RESULTS

Gating of GIRK1/GIRK2 Heteromeric Channels by Intracellular Na⁺ and PIP2—We measured the effects of raising internal [Na⁺] on GIRK1/GIRK2 heteromeric channels heterologously expressed in Xenopus oocytes. Fig. 1A shows a multichannel recording firstly in the cell-attached mode and then following patch excision in a solution lacking ATP, GTP, and Na⁺. Channel activity ran down over a period of 1–2 min and was partly restored by application of 5 mM MgATP and further increased by application of 20 mM Na⁺ in the continued presence of ATP. In contrast, application of 20 mM Na⁺ in the absence of MgATP had little effect on channel activity (Fig. 1B), and 5 mM AMP-PNP was unable to substitute for ATP in activating the channel. Even in the presence of 100 μM GTPγS plus 5 mM MgATP, application of 20 mM Na⁺ further increased channel activity (Fig. 1C), and the effects of Na⁺ were not blocked by 100 μM GDPβS (Fig. 1D). Thus, Na⁺-dependent activation of GIRK1/GIRK2 channels appears to require ATP hydrolysis, but is independent of G proteins. Sui et al. (6) suggested that the production of PIP2 by hydrolysis of MgATP is responsible for the ATP dependence of GIRK1/GIRK4 channel activity. They applied 1 μM PIP2 in the absence of Na⁺ or Gβγ and showed little change in GIRK1/GIRK4 channel opening frequency, but a large increase in channel activity upon subsequent application of 20 mM Na⁺. We applied 50 μM PIP2 for 5 min in the absence of Na⁺ or Gβγ and saw a dramatic increase in the opening frequency of the channel (Fig. 1E), with no apparent change in the single channel conductance. Subsequent application of 20 mM Na⁺ further increased the open probability (Popen) by ~2-fold (1.93 ± 0.16, n = 3). Thus PIP2 appears to be an activator of GIRK channels in the absence of either Na⁺ or Gβγ, but the actions of PIP2 do not preclude further activation by Na⁺.

Fig. 2 shows the dose-response relationship for Na⁺-activation of GIRK1/GIRK2, GIRK2, and GIRK1/GIRK4 channels in the presence of 5 mM MgATP. The data were fitted with the Hill equation:

\[ Y = \frac{Y_{\text{max}}}{1 + (\frac{K_{\text{d}}}{[\text{Na}^+])^n}} \]

(arrow). Application of 5 mM MgATP and 20 mM Na⁺, for the duration indicated by the bars, re-activated GIRK1/GIRK2 channels. B, application of 20 mM Na⁺ alone and together with 5 mM AMP-PNP was unable to reactivate GIRK channels. C, 100 μM GTPγS in the presence of 5 mM MgATP maintained channel activity in an inside-out patch; subsequent application of 20 mM Na⁺ further enhanced channel activity. D, channel activation by 20 mM Na⁺ was not blocked by 100 μM GDPβS. E, recordings from an inside-out patch, before and after application of 50 μM purified PIP2. PIP2 dramatically increased the channel opening frequency in the absence of Na⁺ or Gβγ. Subsequent application of 20 mM Na⁺ further increased the channel open probability. The holding potential was ~80 mV, and the solution in the bath and in the pipette contained 96 mM K⁺.
Na$^+$-dependent Activation of GIRK Channels

Fig. 2

A
GIRK1/GIRK2

B

Normalized mean current

[Na$^+$]

C
1211

D

Normalized mean current

[Na$^+$]

E
1211

F
1211

G
GIRK1/GIRK2

H

Normalized mean current

[Na$^+$]

FIG. 2


**Na⁺-dependent Activation of GIRK Channels**

A summary of the Na⁺ sensitivity of wild type and mutant GIRK channels

| Channel               | EC₅₀ (mm) | IC₅₀ (mm) | Hill coefficient | n   |
|-----------------------|-----------|-----------|------------------|-----|
| **Group I**           |           |           |                  |     |
| GIRK1/GIRK2           | 27        | 1.7       | 7                | 7   |
| GIRK1/GIRK4           | 44        | 2.0       | 5                | 5   |
| GIRK1/1222            | 43        | 2.3       | 4                | 4   |
| GIRK1/1221            | 28        | 1.6       | 4                | 4   |
| GIRK1/GIRK2E234S      | 30        | 1.6       | 6                | 6   |
| GIRK1N1217D/GIRK2D226N| 32        | 1.7       | 5                | 5   |
| **Group II**          |           |           |                  |     |
| GIRK2                 | 37        | 4.2       | 5                | 5   |
| 1221                  | 37        | 3.8       | 4                | 4   |
| GIRK2E234S            | 42        | 4.0       | 5                | 5   |
| 1211N217D             | 37        | 3.6       | 4                | 4   |
| **Group III**         |           |           |                  |     |
| 1211                  | 23        | 0.9       | 5                | 5   |
| 1212                  | 17        | 1.1       | 3                | 3   |
| GIRK2D226N            | 18        | 0.9       | 6                | 6   |
| GIRK1/1212            | 16        | 1.0       | 6                | 6   |
| GIRK1/GIRK2D226N      | 29        | 1.3       | 4                | 4   |

a Group I is Na⁺-activated heteromeric channels.
b Group II is Na⁺-activated homomeric channels.
c Group III is Na⁺-inhibited channels.

To locate the Na⁺-sensing region of GIRK2 we looked at the effects of Na⁺ on the 1222 chimeric subunit. This differs from chimeras 1211 in that it has the GIRK2 C-terminal tail and only the N-terminal tail of GIRK1. 1222 subunit (previously called 122) does not appear to form functional homomeric channels but does form functional heteromeric channels when coexpressed with GIRK1 in *Xenopus* oocytes (15). Increasing internal Na⁺ activated this channel with an EC₅₀ of ~43 mm and a Hill coefficient of 2.3 (Fig. 3, A and B), suggesting that the C-terminal tail of GIRK2 is sufficient to restore the Na⁺-dependent activation of GIRK channels. To further narrow down the region crucial for Na⁺ gating we generated two additional chimeric subunits, 1221 and 1212, which divided up the GIRK2 C terminus into two segments: amino acids 199 to 369, which are highly conserved within the GIRK family, and the poorly conserved region downstream of residue 369 (Fig. 3C). Two electrode voltage clamp measurements showed that both of these chimeric subunits produced much larger whole cell currents when coexpressed with GIRK1 than when expressed alone, indicating that they formed heteromeric complexes with GIRK1. We compared the effects of Na⁺, in the presence of MgATP, on the homomeric chimeric channels and the heteromeric channels. The 1221 and GIRK1/1211 channels were both reversibly activated by Na⁺, whereas the 1212 and GIRK1/1212 channels were inhibited (Fig. 3). The Na⁺ dose-response curve for 1221 had a Hill coefficient of 3.8, whereas the curve for GIRK1/1212 had a Hill coefficient of 1.6 (Table I). Thus, the region of the GIRK2 C-terminal tail that lies proximal to TM2 appears to be responsible for the Na⁺-dependent activation of GIRK1/GIRK2 channels.

**Aspartate 226 Is Important for Na⁺-dependent Activation of GIRK2 and GIRK1/GIRK2 Channels**—We compared the sequences of GIRK1, GIRK2, and GIRK4 within the proximal C-terminal region and looked for positions where there is a negative charge in GIRK2 and GIRK4 that is not present at the equivalent position in GIRK1 (Fig. 4A). There are 7 acidic residues conserved between GIRK2 and GIRK4 but not GIRK1. Two of these residues are located within the first 45 amino acid segment downstream of the TM2 region (Fig. 4A). There is an aspartate at position 226 in GIRK2, which is also present in GIRK4, but is an asparagine in GIRK1. There is a glutamate at position 234, where GIRK1 has a serine. We generated two GIRK2 mutants, GIRK2D226N and GIRK2E234S, and expressed these individually and together with GIRK1, plus the m2-muscarinic receptor. The mutants displayed similar characteristics to wild type GIRK2 in that they produced small whole cell currents when expressed individually but much larger currents when coexpressed with GIRK1 indicating the formation of functional heteromeric channels (Fig. 4B). The time course of the GIRK1/GIRK2E234S and GIRK1/GIRK2 channels is similar to the wild type.
**Fig. 3.** The proximal region of the GIRK2 C-terminal tail is required for Na\(^+\)-dependent activation. A, activation of GIRK1/1222 heteromeric channels by internal Na\(^+\) in the presence of 5 mM MgATP. B, the dose-response relationship fitted with a Hill equation with EC\(_{50}\) of
GIRK2D226N heteromeric currents and their degree of inward rectification were indistinguishable from wild type GIRK1/GIRK2 currents (Fig. 4C and Ref. 15). The mutant GIRK2 homomeric currents also rectified strongly, suggesting that neither glutamate 234 nor aspartate 226 plays a key role in determining the rectification properties of these channels.

43 mm and Hill coefficient of 2.3 (n = 4). C, diagram to illustrate the structure of the chimeric subunits. D, the mean amplitudes of the Ba²⁺-sensitive basal and CCh-induced currents at –80 mV, recorded by two-electrode voltage clamp from oocytes injected with the cRNAs indicated. All of the values shown were obtained from the same batch of oocytes and represent the mean ± S.E. for at least six oocytes.
FIG. 5

Na⁺-dependent Activation of GIRK Channels

A

GIRK2E234S

2 pA

Na⁺

MgATP

60 s

B

10.0

0.2

GIRK2E234S

GIRK1/GIRK2E234S

Normalized mean current

C

2 pA

GIRK2D226N

2 pA

20 ms

GIRK1/GIRK2D226N

10 pA

D

1.0

0.9

GIRK2D226N

GIRK1/GIRK2D226N

Normalized mean current

E

1 pA

1211N217D

2 pA

20 ms

F

1.0

0.4

1211N217D

Normalized mean current

G

5 pA

GIRK1N217D/GIRK2D226N

H

1.0

0.2

GIRK1N217D/GIRK2D226N

Normalized mean current

FIG. 5
GIRK2E234S and GIRK1/GIRK2E234S channels were activated by increasing internal Na\(^+\), similar to the wild type channels; the dose-response relationships were fitted with Hill functions with EC\(_{50}\) values of \(-42\) and \(-30\) mm, respectively, and Hill coefficients of 4.0 and 1.6, respectively (Fig. 5, A and B). In contrast both GIRK2D226N channels and GIRK1/GIRK2D226N channels were inhibited by increasing internal Na\(^+\) (Fig. 5, C and D), similar to the 1211 channels. Thus the aspartate at position 226 in GIRK2 appears to play a crucial role in Na\(^+\)-dependent activation of both GIRK2 homomeric and GIRK1/GIRK2 heteromeric channels. Two other mutations at this position, D226K and D226E, failed to produce functional channels, either when expressed alone or coexpressed with GIRK1. As a result, we were not able to further investigate the relationship between the structure of the side group of this residue and activation by Na\(^+\).

The reverse mutation in the 1211 chimera, N217D, was able to confer Na\(^+\)-dependent activation to this channel. The dose-response relationship was best fitted with a Hill coefficient with an EC\(_{50}\) of \(-37\) mm and a Hill coefficient of 3.6 (Fig. 5, E and F, and Table I). This is very similar to the dose-response relationship for Na\(^+\)-dependent activation of the GIRK2 homomeric channel. The reverse mutation in the GIRK1 subunit, N217D, was also sufficient to recover Na\(^+\)-dependent activation to GIRK1N217D/GIRK2D226N heteromeric channels (Fig. 5, G and H, and Table I).

Single channel records of the wild type and mutant channels in the cell-attached configuration are shown in Fig. 6. The E234S mutation, while not affecting the Na\(^+\)-dependent activation of the channel, did alter the intrinsic gating of the GIRK2 homomeric channels; all of the traces show a rapid flickering between the open and closed states of the channel. However, the D226N mutation did not appear to change either the kinetic behavior or the unitary conductance of the homomeric and heteromeric channels. GIRK2 and GIRK2D226N openings were very brief; the open time distributions were fitted with single exponential with time constants of 0.51 and 0.50 ms, respectively. The heteromeric channels displayed longer openings; there was an additional slower component to the open time distribution with a time constant of 2.8 ms (36%) for GIRK1/GIRK2D226N and 3.8 ms (23%) for GIRK1/GIRK2. Thus the loss of Na\(^+\)-dependent activation of the mutant channels does not appear to be caused by a change in their intrinsic gating behavior. Interestingly the reverse mutation in the 1211 chimera, N217D, did alter the intrinsic gating of the channel. In the cell-attached recording mode, this mutant displayed very brief opening events (\(\tau = 0.21\) ms), and correspondingly very small whole cell currents (Fig. 4B), unlike the 1211 chimera, which produces large whole cell currents (15) and displayed much longer openings; \(\tau_1 = 0.85\) (61%), \(\tau_2 = 2.5\) ms (39%).

**DISCUSSION**

We have shown that the aspartate residue at position 226 within the proximal C-terminal region of GIRK2 plays a crucial role in the Na\(^+\)-dependent activation of GIRK1/GIRK2 heteromeric channels and GIRK2 homomeric channels. When D226 in GIRK2 was substituted for an asparagine, activation of GIRK2D226N homomeric channels and GIRK1/GIRK2D226N heteromeric channels by Na\(^+\) was lost, and instead \(P_{\text{open}}\) decreased with increasing internal [Na\(^+\)]. The wild type GIRK1 subunit was not able to support Na\(^+\)-dependent activation of the heteromeric channel. The reverse mutation, N217D, in the GIRK1 subunit was sufficient to recover Na\(^+\)-dependent activation of the GIRK1N217D/GIRK2D226N channel, and similarly this mutation in the 1211 chimera introduced Na\(^+\)-dependent activation to 1211N217D channels. The D226N mutation in GIRK2 did not inhibit the activation of the channel via the m2 receptor, suggesting that G protein gating of GIRKs occurs via a different mechanism to Na\(^+\)-dependent activation.

Substituting asparagine for aspartate 226 in GIRK2 could be disrupting Na\(^+\)-dependent activation of the homomeric and heteromeric channels in at least three different ways: 1) it could reduce the binding affinity of Na\(^+\) to the channel; 2) it could interfere with the mechanism by which Na\(^+\) binding, to either GIRK2 or an accessory protein, is coupled to channel opening; 3) it could disrupt the intrinsic gating of the channel in such a way as to prevent the channel from opening in response to any stimulus. We can rule out the third possibility, because the mutant and chimeric channels that were not activated by Na\(^+\) were still activated by both internal MgATP and external CCh. Also, the single channel properties of GIRK2D226N channels and GIRK1/GIRK2D226N channels in

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**Fig. 5.** Effects of intracellular Na\(^+\) on GIRK2E234S, GIRK2D226N, 1211N217D, and GIRK1N217D mutants. A, C, E, and F, inside-out patches from oocytes injected with the cRNAs indicated, in the presence of increasing concentrations of Na\(^+\) and 5 mM Mg ATP; B, D, F, and H, Na\(^+\) dose-response relationships for the channels indicated. Values for EC\(_{50}\) and Hill coefficients are given in Table I.
the cell-attached recording configuration did not differ substantially from the wild type homomeric and heteromeric channels. Thus, the intrinsic gating of these channels does not appear to have been dramatically altered.

We have no direct evidence that Na⁺ acts by binding to aspartate 226 or anywhere on the GIRK2 subunit. However, Na⁺-dependent activation is observed in isolated membrane patches, indicating that other cytosolic proteins are not required for mediating its actions. Also, other negatively charged residues within the proximal C-terminal region of Kir subunits are involved in the binding of Mg²⁺ and polyamines (16, 17), indicating that this region of Kir subunits is accessible to internal cations. Aspartate residues have also been shown to mediate the allosteric actions of internal Na⁺ on other transmembrane proteins, for example the D₂-dopamine receptor (18).

Aspartate 226 in GIRK2 and aspartate 223 in GIRK4 are only 7 residues downstream of the cysteine that appears to be crucial for Gβγ-mediated activation of GIRK4 and GIRK1/GIRK4 channels (13). A peptide from this region of GIRK4 has also been shown to compete with binding of the Gβγ to the native channel (13). The binding site for PIP₂ appears also to be located within this proximal C-terminal region. PIP₂ regulates the activity of several of the inward rectifiers, including ROMK1 (Kir1.1) and the ATP-sensitive K⁺ channel (SUR/Kir6.2), as well as GIRK channels. Neutralization of the arginines at positions 176 and 177 in Kir6.2, and position 188 in Kir1.1, reduce PIP₂ sensitivity (19, 7, 20). Thus, the proximal C-terminal region immediately following the second transmembrane segment appears to be an important domain for mediating the effects of internal ligands.

Huang et al. (7) suggested that Gβγ activates GIRK channels by stabilizing interactions between PIP₂ and the channel. They showed that in the presence of Gβγ, the rate of channel activation by PIP₂ was increased, and the rate of dissociation of PIP₂ was decreased. They also showed that activation of GIRK channels by PIP₂, in the presence of 20 mM Na⁺, precluded further activation by Gβγ. One possible mechanism for Na⁺-dependent activation of GIRK channels is that Na⁺ interacts with aspartate 226 (or aspartate 223 in GIRK4) to promote the binding of the anionic PIP₂ to a nearby region of the C terminus. However, the results of Sui et al. (6) suggest that PIP₂ and Na⁺ activate GIRK channels via different mechanisms. They reported that 1 μM PIP₂ increased the mean open time of GIRK1/GIRK4 channels, whereas subsequent application of Na⁺ specifically increased the opening frequency. In our experiments, a 5-min application of 50 μM PIP₂ produced a dramatic increase in the apparent open frequency of GIRK1/GIRK2 channels, but it did not preclude further activation of the channels by Na⁺. Thus, we propose that Na⁺ increases the Popen of PIP₂-bound channels. It remains to be tested whether or not it also increases the affinity of PIP₂ binding to GIRK channels.

For those mutant GIRK channels lacking aspartate 226, the decrease in the open probability as a result of increasing internal [Na⁺] might be caused by a reduction in PIP₂ binding. Shyng and Nichols (20) compared the ability of different phospholipids to activate ATP-sensitive K⁺ channels (SUR/Kir6.2) and concluded that a negatively charged head and a lipid tail are necessary to stimulate these channels. Screening of negative charges by application of polyoxalanes, such as Ca²⁺ or polylysine, inhibited PIP₂-stimulated ATP-sensitive K⁺ channel activity. In our experiments, increasing internal [NMDG] appeared to be as effective as increasing [Na⁺] in reducing the open probability of these mutant GIRK channels, and it also inhibited GIRK1/GIRK2 currents. The Na⁺ and NMDG were added to the bath solution without compensation for changes in ionic strength. The resultant increase in the ionic strength of the internal solution might have reduced long range electrostatic interactions between PIP₂ in the membrane and the GIRK channels.

Neither the D226N nor E234S mutation in GIRK2 appeared to change the rectification of the whole cell currents. The E234S mutation did alter the channels gating characteristics, causing it to flicker rapidly between an open and shut state. Mutating the equivalent glutamate in IRK1 channels has been shown to produce a similar flickering behavior, but it also decreases the sensitivity of IRK1 to the internal blockers Mg²⁺ and polyamines (16, 17), thus reducing rectification. Our results, together with the fact that GIRK2 does not possess a negatively charged residue within its TM2 region, suggest that the residues involved in determining the rectification of GIRK2 differ from those shown to be important in the rectification of other inward rectifier channels.

GIRK channels are located in postsynaptic neurons in several areas of the central nervous system (21, 11), where the influx of Na⁺ occurs through both ligand-gated receptors and voltage-gated Na⁺ channels (22). During periods of rapid firing, large increases in subplasmalemmal [Na⁺] are likely to occur in the vicinity of these channels. Yu et al. (23) recently reported that NMDA receptors are activated by [Na⁺], over a similar range of concentrations as required for the activation of GIRK channels. They showed in hippocampal neurons that Na⁺ entry through either NMDA receptors or voltage-gated Na⁺ channels could activate neighboring channels that were isolated within a cell-attached membrane patch. Thus, it seems likely that GIRK channels will be activated by Na⁺ influx in dendrites under physiological conditions, and this may provide a negative feedback mechanism for suppressing neuronal firing following periods of high activity.

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