THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 10, Issue of March 8, pp. 5866–5870, 1996

Printed in U.S.A.

Inhibitory Interactions between Two Inward Rectifier K^+ Channel Subunits Mediated by the Transmembrane Domains*

(Received for publication, October 10, 1995, and in revised form, December 20, 1995)

Stephen J. Tucker‡, Chris T. Bond, Paco Herson, Mauro Pessia, and John P. Adelman§

From the Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon 97201

Inwardly rectifying K^+ channel subunits may form homomeric or heteromeric channels with distinct functional properties. Hyperpolarizing commands delivered to *Xenopus* oocytes expressing homomeric K_{ir} 4.1 channels evoke inwardly rectifying K^+ currents which activate rapidly and undergo a pronounced decay at more hyperpolarized potentials. In addition, K_{ir} 4.1 subunits form heteromeric channels when coexpressed with several other inward rectifier subunits. However, coexpression of K_{ir} 4.1 with K_{ir} 3.4 causes an inhibition of the K_{ir} 4.1 current. We have investigated this inhibitory effect and show that it is mediated by interactions between the predicted transmembrane domains of the two subunit classes. Other subunits within the K_{ir} 3.0 family also exhibit this inhibitory effect which can be used to define subgroups of the inward rectifier family. Further, the mechanism of inhibition is likely due to the formation of an "inviable complex" which becomes degraded, rather than by formation of stable nonconductive heteromeric channels. These results provide insight into the assembly and regulation of inwardly rectifying K^+ channels and the domains which define their interactions.

Inwardly rectifying potassium channels (K_{ir})[1] are found in a wide variety of tissues and cell types where they are involved in the maintenance of the resting membrane potential and control of excitability (1–6). The diversity of these channels can at least in part be explained by the growing number of cloned inward rectifier subunits (7–14). In addition, as with voltage-dependent potassium channels (K_v), diversity is enhanced by the ability of inward rectifier subunits to form homomeric or heteromeric channels. For example, coexpression of K_{ir} 4.1 (BIR10; Ref. 10) with K_{ir} 1.1 (ROMK1; Ref. 9) or K_{ir} 5.1 (BIR9) results in heteromeric channels distinct from either homomeric parental channel (15).[2] Also, coexpression of different members of the K_{ir} 3.0 subfamily has profound effects. For instance, coexpression of either K_{ir} 3.2 (GIRK2; Refs. 12 and 14), 3.3 (GIRK3; Ref. 12), or 3.4 (CIR, Refs. 16 and 17) with K_{ir} 3.1 results in significant G-protein stimulated channel activity (16, 18, 19). Also, an inhibitory effect of K_{ir} 3.3 upon K_{ir} 3.2 channel activity has been reported although the mechanism has not been determined (19).

In this study, we have investigated the effects of coexpression of K_{ir} 3.4 with K_{ir} 4.1. In this case, the effect is neither a potentiation nor a modification of channel activity, rather an inhibitory "dominant-negative" effect upon K_{ir} 4.1 currents. We show that this effect on K_{ir} 4.1 is also endowed by other members of the K_{ir} 3.0 family, and that the TMs are the structural elements which mediate the inhibitory interactions. Further analysis suggests that the inhibitory interactions occur shortly after translation and that the resulting complexes are degraded rather than processed as nonconducting complexes to the plasma membrane.

EXPERIMENTAL PROCEDURES

Electrophysiology—Xenopus laevis care and handling were in accordance with the highest standards of institutional guidelines. Frogs underwent no more than two surgeries, separated by at least 3 weeks. Frogs were anesthetized with an aerated solution of 3-aminobenzoic acid ethyl ester. Standard recording solution contained 90 mM KCl, 3 mM $MgCl_2$, 10 mM HEPES (pH 7.4) unless otherwise stated. Microelectrodes were filled with 3 M KCl and had resistances of 0.1–0.5 MΩ. Recordings were performed at 22 °C, 24–36 h after injection using a Geneclamp 500 amplifier (Axon Instruments) interfaced to a Macintosh Quadra 800 computer. Currents were evoked by voltage commands from a holding potential of −5 mV, delivered in −10-mV increments from 40 mV to −100 mV, unless otherwise stated. Data collection and analyses were performed using Pulse, PulseFit (Heka), and IGOR (Wavemetrics) software. Values for the average whole cell current were obtained by measuring the steady state current at −100 mV. All data are presented as the mean ± S.E. for groups of at least six oocytes (actual numbers used in each group are indicated above the bars) and are expressed as the percentage of control current observed for an identical group of oocytes injected with an equivalent amount of K_{ir} 4.1 mRNA. In all cases, the amount of K_{ir} 4.1 mRNA and the volume injected per oocyte were held constant at approximately 0.1 ng in 50 nl, which would typically result in the 10–15 μA of current 24–36 h after injection. All coinjected mRNAs were varied according to the ratios described in the text, *e.g.* a 10-fold excess of K_{ir} 3.4 (10:1 ratio) was obtained by coinjecting 1 ng of K_{ir} 3.4 + 0.1 ng of K_{ir} 4.1 mRNA per oocyte.

Molecular Biology—All channel subunits were subcloned into the oocyte expression vector pBF (graciously provided by Dr. B. Fakler)[3] which provides 5′- and 3′-untranslated regions from the *Xenopus* β-globin gene flanking a polylinker containing multiple restriction sites. *In vitro* mRNAs were generated using SP6 polymerase (Life Technologies, Inc.); following synthesis, mRNAs were evaluated spectrophotometrically and by ethidium bromide staining after agarose gel electrophoresis. Chimeras were generated by a method described by Horton *et al.*(20) in which the chimeric junctions were generated by overlap extension of PCR primers which encoded the desired sequence. The subunit domains are defined by the following amino acids: K_{ir} 3.4, N terminus, amino acids 1–82; TM1, amino acids 83–116; pore, amino acids 117–164; TM2, amino acids 165–192; C terminus, amino acids 192–419; K_{ir} 4.1, N terminus, amino acids 1–60; TM1, amino acids 61–93; pore, amino acids 94–143; TM2, amino acids 144–170; C terminus, amino acids 171–379. To engineer the FLAG epitope onto the C terminus of K_{ir} 4.1, an oligonucleotide which deleted the K_{ir} 4.1 stop

* This work was supported by National Institutes of Health grants (to J. P. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Wellcome Trust International Prize Travelling Fellow.

§ To whom correspondence should be addressed: Vollum Institute, OHSU/L-474, 3181 S. W. Sam Jackson Park Rd., Portland, OR 97201. Tel.: 503-494-5450; Fax: 503-494-4976; E-mail: adelman@ohsu.edu.

[1] The abbreviations used are: K_{ir}, inward rectifier potassium channel; K_v, voltage-dependent potassium channel; TM, transmembrane domain.

[2] J. P. Adelman, submitted for publication.

[3] B. Fakler, unpublished data.

codon and added 24 nucleotides encoding the 8-amino acid epitope and a translational stop codon was employed in the polymerase chain reaction. All polymerase chain reactions were performed using VENT polymerase (New England Biolabs) according to the manufacturer's instructions. The nucleotide sequences of all chimeric junctions and polymerase chain reaction products were verified before use. Oligonucleotides were synthesized on an Applied Biosystems 391 DNA synthesizer.

Membrane Preparation and Western Blot Analysis—Total oocyte membranes were prepared using a method modified from Geering *et al.* (21). 25 oocytes were suspended in 1 ml of phosphate-buffered saline (50 mM phosphate, 150 mM NaCl, pH 8.0) containing 0.1 mM phenylmethylsulfonyl fluoride and 5 μg/ml each of leupeptin, aprotinin, and pepstatin A and homogenized, first by 5 passages through an 18-gauge needle and then one passage through a 27-gauge needle. The homogenates were centrifuged repeatedly at 1,000 $\times$ *g* for 10 min at 4 °C until all yolk granules and melanosomes were pelleted, typically 3–4 times. The final supernatant was pelleted at 165,000 $\times$ *g* for 30 min to generate a total membrane fraction devoid of yolk granules. This membrane pellet was resuspended in 25 μl of phosphate-buffered saline, and samples were subjected to SDS-polyacrylamide gel electrophoresis using a 10% resolving gel and 3% stacking gel in a Bio-Rad Miniprotean II apparatus. 20% of each sample, representing 4 oocytes, was loaded in each lane. Proteins were transferred to nitrocellulose filters, and Western blot analysis was performed using the m2-FLAG monoclonal antibody (IBI, Eastman Kodak Co.). Antibodies were detected using the Amersham ECL-detection system according to the manufacturer's instructions.

RESULTS

Inhibition of K_{ir} 4.1 by K_{ir} 3.4 and Other Members of the K_{ir} 3.0 Family—Injection of mRNA encoding K_{ir} 4.1 into *Xenopus* oocytes results in hyperpolarization-activated, inward potassium currents which have a time-dependent decay at more negative potentials (Fig. 1*a*; Refs. 10 and 22). In contrast, hyperpolarizing commands delivered to oocytes injected with K_{ir} 3.4 mRNA do not evoke macroscopic channel activity different from control oocytes (Fig. 1*b*; Ref. 16). However, coexpression of K_{ir} 3.4 with K_{ir} 4.1 results in a reduction of K_{ir} 4.1 whole cell currents that is proportional to the ratio of K_{ir} 3.4 to K_{ir} 4.1 mRNA injected (Fig. 1, *c* and *e*). Indeed, when K_{ir} 3.4 mRNA is coinjected in a 10-fold excess to K_{ir} 4.1, all current is abolished (Fig. 1, *d* and *e*). Inhibition of the K_{ir} 4.1 current is not due to effects of excess mRNA on translation because coinjection of K_{ir} 4.1 with an equivalent excess of mRNA for another membrane protein, the dopamine D2 receptor, does not affect K_{ir} 4.1 current amplitudes (Fig. 1*e*; Ref. 23).

The reduced K_{ir} 4.1 currents evoked from coinjected oocytes were not different from K_{ir} 4.1 currents recorded from oocytes injected only with K_{ir} 4.1 mRNA. Fitting the time-dependent component of the whole cell current trace recorded at −100 mV with a double exponential yielded time constants of τ_f = 57.7 ± 2.5 ms and τ_s = 366.0 ± 11.0 ms (A_f = 76.6%, n = 6) for oocytes injected only with K_{ir} 4.1, and of τ_f = 50.0 ± 1.2 ms and τ_s = 277.2 ± 9.9 ms (A_f = 85.1%, n = 6) for oocytes injected with a 1:1 ratio of K_{ir} 4.1 and K_{ir} 3.4 mRNAs. These results suggest a specific inhibitory effect of K_{ir} 3.4 upon K_{ir} 4.1.

To determine if other members of the K_{ir} 3.0 subfamily had a similar effect on K_{ir} 4.1, K_{ir} 3.1 (8, 24), and K_{ir} 3.2 (14, 12) were coexpressed with K_{ir} 4.1. As shown in Fig. 2, both of these K_{ir} 3.0 subfamily members had similar inhibitory effects upon K_{ir} 4.1 current amplitudes. To test whether K_{ir} 3.4 inhibits other inward rectifier subunits, K_{ir} 1.1 (9), a subunit closely related to K_{ir} 4.1, was coexpressed with K_{ir} 3.4. Currents evoked following coexpression of K_{ir} 1.1 and K_{ir} 3.4 were reduced compared to oocytes expressing only K_{ir} 1.1, similar to the effects on K_{ir} 4.1 (currents reduced to <5% of controls; not shown).

Inhibition Is Mediated by the Transmembrane Domains—To localize structural elements responsible for the inhibitory interactions between members of these two inward rectifier subfamilies, a panel of chimeras between K_{ir} 3.4 and K_{ir} 4.1 was constructed (Fig. 3; see "Experimental Procedures"). When expressed alone, chimeras 1413, 1414, and 1415, all containing the TM/pore region of K_{ir} 3.4, did not produce currents different from control oocytes. In contrast, chimeras 1407, 1408, and 1409 which share a common structural domain, the TM/pore region of K_{ir} 4.1, did yield significant channel activity resembling K_{ir} 4.1 currents (Fig. 3).

To determine which domains mediate the inhibitory effect of K_{ir} 3.4 upon K_{ir} 4.1, chimeras 1413, 1414, and 1415 were coexpressed in a 10-fold excess to K_{ir} 4.1 and chimeras 1407,

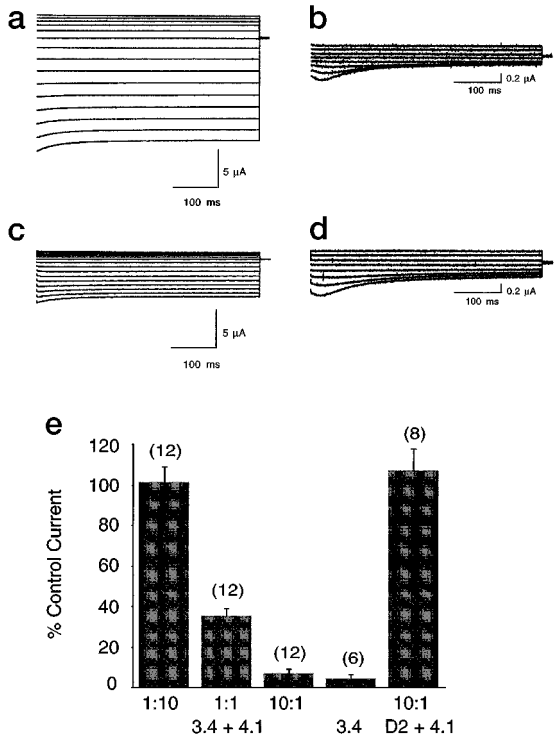


FIG. 1. **Inhibition of K_{ir} 4.1 currents by K_{ir} 3.4.** Currents recorded from oocytes injected with K_{ir} 4.1 mRNA (*a*), K_{ir} 3.4 mRNA (*b*), K_{ir} 3.4 and K_{ir} 4.1 mRNAs in a 1:1 ratio (*c*), and K_{ir} 3.4 and K_{ir} 4.1 mRNAs in a 10:1 ratio (*d*). The currents observed following injection of K_{ir} 3.4 mRNA alone or a 10:1 ratio of K_{ir} 3.4 to K_{ir} 4.1 mRNAs were indistinguishable from mock-injected oocytes. Current families were evoked by 500-ms voltage steps from a holding potential of −5 mV to potentials from 40 mV to −100 mV in −10-mV increments. *e*, averaged current amplitudes were recorded at −100 mV from oocytes injected with a constant amount of K_{ir} 4.1 mRNA and varying ratios of K_{ir} 3.4 or D2 receptor mRNAs. Currents were normalized relative to current amplitudes recorded from oocytes injected with only K_{ir} 4.1 mRNA. *Error bars* represent ± S.E.

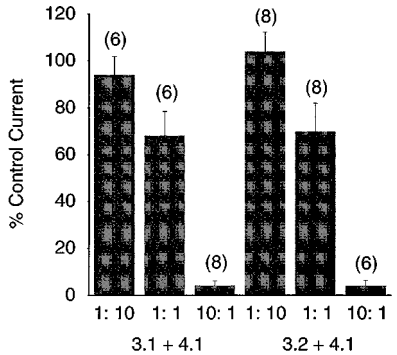
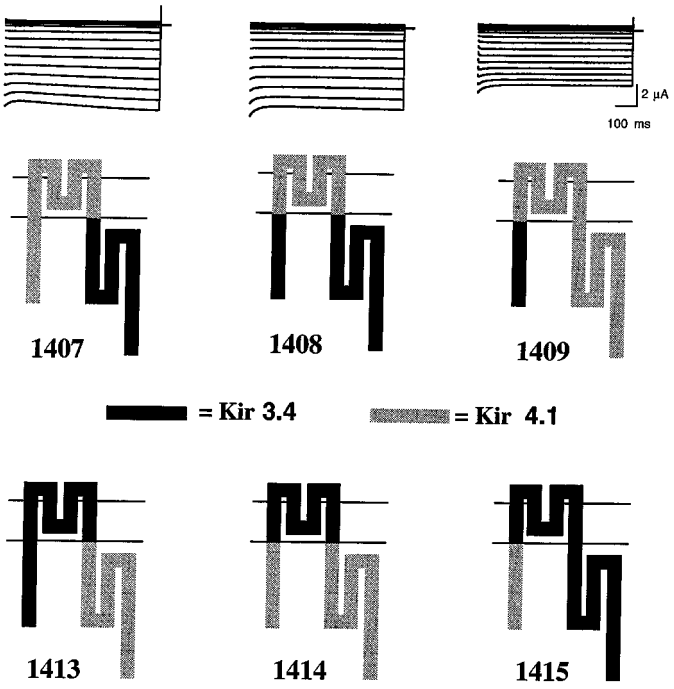


FIG. 2. **Inhibition of K_{ir} 4.1 currents by other members of the K_{ir} 3.0 subfamily.** Averaged current amplitudes recorded at −100 mV from oocytes coinjected with a constant amount of K_{ir} 4.1 mRNA and varying amounts of either K_{ir} 3.1 or K_{ir} 3.2.



	1407	1408	1409	1413	1414	1415
INHIBITS 4.1	N.D.	N.D.	N.D.	YES	YES	YES
INHIBITED BY 3.4	YES	YES	YES	N.D.	N.D.	N.D.
DETECTABLE CURRENTS	YES	YES	YES	NO	NO	NO

FIG. 3. **Chimeras between K_{ir} 4.1 and K_{ir} 3.4 suggest the inhibitory interaction resides within the TM/pore region.** *Top*, current families recorded from oocytes injected with mRNAs encoding chimeras 1407, 1408, and 1409; no currents different from control oocytes were detected following injection of mRNAs for chimeras 1413, 1414, or 1415 (not shown). Diagrammatic representations of the chimeric subunits are shown below. *Bottom table*, activity of the chimeras presented above when injected alone and their effects upon coexpression with K_{ir} 4.1 or K_{ir} 3.4 (*N.D.* = not determined).

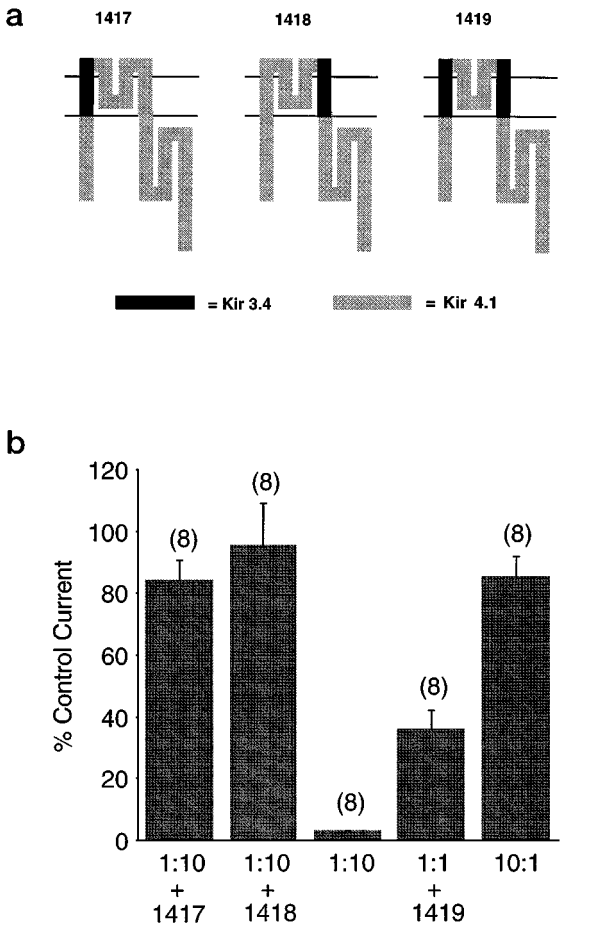


FIG. 4. **Both TMs are required for the inhibition.** *a*, chimeras of K_{ir} 4.1 containing either the first, second. or both putative TMs of K_{ir} 3.4. *b*, averaged current amplitudes recorded at −100 mV from oocytes coinjected with K_{ir} 4.1 mRNA and the chimera mRNAs in the indicated ratios.

1408, and 1409 with a 10-fold excess of K_{ir} 3.4 mRNA. The table in Fig. 3 shows that those chimeras with the TM/pore domains of K_{ir} 3.4 (1413, 1414, and 1415) had an inhibitory effect on K_{ir} 4.1, while chimeras 1407, 1408, and 1409, which contain the TM/pore region of K_{ir} 4.1, were inhibited by coexpression with K_{ir} 3.4. Therefore, the structural elements which mediate inhibition reside within the TM/pore domain.

To further localize the structural elements responsible for the inhibitory interactions, three more chimeras were constructed in which K_{ir} 4.1 contained either the first, second, or both transmembrane domains of K_{ir} 3.4 (Fig. 4*a*). None of these three chimeras was functional when expressed alone, and coexpression of K_{ir} 4.1 and 1417 or 1418, the chimeras containing either one or the other of the K_{ir} 3.4 TMs, had no significant effect upon K_{ir} 4.1 currents. In contrast, coexpression of K_{ir} 4.1 and 1419, the chimera with both K_{ir} 3.4 TM domains, inhibited K_{ir} 4.1 currents, similar to the inhibition by wild type K_{ir} 3.4 (Fig. 4*b*). These results demonstrate that both TMs are necessary and sufficient for inhibition of K_{ir} 4.1 channel activity.

Inhibition Results in Subunit Degradation—To investigate the mechanism of inhibition, the 8-amino acid FLAG epitope tag was engineered onto the C terminus of the K_{ir} 4.1 subunit (K_{ir} 4.1-F), permitting immunological detection with a monoclonal antibody (m2-FLAG ab). Currents recorded from oocytes injected with K_{ir} 4.1-F mRNA were indistinguishable from those recorded from oocytes injected with wild type K_{ir} 4.1 mRNA and, when coinjected with K_{ir} 3.0 mRNA, currents were reduced similar to coexpression with unmodified K_{ir} 4.1 (not shown).

Total membranes were prepared from oocytes injected with either K_{ir} 4.1-F mRNA alone or from oocytes coinjected with K_{ir} 4.1-F mRNA plus a 10-fold excess of test mRNAs. The membrane fractions were prepared as a Western blot and probed with the m2-FLAG antibody (Fig. 5). The K_{ir} 4.1-F protein was detected by the m2-FLAG antibody as a protein of approximately 40 kDa, in close accord with its predicted molecular mass (41.1 kDa); the fainter bands of higher molecular weight likely represent aggregates of the K_{ir} 4.1-F protein. These bands were not detected from mock injected oocytes (not shown) or oocytes coinjected with an excess of K_{ir} 3.4, 1419, K_{ir} 3.1, or K_{ir} 3.2 mRNAs, in which K_{ir} 4.1-F currents were completely inhibited (<6% of the control current). However, the K_{ir} 4.1-F protein was detected in oocytes coinjected with mRNAs encoding K_{ir} 4.1-F and the D2 receptor, K_{ir} 1.1, or the two transmembrane chimeras which do not inhibit K_{ir} 4.1 (1417 and 1418); currents from these oocytes were not reduced compared to control oocytes expressing K_{ir} 4.1-F (Fig. 5).

When injected at a 10-fold excess, all members of the K_{ir} 3.0 family tested, as well as chimera 1419, abolish the K_{ir} 4.1 current and result in undetectable levels of the K_{ir} 4.1-F protein, suggesting that they act through a common mechanism. Because the membrane preparations contained intracellular as well as plasma membrane compartments, it is likely that co-

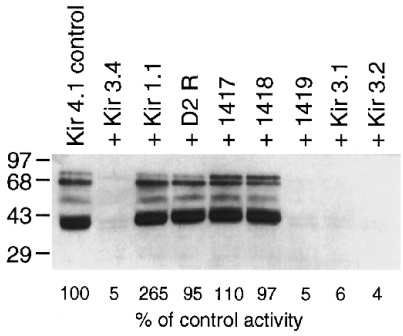


FIG. 5. **Coexpression of K_{ir} 4.1 with K_{ir} 3.0 results in K_{ir} 4.1 subunit degradation.** Western blot of total oocyte membranes probed with the m2-FLAG antibody. Oocytes were coinjected with a constant amount of K_{ir} 4.1-F mRNA and a 10-fold excess of the indicated test mRNAs. The immunoreactive higher molecular mass bands likely represent aggregates of K_{ir} 4.1-F because they are not detected in control oocytes and because heating of the samples above 45 °C prior to loading results in disappearance of the major 40-kDa band and increased intensity of the higher molecular mass bands. Shown below each lane is the normalized current amplitude recorded at −100 mV, from each group of oocytes prior to membrane preparation.

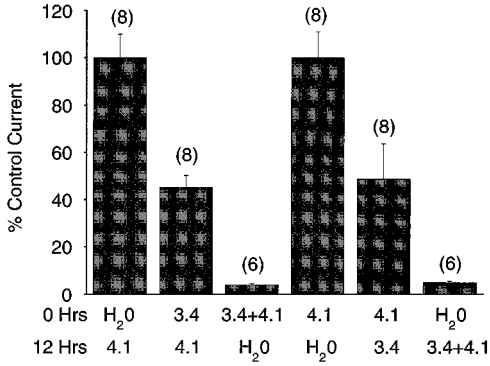


FIG. 6. **Temporal separation of K_{ir} 4.1 and K_{ir} 3.4 expression results in reduced inhibition.** Normalized current amplitudes recorded from oocytes injected with K_{ir} 4.1 and a 10-fold excess of K_{ir} 3.4; the injection order at either time 0 or 12 h later is shown below each *bar*.

expression of K_{ir} 4.1 and K_{ir} 3.4 results in degradation of heteromeric complexes. If this is the case, then temporally separating the expression of K_{ir} 4.1 and K_{ir} 3.4 might separate coassembly of these subunits and allow K_{ir} 4.1 channels to reach the plasma membrane. To test this hypothesis, oocytes were injected with a 10-fold excess of K_{ir} 3.4 mRNA either 12 h before or 12 h after injection of a constant amount of K_{ir} 4.1 mRNA. Fig. 6 shows that if the expression of either subunit is delayed by 12 h then approximately 50% of the control K_{ir} 4.1 current is observed. However, if the two are simultaneously coinjected, then K_{ir} 4.1 currents are abolished. These results suggest that coassembly of K_{ir} 4.1 and K_{ir} 3.4 subunits occurs shortly after translation, and temporally separating translation of the two classes of mRNAs reduces the likelihood of heteromer formation.

DISCUSSION

Coexpression of members of the K_{ir} 3.0 family with K_{ir} 4.1 inhibits K_{ir} 4.1 currents, an effect which is likely due to cotranslational subunit assembly and subsequent degradation of the heteromeric complexes. The structural motifs which mediate the inhibitory interactions of K_{ir} 3.4 with K_{ir} 4.1 reside within the putative TMs, and, while these are the only necessary structural motifs, both are required for inhibition.

The inhibition of K_{ir} 4.1 currents by K_{ir} 3.4 is not due to nonspecific effects on translation, because coexpression of K_{ir} 4.1 with an equivalent amount of additional mRNA encoding another membrane protein, such as the dopamine D2 receptor, is without effect. Further, K_{ir} 3.4 has a similar inhibitory effect upon K_{ir} 1.1 (9), another inward rectifier which is closely related to K_{ir} 4.1. The inhibition of K_{ir} 4.1 and related subunits by members of the K_{ir} 3.0 family thus provides an additional criterion for inward rectifier subunit classification (25).

The observation that both TMs are required for the inhibitory interaction suggests that when a single TM is swapped for that of another family, the subunits cannot be coassembled. Chimera 1419 which has both K_{ir} 3.4 TMs is capable of interacting with K_{ir} 4.1 subunits and inhibiting the current similar to K_{ir} 3.4. Although the structural domains which mediate inhibitory interactions between members of the K_{ir} 3.4 subfamily and K_{ir} 4.1 are different from the domains which mediate coassembly of distinct K_v subunits, both K_{ir} and K_v subunit coassembly may occur cotranslationally (26).

The mechanism of inhibition appears to act through sequestration of heteromeric complexes into a degradative pathway soon after translation and before insertion in the plasma membrane. If heteromeric channels are processed to the plasma membrane as inactive complexes or if subunit coassembly occurs by association within the plasma membrane, then the K_{ir} 4.1-F subunit should be detected in oocytes coinjected with K_{ir} 4.1-F and K_{ir} 3.4 mRNAs. However, K_{ir} 4.1-F subunits were not detected by Western blot, even though the preparations did not separate intracellular and plasma membrane compartments. In addition, the inhibitory interactions were uncoupled by temporally separating expression of the two different subunit types. Thus, when K_{ir} 4.1 subunits are allowed to assemble together before interference from K_{ir} 3.4 subunits, they are processed to the plasma membrane as functional channels, and, conversely, when K_{ir} 3.4 subunits have already assembled together, subsequent inhibition of K_{ir} 4.1 is reduced. However, when they are simultaneously expressed, as shown above, K_{ir} 4.1 currents are abolished. The fact that other members of the K_{ir} 3.0 family also had the same effect implies that the mechanism of inhibition by members of this family is the same.

There are several tissues, including the heart and brain, where members of the K_{ir} 3.0 family and the subunits they inhibit are coexpressed. In atrial myocytes for example, K_{ir} 3.4 and K_{ir} 3.1 coassemble to form the channel underlying I_{KAch} (16), but K_{ir} 4.1 is also expressed in this tissue (10). It is possible that the inhibitory interaction described here between the K_{ir} 3.4 and K_{ir} 4.1 provides a way for the cell to prevent either inactive or possibly disruptive heteromeric complexes from reaching the plasma membrane, reflecting an additional physiological mechanism which regulates the array of distinct inward rectifier subtypes.

Acknowledgments—We thank Amela Brankovic for oocyte preparation and extreme patience and Thanos Tzounopoulos and Armando Lagrutta for helpful comments and interactions.

REFERENCES

1. Sakmann, B., and Trube, G. (1984) *J. Physiol. (Lond.)* **347,** 641–657
2. Constanti, A., and Galvan, M. (1983) *J. Physiol. (Lond.)* **335,** 153–178
3. Katz, B. (1949) *Arch. Sci. Physiol.* **2,** 285–289
4. Mihara, S., North, R. A., and Surprenant, A. (1987) *J. Physiol.* **390,** 335–355
5. Barres, B. A. (1991) *Curr. Opin. Neurobiol.* **1,** 354–359
6. McKinney, L. C., and Gallin, E. K. (1988) *J. Membr. Biol.* **103,** 41–53
7. Kubo, Y., Baldwin, T. J., Jan, Y. N., and Jan, L. Y. (1993) *Nature* **362,** 127–133
8. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N., and Jan, L. Y. (1993) *Nature* **364,** 802–806
9. Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., Kanazirska, M. V., and Hebert, S. C. (1993) *Nature* **362,** 31–37
10. Bond, C. T., Pessia, M., Xia, X. M., Lagrutta, A., Kavanaugh, M. P., and Adelman, J. P. (1994) *Recept. Channels* **2,** 183–191
11. Morishige, K.-I., Takahashi, N., Findlay, I., Koyama, H., Zanelli, J. S., Peterson, C., Jenkins, N. A., Copeland, N. G., Mori, N., and Kurachi, Y. (1993) *FEBS Lett.* **336,** 375–380
12. Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M., and Hugnot, J.-P. (1994) *FEBS Lett.* **353,** 37–42
13. Tang, W., and Yang, X. C. (1994) *FEBS Lett.* **348,** 239–243

14. Bond, C. T., Ammala, C., Ashfield, R., Blair, T. A., Gribble, F., Khan, R. N., Lee, K., Proks, P., Rowe, I. C. M., Sakura, H., Ashford, M. J., Adelman, J. P., and Ashcroft, F. M. (1995) *FEBS Lett.* **367,** 61–66
15. Glowatzki, E., Fakler, G., Brandle, U., Rexhausen, U., Zenner, H.-P., Ruppersberg, J. P., and Fakler, B. (1995) *Proc. R. Soc. Lond. B Biol. Sci.* **261,** 251–261
16. Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D. E. (1995) *Nature* **374,** 135–141
17. Ashford, M. L. J., Bond, C. T., Blair, T. A., and Adelman, J. P. (1994) *Nature* **370,** 456–459
18. Duprat, F., Lesage, F., Guillemare, E., Fink, M., Hugnot, J.-P., Bigay, J., Lazdunski, M., Romey, G., and Barhanin, J. (1995) *Biochem. Biophys. Res. Commun.* **212,** 657–663
19. Kofuji, P., Davidson, N., and Lester, H. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6542–6546
20. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) *Gene (Amst.)* **77,** 61–68
21. Geering, K., Theulaz, I., Verrey, F., Hauptle, M. T., and Rossier, B. C. (1989) *Am. J. Physiol.* **257,** C851–C858
22. Pessia, M., Bond, C. T., Kavanaugh, M. P., and Adelman, J. P. (1995) *Neuron* **14,** 1039–1045
23. Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A., and Civelli, O. (1988) *Nature* **336,** 783–787
24. Dascal, N., Schreibmayer, W., Lim, N. F., Wang, W., Chavkin, C., DiMagno, L., Labarca, C., Kieffer, B. L., Gaveriaux-Ruff, C., Trollinger, D., Lester, H. A., and Davison, N. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 10235–10239
25. Doupnik, C. A., Davidson, N., and Lester, H. A. (1995) *Curr. Opin. Neurobiol.* **5,** 268–277
26. Deal, K. K., Lovinger, D. M., and Tamkun, M. M. (1994) *J. Neurosci.* **14,** 1666–1676