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Inhibition of function in *Xenopus* oocytes of the inwardly rectifying G-protein-activated atrial K channel (GIRK1) by overexpression of a membrane-attached form of the C-terminal tail

NATHAN DASCAL*†, CRAIG A. DOUPNIK†‡, TATIANA IVANINA*, SUZANNE BAUSCH§, WEIZHEN WANG‡, CATHERINE LIN‡, JUSTINE GARVEY‡, CHARLES CHAVKIN§¶, HENRY A. LESTER‡, AND NORMAN DAVIDSON‡‖

*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel; ‡Division of Biology, 156-29 and ¶Program in Computation and Neural Systems, 139-74, California Institute of Technology, Pasadena, CA 91125; and §Department of Pharmacology, University of Washington, Seattle, WA 98195

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ABSTRACT **Coexpression in *Xenopus* oocytes of the inwardly rectifying guanine nucleotide binding (G)-protein-gated K channel GIRK1 with a myristoylated modification of the (putative) cytosolic C-terminal tail [GIRK1 aa 183–501 fused in-frame to aa 1–15 of $p60^{src}$ and denoted src^+(183–501)] leads to a high degree of inhibition of the inward G-protein-gated K^+ current. The nonmyristoylated segment, src^-(183–501), is not active. Although some interference with assembly is not precluded, the evidence indicates that the main mechanism of inhibition is interference with functional activation of the channel by G proteins. In part, the tail functions as a blocking particle similar to a "Shaker ball"; it may also function by competing for the available supply of free $G\beta\gamma$ liberated by hormone activation of a seven-helix receptor. The non-G-protein-gated weak inward rectifier ROMK1 is less effectively inhibited, and a Shaker K channel was not inhibited. Immunological assays show the presence of a high concentration of src^+(183–501) in the plasma membrane and the absence of any membrane forms for the nonmyristoylated segment.**

The inwardly rectifying guanine nucleotide binding (G)-protein-gated atrial K channel [initially denoted GIRK1 (1) or KGA (2); we used GIRK1 herein] was the first member of the expanding GIRK subfamily of inwardly rectifying K (Kir) channels to be cloned (for review, see ref. 3). Of all known Kir channels, GIRK1 has the longest C-terminal cytosolic tail (see, for example, figure 1 of ref. 2), spanning aa 183–501. Regions of significant sequence homology with non-G-protein-activated inward rectifiers, such as ROMK1 and IRK1, end around aa 360. There is also considerable sequence homology within the region of aa 183–360 with two additional members of the G-protein-gated inward rectifier family, mbGIRK2 and mbGIRK3, which are expressed in mouse brain (4).

The relatively large length of the GIRK1 C-terminal segment and the presence of regions of homology and nonhomology with other members of the Kir family suggest that this segment may play one or several roles in channel function, including interactions with G proteins and with the conducting pore. Furthermore, several recent studies of other IRKs have indicated interactions between the C terminus and the respective channel pores (5, 6). The experiments described herein are an initial study of the possible roles in gating of the segment of aa 183–501. Specifically, we have coexpressed this segment in oocytes as a free cytosolic segment or with an N-terminal myristoylation signal, providing peripheral membrane attachment. We find that overexpression of the membrane-attached form relative to GIRK1 leads to a marked decrease in hormone-evoked GIRK1 currents. In effect, the myristoylated segment of aa 183–501 can act as a dominant negative truncation mutant. Our results are consistent with the hypothesis that the segment of aa 183–501 functions as a blocking particle, analogous, for example, to the N-terminal peptide of certain rapidly inactivating Shaker-type K channels (7); and, in addition, it may inhibit gating of the channel by competitive binding of activated G-protein subunits (probably $G\beta\gamma$; see *Discussion*). A preliminary note describing this work has appeared (8).

MATERIALS AND METHODS

Transcription Templates. GIRK1 complementary RNA (cRNA) was prepared by *in vitro* transcription of the *Xho* I-linearized pBluescript plasmid as described (2). The cRNA for the segment of aa 183–501 [denoted src^-(183–501)] was generated from a cDNA derived by a two-step PCR procedure, using primers that added to the 5′ end a T7 promoter sequence and a segment of the 5′ untranslated region of alfalfa mosaic virus plus a Kozak box and a poly(dA) tail to the 3′ end of aa 183–501 (9, 10). For the myristoylation signal, the forward PCR primer also provided the first 15 aa of $p60^{src}$ (11) fused in-frame to aa 183 [denoted src^+(183–501)].

Oocytes and Electrophysiology. These standard procedures as practiced in our laboratories have been described (2). Injected oocytes were incubated 3–8 days in ND96 solution (96 mM NaCl/2 mM KCl/1 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.5/2.5 mM sodium pyruvate/gentamycin at 50 μg/ml). For most of the electrophysiology experiments, oocytes clamped at −80 mV were perfused with ND96 without pyruvate and antibiotics, which was then changed to hK solution (96 mM KCl/1 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.5). Current/voltage curves were recorded by voltage ramps lasting 1 sec.

Immunological Methods. The peptide KTRMEGNLPAKLRKMNSD, corresponding to aa 482–498 of GIRK1 with an added N-terminal lysine, was synthesized, purified, and characterized by the Caltech Microchemical Facility. The peptide was coupled to bovine serum albumin and several antisera raised in rabbits. Affinity-purified antibody was then prepared by chromatography to the peptide coupled to CNBr-activated Sepharose 4B.

For immunological localization, 20-μm sections were cut from injected and uninjected oocytes that had been fixed in 2%

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Abbreviations: cRNA, complementary RNA; m2AChR, m2 muscarinic acetylcholine receptor; I_{hK}, high K current; I_{ACh}, acetylcholine-induced current; G protein, guanine nucleotide binding protein.

†N.D. and C.A.D. have contributed equally to this work.

‖To whom reprint requests should be addressed.

(wt/vol) paraformaldehyde and frozen in OCT embedding compound. After incubation with antibody, sites of binding were amplified by using the biotin amplification procedure, visualized with lissamine rhodamine-conjugated streptavidin, and photographed by using confocal microscopy (12).

Location and relative amounts of antigen were also measured by immunoprecipitation/gel electrophoretic methods. As a control for the properties of the antibody with known sources of antigen, reaction of the antibody with SDS-solubilized membranes from rat atria, ventricle, and brain were observed by Western blot analysis. Experiments on oocytes were then carried out as described (13). Briefly, injected oocytes were labeled with [^{35}S]methionine from days 1 to 4 after cRNA injection. Plasma membranes were manually separated from the remainder of the oocyte, which is referred to as the "internal" fraction. The plasma membrane and internal fractions were solubilized; antigen was immunoprecipitated and analyzed by gel electrophoresis and autoradiography. In a second set of experiments, a total membrane pellet was separated from the internal fractions; the soluble and membrane bound fractions were subjected to gel analysis.

RESULTS

Suppression of GIRK1 Currents by src$^+$(183–501). In our standard paradigm for studying the electrophysiological response of GIRK1, control oocytes were coinjected with cRNAs encoding GIRK1 and a seven-helix receptor [for example, the m2 muscarinic acetylcholine receptor (m2AChR)]; a parallel group of oocytes (from the same batch) were injected with these cRNAs and one encoding src$^+$(183–501) or src$^-$(183–501). Similarly, the effects of src$^+$(183–501) on ROMK1 and Shaker (Δ6–46, inactivation removed) (7) were also examined.

With control oocytes clamped at −80 mV, the bath medium was shifted from ND96 to a high potassium (hK) medium, with its major constituent being 96 mM KCl. The current induced by this shift, denoted I_{hK}, was due in part to the injected exogenous GIRK1 and in part to a small but variable oocyte K^+ current. With GIRK1 and m2AChR but no src$^+$(183–501) cRNAs injected, addition of agonist (5 μM acetylcholine) induced the relatively large inward K current of principal interest here, I_{ACh}. For the present experiments I_{ACh} varied from 100 nA to 1 μA, depending on the amount and batch of the injected cRNA, oocyte batch, and time of incubation (Fig. 1*A*).

When the non-G-protein-gated inward rectifier ROMK1 was used, the m2AChR was omitted. In this case I_{hK} was large (0.4–1.5 μA) and mainly due to ROMK1. The net I_{hK} is calculated as the change from ND96 to hK medium (Fig. 1*B*). Compared to most other IRs, ROMK1 is weakly rectifying, i.e., more permeable for outward K currents (data not shown; see ref. 14).

Our principal results are presented as the averaged data from many experiments on different oocyte batches in Fig. 1*C*. A typical example of the reduced signal due to src$^+$(183–501) is shown in Fig. 1*A*. In summary, coinjection of src$^+$(183–501) cRNA in excess over the amount of GIRK1 cRNA (and with m2AChR present) resulted in a decrease of I_{ACh} to 27 ± 2.7% (mean ± SEM) of that observed in controls with GIRK1 and m2AChR. However, coinjection of 5 ng of cRNA for the presumed cytosolic product src$^-$(183–501) did not reduce the level of I_{ACh} (96.6 ± 17.5%). The inhibition of GIRK1 current caused by src$^+$(183–501) could be largely overcome by increasing the amount of GIRK1 cRNA to 5 ng (Fig. 1*C*). Additional experiments confirmed that at least 0.5 ng of src$^+$(183–501) cRNA and an excess over the amount of GIRK1 cRNA was needed for significant inhibition (data not shown).

The question of whether src$^+$(183–501) affects currents from other K channels was addressed by studying its effect

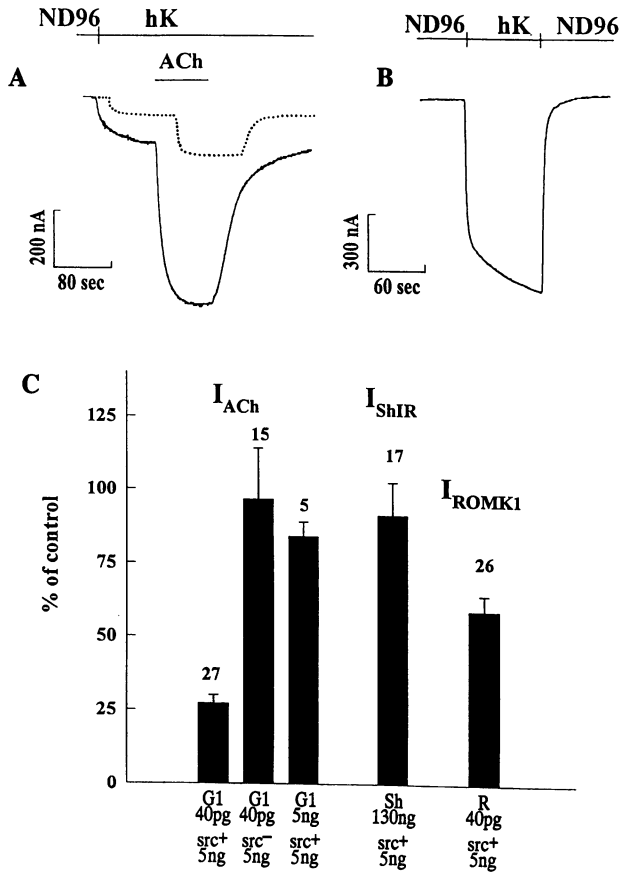

FIG. 1. Typical currents for injection into oocytes of cRNAs. (*A*) Solid line, GIRK1 (40 pg) and m2AChR (200 pg); dotted line, GIRK1 (40 pg), m2AChR (200 pg), and src$^+$(183–501) (2 ng) (this tracing has been displaced slightly to the right for clarity). (*B*) ROMK1 (50 pg). For this case, a tracing in the presence of src$^+$(183–501) with a typical 40% inhibition is not shown. (*C*) Averaged data (numbers of oocytes indicated over the bars; usually four or five tracings per oocyte) of the effects of coinjection of src$^+$(183–501) or of src$^-$(183–501) on the various channels. For GIRK1, m2AChR was always coinjected. For each channel, the control (100% value) was based on experiments in the same batch and with a comparable number of oocytes injected with the channel but with neither src$^+$(183–501) nor src$^-$(183–501). In *C* and Fig. 3, the abbreviations are as follows: G1, GIRK1; src$^+$, src$^+$(183–501); src$^-$, src$^-$(183–501); Sh, Shaker IR; R, ROMK1. [The amount of Shaker IR RNA used actually varied between 75 and 200 pg, with an average of 130 pg (for further details, see text).]

on the voltage-gated Shaker channel and on the weak IR ROMK1. Shaker currents were not significantly changed by coexpressed src$^+$(183–501) (91 ± 11% of control). However, src$^+$(183–501) cRNA did significantly reduce the level of ROMK1 current to 59 ± 5% of control.

The effect of the ratio of src$^+$(183–501) to GIRK1 cRNAs was studied in a separate set of experiments. With the amount of GIRK1 cRNA fixed at 0.5 ng, a 1:1 ratio of src$^+$(183–501)/GIRK1 had no effect; 5:1 and 10:1 ratios caused 33% and 94% inhibition, respectively.

These experiments indicate that inhibition by src$^+$(183–501) depends on having a sufficiently high ratio of src$^+$(183–501) to GIRK1 products in the plasma membrane, as expected for inhibition by overexpression for any dominant negative mutant; since the translation efficiencies to give functional protein (i.e., in the plasma membrane) are unknown, a numerical ratio cannot be stated. As shown in the immunohistochemical experiments reported below, this ratio is in fact quite high.

One possible interpretation of inhibition of GIRK1 is that the src$^+$(183–501) segment interferes with assembly and/or transport to the membrane of the presumed GIRK1 tetramer.

That possibility was addressed in a sequential injection experiment. As shown in Fig. 2, when GIRK1, m2AChR, and src$^+$(183–501) cRNAs were injected on day 0, there was inhibition of I_{ACh} by ≈70% on day 4. When GIRK1 and m2AChR were injected alone, the signal was increased only slightly by day 8. However, the src$^+$(183–501) inhibition was quite small by day 8, suggesting that much of the src$^+$(183–501) mRNA and protein had decayed by this time. However, when injection of src$^+$(183–501) cRNA was delayed until 4 days after the injection of GIRK1 and m2AChR, there was inhibition by ≈60% on day 8. We interpret this result to indicate that the functional preexisting GIRK1 present on day 4 has been inhibited by the src$^+$(183–501) synthesized from days 4 to 8. From these experiments, we conclude that inhibition is not mainly due to interference with assembly of functional GIRK1 channels on the plasma membrane.

Immunochemical Localization of GIRK1, src$^+$(183–501), and src$^-$(183–501). Fig. 3 exhibits results by one of the two methods that has been used for immunological analyses of the levels and locations of GIRK1, src$^+$(183–501), and src$^-$(183–501) proteins within the oocyte. All experiments were performed with an antiserum raised in rabbits to a peptide corresponding to aa 482–498 of GIRK1.

Fig. 3*C* is a Western blot showing that a strong band of the expected molecular mass of 56 kDa for GIRK1 was observed in atrial membranes with a weaker band for brain membranes and none for ventricles. A cross-reacting band of unknown identity at 40 kDa was observed in atria and ventricles. Fig. 3*D* is an autoradiogram for an *in vitro* translation for GIRK1, src$^+$(183–501), and src$^-$(183–501) cRNAs, showing that the main protein bands have the expected molecular masses of ≈55, 37, and 35 kDa. Thus, the results in Fig. 3 *C* and *D* partially confirm the specificity of the antiserum and the identity of the gene product encoded by the two cRNAs.

For Fig. 3 *A* and *B*, injected oocytes were labeled with [^{35}S]methionine. Plasma membranes were manually isolated; the remainder of the oocyte is labeled internal and consists principally of internal membranes and cytosol (13). Immunoprecipitates from each fraction were analyzed by SDS/PAGE and autoradiography. In the plasma membrane fraction (3B), src$^+$(183–501) gave two bands of molecular mass 44 and 42 kDa; as expected, no bands were detected for src$^-$(183–501). In the internal fraction (Fig. 3*A*), src$^+$(183–501) showed the same bands at 44 and 42 kDa and two bands at 39 and 37 kDa, whereas src$^-$(183–501) showed three bands at 40, 39, and 37 kDa. We surmise that the band heterogeneity is due to unknown proteolytic or processing events and that the lower molecular mass bands for src$^+$(183–501) are nonmyristoylated forms, similar or identical to the src$^-$(183–501) bands.

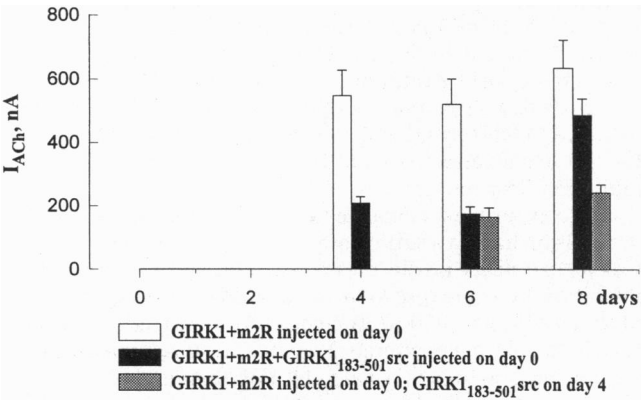

FIG. 2. Sequential injection experiment to test effect of later injection of src$^+$(183–501) on preexisting GIRK1 channels (for further details and discussion, see text). m2R, m2AChR.

For GIRK1 itself, it was necessary to inject 2.5–5 ng of cRNA to obtain a strong signal from the 10–20 oocytes used (Fig. 3 *A* and *B*, lanes 5). For both the plasma membrane and internal fractions, there was a doublet at the expected molecular mass of ≈55 kDa and a fainter higher molecular mass smear with a suggestion of discrete bands. Fig. 3 *A* and *B*, lanes 4, shows extracts from oocytes injected with 40 pg of GIRK1 and 5 ng of src$^+$(183–501); lanes 6 were for 40 pg of GIRK1 only. The GIRK1 signal was barely detectable, showing that under the conditions used for the electrophysiological inhibition experiments, the ratio of src$^+$(183–501) to GIRK1 was high in both plasma membrane and internal fractions.

A second set of gel electrophoretic analyses of ^{35}S-labeled proteins from oocytes were carried out to estimate the relative amounts of the several antigens in the internal fraction that were in internal membrane and soluble fractions. After physical removal of the plasma membrane, the internal fraction was divided into a crude membrane pellet and the cytosol fraction (Fig. 3*F*). Immunoprecipitated antigens were analyzed. The results (Fig. 3 *E* and *F*) show that little or no src$^-$(183–501) was in the membrane pellet. For src$^+$(183–501), the lower molecular mass bands, which we suggest above are nonmyristoylated, were primarily in the cytosol fraction. The 44- and 42-kDa myristoylated isoforms were present in significant amounts in both the membrane fraction and the cytosol. This result indicates that, at least for a highly expressed molecule, myristoylation is not sufficient to cause 100% membrane attachment.

In an alternative immunohistochemical approach to subcellular localization of expression, 20-μm slices were made from fixed and frozen oocytes and stained with antibody. Fig. 4*A* shows the weak background staining in uninjected oocytes. Oocytes injected with 5 ng of GIRK1, src$^+$(183–501), or src$^-$(183–501) are shown in Fig. 4 *B*, *D*, and *C*, respectively. There was a high concentration of antigen in the plasma membrane for src$^+$(183–501) and a clear but lower concentration for GIRK1. In each case, there was considerable expression of antigen internally in the oocyte. For src$^-$(183–501), there was no antigen in the plasma membrane but diffuse expression internally.

DISCUSSION

Our principal result is that overexpression of the myristoylated membrane-attached form of the GIRK1 segment of aa 183–501, which is the putative cytosolic tail, relative to the amount of GIRK1 protein in the plasma membrane, causes inhibition of the G-protein-gated signal (I_{ACh} in the present instance using the m2AChR as the G-protein-coupled receptor) of the full-length channel. Thus the membrane bound (aa 183–501) segment functions as a dominant negative truncation mutant.

Many patch-clamp experiments have shown that G-protein gating of the natural GIRK1 channel is a membrane-delimited process; it is now generally accepted that Gβγ heterodimers can bind to an internally exposed epitope(s) of GIRK1 and induce channel opening. Experiments by J.G. (unpublished data) and by C. Dessauer (University of Texas Southwestern Medical Center, Dallas) (personal communication) indicate (15, 16) that the segment of aa 183–501 when expressed by *in vitro* translation in a rabbit reticulocyte system or as a glutathionine *S*-transferase fusion in *Escherichia coli* can bind to bovine $G\beta_{1\gamma 2}$.

The fact that the segment of aa 183–501 can suppress ROMK1 currents suggests that this segment functions in part as a blocking particle, similar to a "Shaker ball" for rapidly inactivating voltage-gated K channels (7). The blocking effect hypothesis is consistent with our observation (17) that the peptide corresponding to aa 485–501 of GIRK1 blocks the channel in inside-out patches. We think it plausible, in view of the direct biochemical evidence for binding, that suppression

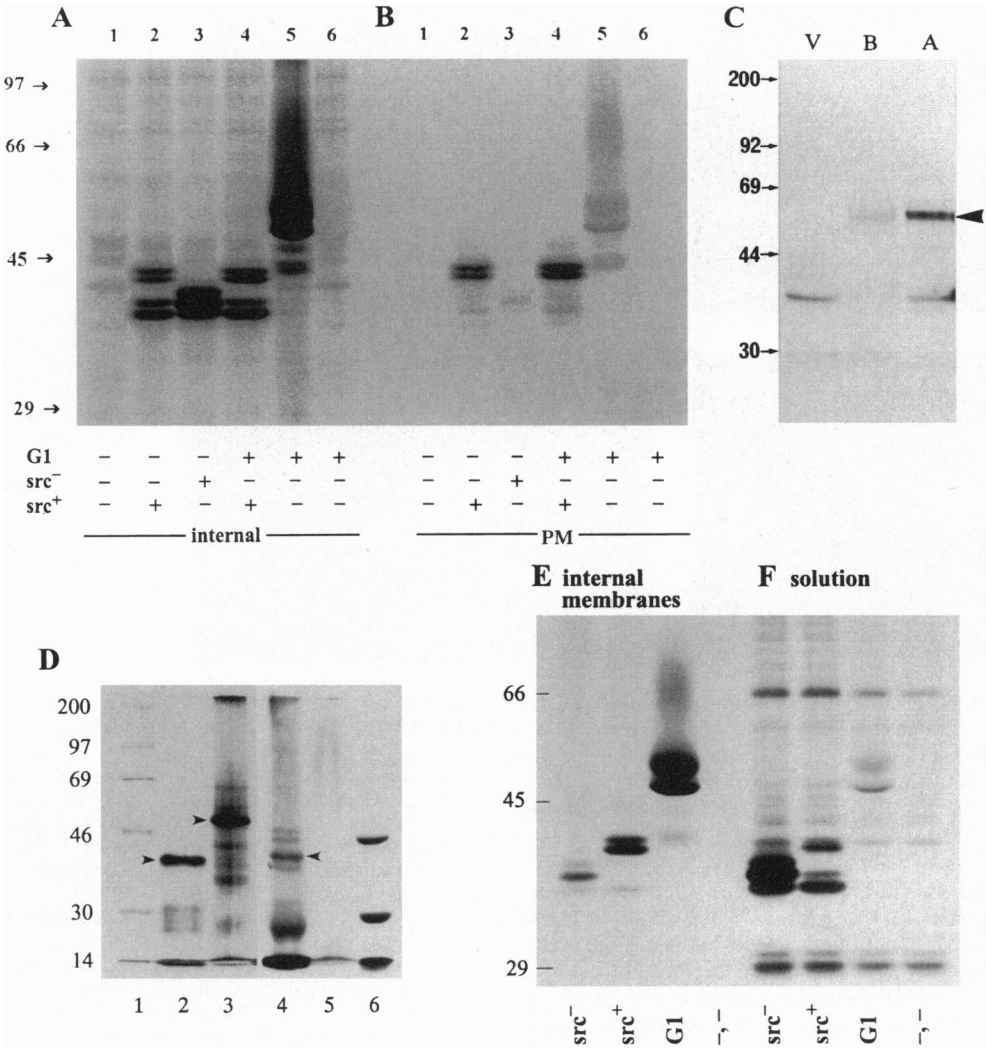

FIG. 3. Immunochemical/gel electrophoretic analyses of expression of GIRK1, src$^+$(183–501), and src$^-$(183–501) in membranes and cytosol fractions of injected oocytes. (*C*) Western blot of membranes (50 μg of protein per lane) from rat heart ventricles (V) and atria (A) and rat brain (B) reacted with the GIRK1 antiserum. (*D*) *In vitro* translation in a rabbit reticulocyte system of the cRNAs for GIRK1, src$^+$(183–501), and src$^-$(183–501). Lanes: 1 and 6, ^{14}C and the rainbow molecular weight markers (Amersham); 2, src$^-$(183–501); 3, GIRK1; 4, src$^+$(183–501) (for lanes 2–4, the principal band is identified by an arrow); 5, control translation with no added cRNA. The gel electrophoretic pattern for GIRK1 was essentially the same in the presence of dog pancreas microsomal membranes; in this case, however, most of the translated protein could be pelleted with the membranes (data not shown). (*A, B, E,* and *F*) Injected oocytes were metabolically labeled with [^{35}S]methionine. The several fractions were prepared by using 10–20 oocytes, each injected with 5 ng of GIRK1 and/or 2.5–5 ng of src$^+$(183–501) or src$^-$(183–501); except that in *A* and *B*, lanes 4 and 6, 40 pg of GIRK1 was used. Extracted fractions were immunoprecipitated, gel-separated, and analyzed by autoradiography. (*A*) Manually dissected plasma membranes (PM). (*B*) The remainder of the oocytes, i.e., cytosol plus internal organelles labeled internal. (*E* and *F*) Plasma membranes were manually removed; the remaining internal fraction was divided into a soluble (true cytosol) fraction (*F*), labeled solution, and a membrane pellet fraction (*E*), labeled internal membrane. For all these experiments, antiserum rather than affinity-purified antibody was used. G1, GIRK1; src$^-$ and src$^+$, src$^-$(183–501) and src$^+$(183–501), respectively.

by the segment of aa 183–501 also involves competitive binding of hormone-liberated G$\beta\gamma$ by the segment of aa 183–501 under conditions when the latter is relatively abundant at the membrane compared to GIRK1. [Coexpression of Gβ1 and Gγ2 in oocytes with GIRK1 converts a major fraction of I_{ACh} to I_{hK} (16, 18)]. However, our attempts to demonstrate competition by overriding src$^+$(183–501) suppression by Gβ plus Gγ expression were not successful (data not shown). We regard this experiment as inconclusive because of lack of knowledge of the amount of free G$\beta\gamma$ in the oocyte under the conditions used. Thus, although we believe it to be likely that the inhibition phenomenon is in part due to competitive binding of G$\beta\gamma$, there is no direct experimental evidence to support the hypothesis.

Both the gel electrophoretic (Fig. 3) and the immunohistochemical (Fig. 4) experiments indicate that under the conditions where src$^+$(183–501) inhibits GIRK1 currents [i.e., 2.5–5 ng of src$^+$(183–501) cRNA and 0.5 ng or less (usually 0.04 ng) of GIRK1], there is a high ratio of src$^+$(183–501) protein to GIRK1 protein in the functionally important plasma membrane. This is consistent with the general interpretation of dominant negative inhibition.

In several instances, truncated subunits of an oligomeric channel may coassemble with intact subunits to give nonfunctional heterooligomers and thus function as dominant negative mutants. This has been reported for short N-terminal domains of the mammalian delayed rectifier Kv1.1 channel, which include the S1 transmembrane segment (19). Similarly, expression of fragments of the α, δ, or γ subunits of the mouse muscle acetylcholine receptor consisting solely of the extracellular N-terminal domain was sufficient to form complexes with intact subunits and thus function as dominant negative mutants (20). Furthermore, it has been shown that the hydrophilic N-terminal domain of a Shaker voltage-gated K channel can

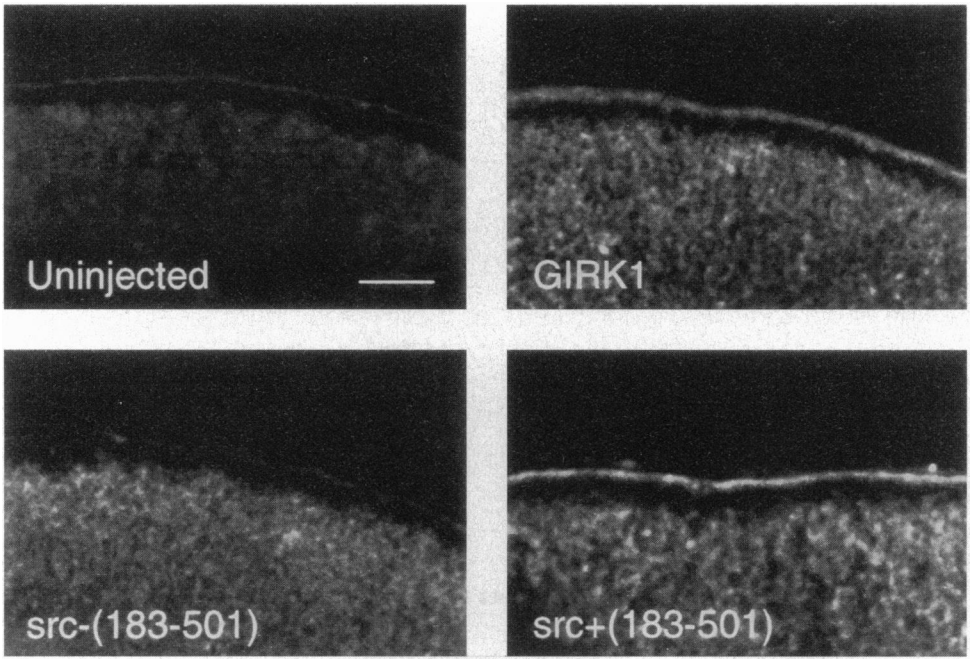

FIG. 4. Immunocytochemical localization with affinity-purified anti-peptide antibody. For each antigen, 5 ng of cRNA was used. (Bar = 25 μm.) For any one batch of oocytes studied, ≈2 of 15 of the uninjected controls showed slightly greater staining of the internal region, in some cases just slightly less than that typically seen for src^-(183–501). Similarly, the photo for src^-(183–501) is typical of that seen for about two-thirds of the oocytes; about one-third were weaker.

coassemble with intact subunits to give nonfunctional channels (21).

The question of whether the myristoylated segment of aa 183–501 functions in this manner was addressed in the delayed injection experiment of Fig. 2. When GIRK1 cRNA was injected on day 0, there was ≈530 nA of I_{ACh} expressed by day 4. On average, this was unchanged by day 6 or 8. However, when src^+(183–501) was injected on day 4, the current was greatly inhibited (70%) by day 6 or 8. While an alternative interpretation of this experiment based on rapid protein turnover and resynthesis is conceivable, the straightforward interpretation is that src^+(183–501) synthesized over the 2- to 4-day period from day 4 to 8 acted as a dominant negative functional inhibitor of preexisting GIRK1.

The observed partial inhibition of ROMK1 current suggests that the membrane-attached myristoylated segment of aa 183–501 can also function as a blocking particle for an inward rectifier K channel. The inhibition of ROMK1 currents is not voltage-dependent, thus indicating no major electrostatic contribution to the blocking action of the segment of aa 183–501 (data not shown); this is unlike the case for Shaker balls (7).

Biochemical experiments using two assays have indicated that the segment of aa 183–501 expressed as an *in vitro* translation product in a rabbit reticulocyte system (J.G., unpublished data) or as a glutathione *S*-transferase fusion in *E. coli* (C. Dessauer, personal communication) can bind to $G\beta1\gamma2$. Since most $G\beta\gamma$ subunits in mammalian cells have peripheral membrane attachment signals, we think it likely that the membrane-attached segment of aa 183–501 can bind free $\beta\gamma$ subunits released by activation of a seven-helix receptor and thus function to interfere with activation of GIRK1, or other enzymes for which $G\beta\gamma$ is an effector, in oocytes and other mammalian cells.

The system described here may provide an additional tool for exploring the sequence requirements in the domain of aa 183–501 for interference with GIRK1 activation.

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1. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N. & Jan, L. Y. (1993) *Nature (London)* **364,** 802–806.
2. 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. & Davidson, N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10235–10239.
3. Doupnik, C. A., Davidson, N. & Lester, H. A. (1995) *Curr. Opin. Neurobiol.* **5,** in press.
4. Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M. & Hugnot, J.-P. (1994) *FEBS Lett.* **353,** 37–42.
5. Taglialatela, M., Wible, B. A., Caporaso, R. & Brown, A. M. (1994) *Science* **264,** 844–847.
6. Yang, J., Jan, Y. N. & Jan, L. Y. (1995) *Biophys. J.* **68,** A362 (abstr.).
7. Zagotta, W. N., Hoshi, T. & Aldrich, R. W. (1990) *Science* **250,** 568–571.
8. Dascal, N., Schreibmayer, W., Ivanina, T., Doupnik, C., Chavkin, C., Lester, H. A. & Davidson, N. (1995) *Biophys. J.* **68,** A362 (abstr.).
9. Kain, K. C., Orlandi, P. A. & Lanar, D. E. (1991) *Biotechniques* **10,** 366–373.
10. Mager, S., Naeve, J., Quick, M., Labarca, C., Davidson, N. & Lester, H. A. (1993) *Neuron* **10,** 177–188.
11. Pellman, D., Garber, E. A., Cross, F. R. & Hanafusa, H. (1985) *Nature (London)* **314,** 374–377.
12. Bausch, S. B., Patterson, T. A., Ehrengruber, M., Lester, H. A., Davidson, N. & Chavkin, C. (1995) *Recept. Channels* **3,** in press.
13. Ivanina, T., Perets, T., Thornhill, W. B., Levin, G., Dascal, N. & Lotan, I. (1994) *Biochemistry* **33,** 8786–8792.
14. Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., Kanazirska, M. V. & Hebert, S. C. (1993) *Nature (London)* **362,** 31–38.
15. Wickman, K. D., Iñiguez-Lluhl, J. A., Davenport, P. A., Taussig, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G. & Clapham, D. E. (1994) *Nature (London)* **368,** 255–257.
16. Reuveny, E., Slesinger, P. A., Inglese, J., Morales, J. M., Iñiguez-Lluhi, J. A., Lefkowitz, R. J., Bourne, H. R., Jan, Y. N. & Jan, L. Y. (1994) *Nature (London)* **370,** 143–146.
17. Schreibmayer, W., Dascal, N., Davidson, N. & Lester, H. A. (1995) *Biophys. J.* **68,** A35 (abstr.).
18. Lim, N. F., Dascal, N., Labarca, C., Davidson, N. & Lester, H. A. (1995) *J. Gen. Physiol.* **105,** 421–439.
19. Babila, T., Moscucci, A., Wang, H., Weaver, F. E. & Koren, G. (1994) *Neuron* **12,** 615–626.
20. Verrall, S. & Hall, Z. W. (1992) *Cell* **68,** 23–31.
21. Li, M., Jan, Y. N. & Jan, L. Y. (1992) *Science* **257,** 1225–1230.