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Atrial G protein-activated K^+ channel: Expression cloning and molecular properties

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ABSTRACT **Activity of several ion channels is controlled by heterotrimeric GTP-binding proteins (G proteins) via a membrane-delimited pathway that does not involve cytoplasmic intermediates. The best studied example is the K^+ channel activated by muscarinic agonists in the atrium, which plays a crucial role in regulating the heartbeat. To enable studies of the molecular mechanisms of activation, this channel, denoted KGA, was cloned from a rat atrium cDNA library by functional coupling to coexpressed serotonin type 1A receptors in *Xenopus* oocytes. KGA displays regions of sequence homology to other inwardly rectifying channels as well as unique regions that may govern G-protein interaction. The expressed KGA channel is activated by serotonin 1A, muscarinic m2, and δ-opioid receptors via G proteins. KGA is activated by guanosine 5′-[γ-thio]triphosphate in excised patches, confirming activation by a membrane-delimited pathway, and displays a conductance equal to that of the endogenous channel in atrial cells. The hypothesis that similar channels play a role in neuronal inhibition is supported by the cloning of a nearly identical channel (KGB1) from a rat brain cDNA library.**

A major signal transduction mechanism in cardiac physiology and neurobiology is the direct coupling of neurotransmitter receptors to ion channels by a membrane-delimited pathway that does not involve cytoplasmic intermediates (for reviews, see refs. 1 and 2). The best studied member of this group is the G protein-activated K^+ channel found in atria of all vertebrates. This channel, which we denote KGA, figured in the original discovery of chemical synaptic transmission (3, 4) and plays a crucial role in regulating the heartbeat. The KGA channel rectifies at the single-channel level, allowing much larger inward than outward currents (5, 6). It is activated by acetylcholine acting on muscarinic m2 receptors via a pathway that includes a pertussis toxin (PTX)-sensitive G protein, probably of the G_i family (1, 7–12). In excised membrane patches, either the activated G_i α subunit or the βγ subunit dimer activates the K^+ channels directly (11–14). The same or a similar K^+ channel can be activated by serotonin type 1A (5HT1A) and/or by γ-aminobutyrate type B ($GABA_B$) receptors in the hippocampus via a PTX-sensitive G protein(s) (2, 15, 16). In the brain, this channel may regulate firing rates, membrane potential, and neurotransmitter responses. It is probable, but not certain, that these ion channels are examples of direct gating of an ion channel by an intracellular ligand; i.e., a G protein binds directly as a ligand to the cytoplasmic side of the channel and induces opening or closing. Cloning of KGA would allow mechanisms of G protein–effector interaction to be studied on the level of a single molecule by use of the powerful blend of molecular biology and electrophysiology approaches.

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By low-stringency hybridization or related PCR methods, a large number of voltage-sensitive K^+ channels have been cloned, starting with sequence data from the original Shaker K^+ channel. However, no member of the inwardly rectifying K^+ channel family has yet been cloned by this approach, suggesting considerable sequence divergence between the inwardly rectifying and most of the voltage-dependent K^+ channels. Recently this expectation was confirmed when cDNAs of two inwardly rectifying K^+ channels were isolated by expression cloning methods (17, 18). The two proteins, ROMK1 and IRK1, are encoded by members of a separate gene family. The prominent features of their predicted secondary structure are a pore-forming region (P) characteristic of all voltage-dependent channels (e.g., refs. 19 and 20), flanked by two hydrophobic membrane-spanning domains, M1 and M2 (in contrast with the members of the voltage-dependent K^+ channel family, which contain six hydrophobic transmembrane domains per subunit), and cytoplasmic N and C termini (17, 18).

However, these two channels, isolated from kidney and macrophage cDNA libraries, respectively, are not known to be G protein-gated and are constitutively active when expressed in *Xenopus* oocytes. Prior to the publication of the results concerning ROMK1 and IRK1, we had independently initiated a cloning project for KGA. The expression cloning of KGA is complicated by the necessity to introduce a seven-helix receptor that can couple to an oocyte's G protein and activate the channel. The natural pathway for activation of KGA (by acetylcholine via the m2 receptor) produced only small inward-rectifier K^+ currents in oocytes injected with atrial mRNA. Coinjection of atrial mRNA and cRNA of the cloned 5HT1A receptor [which has been shown to be able to couple to KGA in atrial cells (21)] gave a much larger signal (22). We report here that this assay has been used for isolating KGA. The sequence of KGA indicates that it is a third member of the ROMK1/IRK1 family. A virtually identical channel (KGB1) is present in the brain, as shown by cloning of its cDNA by homology screening of a brain library.‖

Abbreviations: 5HT, 5-hydroxytryptamine (serotonin); PTX, pertussis toxin; GTP[γS], guanosine 5′-[γ-thio]triphosphate.

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‖The sequences reported in this paper have been deposited in the GenBank database (accession nos. U01071 for KGA and U01141 for KGB1).

MATERIALS AND METHODS

Preparation of RNAs and of cDNA Libraries. Atrial and brain poly(A)$^+$ RNAs were prepared from 19-day-old rats as described (23). Directional cDNA libraries were constructed with the Stratagene Uni-ZAP cDNA synthesis kit, by inserting poly(A)$^+$-primed reverse-transcribed hemimethylated cDNA (size-selected above 1.3 kb) into pBluescript II KS(−) between *Eco*RI and *Xho* I sites. A portion of this cDNA was electroporated into Top 10 F$^-$ electrocompetent cells (Invitrogen), yielding $\approx 3.6 \times 10^5$ clones (atrium) or $\approx 4 \times 10^5$ clones (brain). For expression cloning, atrial cDNA was linearized for transcription by an 8- to 12-cycle PCR using Deep Vent polymerase (New England Biolabs) with 20- and 37-mer primers roughly corresponding to the M13-20 and reverse primers of pBluescript, with the addition of a $(dT)_{20}$ stretch to the 5′ end of the latter. KGB1 was cloned by homology screening of the brain library with the full-length KGA probe by standard procedures (24). KGA and KGB1 cDNAs were sequenced by a nested-deletion (Erase-a-Base, Promega, Madison, WI) dye-terminator protocol (Caltech), and KGA was independently sequenced by a transposon-based strategy (Amgen Biologicals), in both cases with Applied Biosystems equipment. Human 5HT1A receptor cDNA (46) was linearized with *Not* I. cDNA of the δ-opioid receptor from NG-108-15 neuroblastoma–glioma cells (25) was amplified by PCR with primers designed to add an SP6 phage promoter and 26 bp of alfalfa mosaic virus (AMV) 5′ untranslated region at the 5′ end and a $(dT)_{20}$ sequence at the 3′ end (see ref. 26). The human m2 receptor cDNA (27) was linearized with *Hin*dIII. After KGA cDNA was isolated, it was routinely linearized with *Xho* I. All RNAs were transcribed *in vitro* with the corresponding RNA polymerases (23).

Northern blot hybridizations were performed with a digoxygenin-labeled (Boehringer Mannheim) full-length KGA cDNA probe (atrial RNA) or with a KGA cDNA probe lacking the 3′ untranslated region (RNA from other tissues). Membranes were hybridized overnight [atrial samples at 41°C in 50% formamide/5× standard saline citrate (SSC)/0.02% SDS/0.1% sodium lauryl sarcosinate/20 mM sodium maleate; other tissues at 65°C in 5× SSC/0.1% sodium lauryl sarcosinate/0.02% SDS] and washed at intermediate stringency (65°C; 0.5× SSC/0.1% SDS).

cDNA Sequence Analysis. Database screening utilized the FASTA program, and sequence alignment of KGA/IRK1/ROMK1 the PILEUP program, of the Genetics Computer Group package (28). Amino acid similarity was calculated using the GCG default peptide symbol comparison table (29). Hydrophobicity analysis (11-amino acid window; see ref. 30) and transmembrane helix prediction (31) were performed with the PCGENE package (IntelliGenetics).

Oocyte Culture and Electrophysiology. *Xenopus* oocytes were prepared and injected (23) with the following cRNAs: pools derived from the library, 10–100 ng; KGA, 1–2 ng; KGB1, 1–10 ng; 5HT1A receptor, 5–30 ng; m2 receptor, 0.1–1 ng; δ-opioid receptor, 0.1–1 ng. The oocytes were incubated 3–5 days at 22°C in ND96 solution (96 mM NaCl/2 mM KCl/1 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.6) supplemented with gentamicin (50 μg/ml), 2.5 mM sodium pyruvate, and, occasionally, 0.5 mM theophylline.

Two-electrode voltage clamp was performed with a Dagan 8500 (Dagan Instruments, Minneapolis) amplifier as described (32). The oocyte was placed in a chamber perfused with ND96 (pH 7.4–7.5), the holding potential was set at −80 mV, and the solution was changed to hK (96 mM KCl/2 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.4–7.5). Intermediate concentrations of K$^+$ were obtained by mixing ND96 and hK solutions. Current–voltage (*I–V*) characteristics were recorded by using voltage ramps (between −120 or −80 mV and +40 mV) lasting 1 s.

Single-channel recordings (33) were made with an Axopatch 1D amplifier (Axon Instruments, Burlingame, CA) with a capacitive headstage using PCLAMP software (Axon Instruments). Atrial cells were prepared from 9- to 10-week-old rats essentially as described (34). Borosilicate glass pipettes were filled with a 150 mM K$^+$ solution (140 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$/2 mM NaCl/10 mM Hepes/KOH, pH 7.5). In some cases, to inhibit the activity of the stretch-activated channels in oocyte patches, 100 μM $GdCl_3$ was added to the pipette solution. The bath solution contained 150 mM KCl, 1 mM EGTA, 1–4 mM $MgCl_2$, 10 mM Hepes/NaOH (pH 7.5); 5 mM ATP was added to inhibit the activity of ATP-dependent K$^+$ channels in atrial patches. The continuous records were acquired with a chart recorder (e.g., Fig. 3*C*) or with a video cassette recorder (e.g., Fig. 3*D*).

RESULTS AND DISCUSSION

5HT-evoked inward K$^+$ currents were measured in the high-K$^+$ solution (hK) by using the two-electrode voltage clamp. Small (≈1 nA) responses were evoked by 5HT in oocytes coinjected with 5HT1A receptor RNA and cRNA transcribed *in vitro* from cDNA from an atrial library pool of ≈40,000 colonies. Sequential subdivisions of the pool led to appropriately larger responses and, eventually, to identification of a single 2070-base-pair (bp) cDNA containing a 1503-bp (bases 32–1534) open reading frame encoding KGA, a 501 amino acid protein of predicted molecular mass 56,573 Da (Fig. 1*A*). Screening of the GenBank database revealed homology between KGA and only two other proteins, IRK1 (18) and ROMK1 (17) (47% and 40% sequence identity, 66% and 65% similarity, respectively) (Fig. 1*A*). The predicted secondary structure of KGA, like that of IRK1 and ROMK1, includes a pore-forming region (P) flanked by two hydrophobic membrane-spanning domains, M1 and M2 (Fig. 2*B*), and cytoplasmic N and C termini. The pore-forming region displays high homology to that of IRK1 and ROMK1 (72% and 67% identity), but less than that between IRK1 and ROMK1 themselves (89%). The absence of a signal peptide sequence at the N terminus, the presence of a single putative N-glycosylation site between M1 and P (a predicted extracellular stretch), and the presence of two consensus protein kinase A phosphorylation sites [(Lys/Arg)-(Lys/Arg)-Xaa-Xaa-(Ser/Thr); see ref. 35] in the predicted cytoplasmic C-terminal portion of the channel molecule (Fig. 1*A*) support the proposed folding. The putative extracellular stretch following the M1 transmembrane region displays a cell-adhesion sequence, Arg-Gly-Asp (RGD), that is often involved in interaction with extracellular matrix proteins such as integrins (36). At present, there are no data about the involvement of KGA or other inward rectifier channels in phenomena related to the extracellular matrix.

The homology between KGA and the two other members of the family extends well into the C-terminal half of the protein, up to Tyr-356, suggesting common function of this (presumably cytoplasmic) domain in all three channels. Residues in KGA that are C-terminal to Tyr-356 display little or no homology to other known proteins; KGA is also 80 amino acids longer than IRK1 and 111 amino acids longer than ROMK1 (Fig. 1*A*). The first 42 amino acids of KGA also display low homology to ROMK1 and IRK1. The unique N- and C-terminal sequences of KGA are candidates for involvement in the control of gating by the α and/or βγ subunits of G proteins.

Northern blot hybridization analysis of RNA from various rat tissues showed the presence of several RNA species hybridizing to KGA cDNA of approximate sizes 2.3, 2.9, 3.9, and 6.2 kb in atrium and brain (Fig. 1*C*). In lung, RNA species of 3.9, 2.9, 2.3, and 1.8 kb were observed. A single ≈3.8-kb RNA band was found in skeletal muscle; no detectable KGA RNA was found in *Xenopus* oocytes (Fig. 1*C*); faint bands of about 4 and 6.2 kb were seen in some preparations of

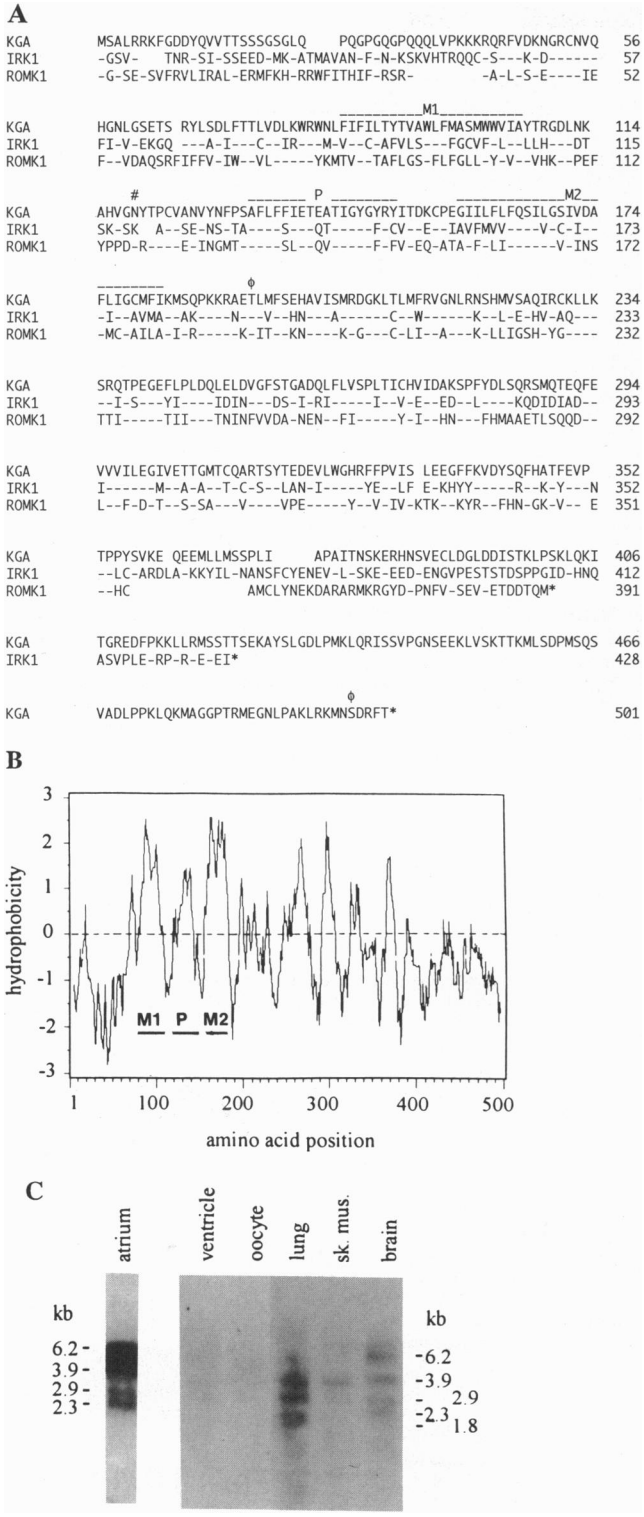

FIG. 1. Structure and tissue distribution of KGA. (*A*) Alignment of the deduced primary structure of the KGA polypeptide with those of IRK1 and ROMK1. Gaps were introduced in all sequences to improve alignment. Dashes denote identical amino acid residues; asterisks show the termination codons. Consensus protein kinase A phosphorylation sites (ϕ), a consensus N-glycosylation site (#), and the predicted transmembrane (M1 and M2) and pore (P) regions (dashed lines) are shown in the KGA sequence. The proposed cytoplasmic parts of the KGA polypeptide contain eight additional putative protein kinase A sites with a lower probability of being phosphorylated [(Lys/Arg)-Xaa-(Ser/Thr); ref. 35] and nine protein kinase C sites (not shown). (*B*) Hydrophobicity plot of the KGA sequence. (*C*) Northern blot hybridization analysis of poly(A)$^+$ RNA from various tissues. Ten micrograms of each RNA was loaded on a 1% agarose gel. Shorter exposures of the atrial RNA autoradiogram showed two clearly resolved bands of larger molecular size, ≈3.9 and ≈6.2 kb.

ventricle RNA (data not shown). It is unlikely that these probes hybridized to IRK1 RNA, which shows a single band of 5.5 kb in most tissues (18), or to ROMK1 RNA, which is expressed mainly in kidney and spleen (17).

The various RNA species hybridizing to the KGA cDNA may represent splice variants or isoforms of KGA or homologous genes encoding inward-rectifier K$^+$ channels. We have screened a brain cDNA library and identified an ≈2.2-kb cDNA that was identical to that of KGA except for an additional stretch of 41 bp within the 5′ untranslated region (8 bp after its beginning; data not shown). This finding suggests the presence in the brain of a G protein-activated K$^+$ channel, KGB1, with a primary amino acid sequence identical to that of KGA. The function of this protein as an inwardly rectifying K$^+$ channel was confirmed by expression of KGB1 cRNA in oocytes coinjected with cRNA of either 5HT1A or δ-opioid receptor (data not shown).

The expressed KGA was activated by three receptor types known to couple to G_i protein: 5HT1A, δ-opioid, and muscarinic m2 (Fig. 2). In each case, the inwardly rectifying K$^+$ current could be activated when both KGA and the receptor were heterologously expressed, but not when only the receptor or KGA cRNA was injected. The expressed receptors displayed the expected pharmacological properties (37, 38): 5HT1A receptor response was elicited by 5HT concentration as low as 1 nM and maximally at 80–100 nM and by a selective 5HT1A agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (data not shown); δ-opioid response was elicited by [Leu^5]-enkephalin and cyclic [2-(D-penicillamine),5-(D-penicillamine)]enkephalin and inhibited by the selective δ-opioid antagonist naltrindole (Fig. 2*B*); and the m2 receptor response was activated by acetylcholine (Fig. 2*C*) and blocked by atropine (data not shown). These results suggest that the KGA (and the identical KGB1) channel in the brain may serve as effectors for a convergent action of many inhibitory neurotransmitters.

In the atrium and in many other excitable cells, the activation of G protein-activated inward-rectifier K$^+$ channels is PTX-sensitive (1, 2). In *Xenopus* oocytes, the coupling between the 5HT1A receptor and KGA channel activation was mediated by an endogenous G protein as shown by sensitivity to guanosine 5′-[β-thio]diphosphate (22); however, the identity of the endogenous G protein is not known, and PTX treatment gave mixed results. We found that PTX treatment (0.5–1 μg/ml, 24–36 hr) did not inhibit the activation of cloned KGA via the δ-opioid receptor in two batches of oocytes and via the 5HT1A receptor in two of four batches. For the two PTX-sensitive batches with 5HT1A, the treatment reduced the 5HT1A response (I_{5HT}) by 65% and 100%. For the PTX-insensitive batches, coexpression of the α subunit of G_{i2} with KGA and 5HT1AR rendered the response PTX-sensitive (>90% inhibition), in accord with the previous findings with atrial poly(A)$^+$ RNA-directed KGA (22). These results confirm that the balance between PTX-sensitive and PTX-insensitive G proteins in the oocytes is batch-dependent (32) and demonstrate that KGA may be activated via G proteins having either PTX-sensitive or PTX-insensitive α subunits. This finding may be relevant to the evidence that βγ subunits play a role in channel activation (13, 14, 39).

The electrophysiological properties of I_{5HT} were further characterized. The *I–V* characteristics of the whole cell current in different external K$^+$ concentrations showed strong inward rectification and displayed gating dependent on external K$^+$ (Fig. 2*D*). The reversal potential of I_{5HT} changed by 56 mV per 10-fold change in K$^+$ concentration (Fig. 2*E*),

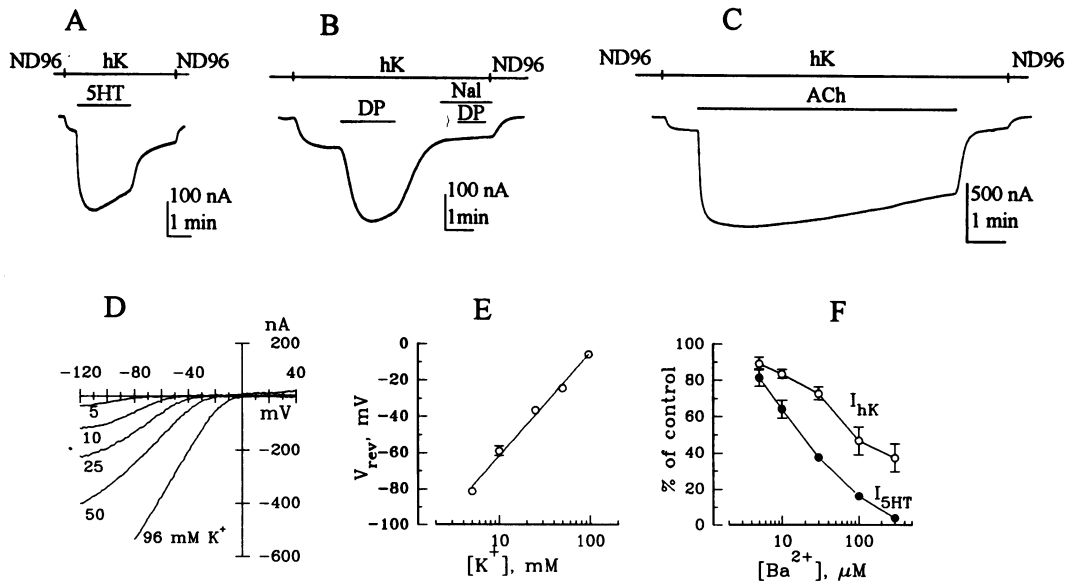

FIG. 2. Functional characterization of KGA in whole oocytes by the two-electrode voltage-clamp technique. Holding potential was −80 mV. (*A–C*) Inward currents evoked by the perfusion of the hK solution (see *Materials and Methods* for composition) (times of solution exchange from ND96 to hK and back are shown on the horizontal line above the current traces), 80 nM 5HT (*A*), 20 nM cyclic [2-(D-penicillinamine),5-(D-penicillinamine)]enkephalin (DP) (*B*), and 5 μM acetylcholine (ACh) (*C*). Note the block of DP response by 1 μM naltrindole (Nal) (*B*). (*D*) *I–V* relations (averaged from two to three cells injected on the same day) of the 5HT1A receptor current response (I_{5HT}) in various concentrations of K^+. (*E*) Dependence of the reversal potential (V_{rev}) of I_{5HT} on external K^+ concentration. Each point represents mean ± SEM of three to seven determinations. (*F*) Ba^{2+} inhibition of the high-K^+-induced current (I_{hK}) (○) and I_{5HT} (●). Each point represents mean ± SEM of three determinations.

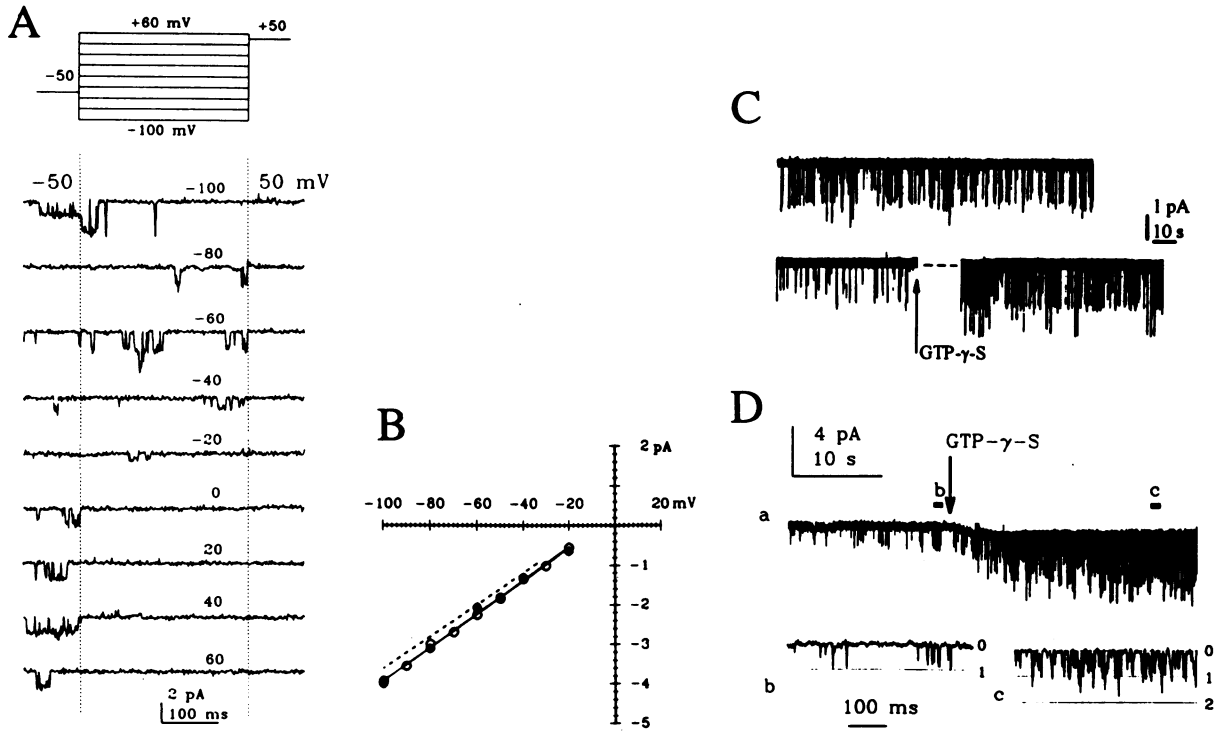

FIG. 3. Single-channel properties of KGA and activation by guanosine 5′-[γ-thio]triphosphate (GTP[γS]) in excised patches in oocytes and atrial cells. (*A*) Representative records of KGA activity in an inside-out patch (same as in *B* and *C*) of an oocyte expressing KGA and 5HT1A receptor. The records were taken ≈10 min after the addition of GTP[γS] (see *C*). The pipette solution contained 50 nM 5HT. The voltage protocol is shown at the top. Only traces with opening are shown. Leak currents were eliminated by subtracting the ≈30% of sweeps with no openings. (*B*) *I–V* relation in the same patch as in *A* in cell-attached configuration (○) and after excision and addition of GTP[γS] to the bath (●). The dashed line shows the *I–V* relation of the endogenous acetylcholine-activated channel in an atrial myocyte for comparison. The single-channel conductance was calculated from the slope of the linear regression curves between −100 and −20 mV. Note that the patch contained at least two channels. (*C*) Continuous records of the channel activity at −50 mV immediately after excision of the patch (upper trace) and 6 min later (lower trace) and the effect of addition of 100 μM GTP[γS] to the bath. The actual duration of GTP[γS] application, during which the mechanical disturbances introduced a large noise, is shown by the dashed line. (*D*) Activation of channels by 100 μM GTP[γS] in an excised patch in an atrial cell at −60 mV. The pipette contained 10 μM carbachol. Traces b and c show portions of record a (before and after application of GTP[γS]) in greater detail. The patch contained at least two channels.

suggesting a high selectivity for K^+ over Na^+. I_{5HT} was blocked by 5–300 μM Ba^{2+}, with 50% inhibition at $\approx$15 μM (Fig. 2*F*). These features are indistinguishable from those of KGA expressed in atrial poly(A)$^+$ RNA-injected oocytes (22) and similar to those in atrial cells (5, 6).

Changing the normal physiological solution (ND96) to the hK solution was accompanied by the development of an inward current (I_{hK}; Fig. 2) reflecting basal activity of inward-rectifier K^+ channels; in native oocytes, I_{hK} ranged between 30 and 100 nA and was not inhibited by Ba^{2+} below 100 μM (*cf.* ref. 22). In a representative oocyte batch, I_{hK} was 37 ± 3 nA (n = 7) in native oocytes, 147 ± 15 nA (n = 7) in KGA-expressing oocytes, and 122 ± 9 nA (n = 19) in oocytes expressing both KGA and 5HT1A receptor. Similar results were observed in >10 other oocyte batches. On many occasions, especially with m2 and δ-opioid receptors, I_{hK} was even higher when high doses of receptor RNA were used (data not shown). The finding that KGA displays basal activity with or without a coexpressed receptor supports the suggestion that the basal activity of G protein-activated K^+ channels contributes to the resting membrane conductance in the absence of an activated receptor (5, 40, 41).

Single-channel recordings in cell-attached and inside-out membrane patches revealed a strongly inwardly rectifying channel in oocytes expressing the KGA channel plus the 5HT1A receptor (Fig. 3*A*) but not in native oocytes (data not shown). The single-channel conductance in cell-attached patches with 150 mM KCl in the pipette was 39.7 ± 1.3 pS (n = 7), and in excised inside-out patches in symmetrical 150 mM KCl it was 39 ± 0.8 pS (n = 5) (Fig. 3*B*). This is similar to 40–45 pS reported under similar conditions in newborn rat atrial myocytes (42) and to what we have recorded in atrial cells of adult rats (38.2 ± 3.2 pS, n = 4, in cell-attached patches; 37.5 ± 5.5 pS, n = 2, in inside-out patches; Fig. 3*B*). With 50–100 nM 5HT in the pipette, the activity of the channel in most cell-attached patches was decreased within several minutes (data not shown), possibly reflecting desensitization also observed in whole-cell recordings (Fig. 2*A*) and reported in the atrium (43, 44). After excision of the patch, the activity of the channel ran down within a few minutes (Fig. 3*C*), or even faster, but was restored, at least partially, by the addition to the bath of 20–100 μM GTP[γS], a nonhydrolyzable analog of GTP (Fig. 3*C*; n = 9), as shown previously in guinea pig and newborn rat atrial cells (e.g., ref. 42). Reactivation by GTP[γS] was also observed in adult rat atrial cells (Fig. 3*D*). Activation of the expressed KGA channel by a GTP analog in excised patches confirms the membrane-delimited pathway of channel activation.

Cloning and expression of KGA and KGB1 confirm that a single protein can serve as an ion channel and a G-protein effector. The existence of regions of sequence that are homologous to those in G protein-independent inward-rectifier K^+ channels, and regions that are unique to KGA, suggest that G protein-interaction and inward-rectification functions reside in separate structural domains of the channel polypeptide. Cloning of KGA may help to answer the outstanding questions about the G protein–ion channel activation pathway, such as the nature of any complexes of the receptor, G protein, and channel; the stoichiometry of the G protein–channel interaction (45); the regulation by other messenger systems; and the secondary and tertiary structure of the K^+ channel protein.

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1. Brown, A. M. & Birnbaumer, L. (1990) *Annu. Rev. Physiol.* **52,** 197–213.
2. North, R. A. (1989) *Br. J. Pharmacol.* **98,** 13–28.
3. Dale, H. (1914) *J. Pharmacol. Exp. Ther.* **6,** 147–190.
4. Loewi, O. (1921) *Pflugers Arch.* **189,** 239–242.
5. Sakmann, B., Noma, A. & Trautwein, W. (1983) *Nature (London)* **303,** 250–253.
6. Horie, M. & Irisawa, H. (1987) *Am. J. Physiol.* **253,** H210–H214.
7. Trautwein, W., Kuffler, S. W. & Edwards, C. (1956) *J. Gen. Physiol.* **40,** 135–145.
8. Hammer, R., Berrie, C. P., Birdsall, N. J. M., Burgen, A. S. V. & Hulme, E. (1980) *Nature (London)* **283,** 90–92.
9. Pfaffinger, P. G., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. (1985) *Nature (London)* **317,** 536–538.
10. Breitwieser, G. E. & Szabo, G. (1985) *Nature (London)* **317,** 538–540.
11. Yatani, A., Codina, J., Brown, A. M. & Birnbaumer, L. (1987) *Science* **235,** 207–211.
12. Yatani, A., Mattera, R., Codina, J., Graf, R., Okane, K., Pardell, E., Iyengar, R., Brown, A. M. & Birnbaumer, L. (1988) *Nature (London)* **336,** 680–682.
13. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. & Clapham, D. E. (1987) *Nature (London)* **325,** 321–326.
14. Ito, H., Tung, R., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ui, M. & Kurachi, Y. (1992) *J. Gen. Physiol.* **99,** 961–983.
15. Andrade, R., Malenka, R. C. & Nicoll, R. A. (1986) *Science* **234,** 1261–1265.
16. Andrade, R. & Nicoll, R. A. (1987) *J. Physiol.* **394,** 99–124.
17. 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.
18. Kubo, Y., Baldwin, T. J., Jan, Y. N. & Jan, L. Y. (1993) *Nature (London)* **362,** 127–132.
19. Catterall, W. A. (1988) *Science* **242,** 50–61.
20. Guy, H. R. & Conti, F. (1990) *Trends Neurosci.* **13,** 201–206.
21. Karschin, A., Ho, B. Y., Labarca, C., Elroy-Stein, O., Moss, B., Davidson, N. & Lester, H. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5694–5698.
22. Dascal, N., Lim, N. F., Schreibmayer, W., Wang, W., Davidson, N. & Lester, H. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6596–6600.
23. Dascal, N. & Lotan, I. (1992) in *Methods in Molecular Biology: Protocols in Molecular Neurobiology*, eds. Longstaff, A. & Revest, P. (Humana, Clifton, NJ), Vol. 13.
24. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1991) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
25. Kieffer, B. L., Befort, K., Gaveriaux-Ruff, C. & Hirth, C. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12048–12052.
26. Mager, S., Naeve, J., Quick, M., Labarca, C., Davidson, N. & Lester, H. A. (1993) *Neuron* **10,** 177–188.
27. Peralta, E. G., Ashkenazi, A., Winslow, J. W., Smith, D. H., Ramachandran, L. & Capon, D. J. (1987) *EMBO J.* **6,** 3923–3929.
28. Genetics Computer Group (1991) Program Manual for the GCG Package (Genetics Computer Group, Madison, WI), Version 7.
29. Schwartz, R. M. & Dayhoff, M. O. (1979) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Washington, DC), pp. 353–358.
30. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
31. Rao, M. J. K. & Argos, P. (1986) *Biochim. Biophys. Acta* **869,** 179–214.
32. Dascal, N., Ifune, C., Hopkins, R., Snutch, T. P., Lubbert, H., Davidson, N., Simon, M. & Lester, H. A. (1986) *Mol. Brain Res.* **1,** 201–209.
33. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflugers Arch.* **391,** 85–100.
34. Bechem, M. & Pott, L. (1985) *Pflugers Arch.* **404,** 10–20.
35. Kennelly, P. J. & Krebs, E. G. (1991) *J. Biol. Chem.* **266,** 15555–15558.
36. Ruoslahti, E. (1991) *J. Clin. Invest.* **187,** 1–5.
37. Hoyer, D. & Schoeffer, P. (1991) *J. Recept. Res.* **11,** 197–214.
38. Portoghese, P. S., Sultana, M. & Takemori, E. E. (1988) *Eur. J. Pharmacol.* **146,** 185–186.
39. Kurachi, Y., Tung, R. T., Ito, H. & Nakajima, T. (1992) *Prog. Neurobiol.* **39,** 229–246.
40. Kaibara, M., Nakajima, T., Irisawa, H. & Giles, W. (1991) *J. Physiol. (London)* **433,** 589–613.
41. Okabe, K., Yatani, A. & Brown, A. M. (1991) *J. Gen. Physiol.* **97,** 1279–1293.
42. Kirsch, G. E., Yatani, A., Codina, J., Birnbaumer, L. & Brown, A. M. (1988) *Am. J. Physiol.* **254,** H1200–H1205.
43. Kurachi, Y., Nakajima, T. & Sugimoto, T. (1987) *Pflugers Arch.* **410,** 227–233.
44. Kim, D. (1991) *J. Physiol. (London)* **437,** 133–155.
45. Kurachi, Y., Ito, H. & Sugimoto, H. (1990) *Pflugers Arch.* **416,** 216–218.
46. Kobilka, B. K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T. S., Francke, U., Lefkowitz, R. J. & Caron, M. G. (1987) *Nature (London)* **329,** 75–78.