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Expression of an atrial G-protein-activated potassium channel in *Xenopus* oocytes

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ABSTRACT **Injection of rat atrial RNA into *Xenopus* oocytes resulted in the expression of a guanine nucleotide binding (G) protein-activated K^+ channel. Current through the channel could be activated by acetylcholine or, if RNA encoding a neuronal 5HT1A receptor was coinjected with atrial RNA, by serotonin (5HT). A 5HT-evoked current (I_{5HT}) was observed in oocytes injected with ventricle RNA fractions (of 2.5–5.5 kb) and 5HT1A receptor RNA. I_{5HT} displayed strong inward rectification with very little conductance above the K^+ equilibrium potential, was highly selective for K^+ over Na^+, and was blocked by 5–300 μM Ba^{2+}. I_{5HT} was suppressed by intracellular injection of the nonhydrolyzable analog of GDP, guanosine 5′-[β-thio]diphosphate, but not by treatment with pertussis toxin (PTX), suggesting coupling of the receptor to the G-protein-activated K^+ channel via a PTX-insensitive G protein, possibly endogenously present in the oocyte. Coexpression of the α subunit of a PTX-sensitive G protein, G_{i2}, rendered I_{5HT} sensitive to PTX inhibition. Native oocytes displayed a constitutively active inwardly rectifying K^+ current with a lower sensitivity to Ba^{2+} block; expression of a similar current was also directed by atrial or ventricle RNA of 1.5–3 kb. *Xenopus* oocytes may be employed for cloning of the G-protein-activated K^+ channel cDNA and for studying the coupling between this channel and G proteins.**

Parasympathetic regulation of the rate of heart contraction is exerted through the release of acetylcholine (ACh), which opens a K^+ channel in the atrium and thus slows the rate of depolarization that leads to initiation of the action potential (1, 2). The coupling between binding of ACh to a muscarinic receptor and opening of the K^+ channel occurs via a pertussis toxin (PTX)-sensitive heterotrimeric guanine nucleotide binding (G) protein, G_k (3–5), probably belonging to the inhibitory G-protein G_i family (6, 7). Activation of this G-protein-activated K^+ (KG) channel by G_k does not require cytoplasmic intermediates (for review, see refs. 8 and 9). However, a long-standing controversy exists as to which G-protein subunit couples to the KG channel. Purified βγ subunit complexes (10, 11) and α subunits of the G_i family (6, 7, 12) activate the KG channel in cell-free inside-out patches of atrial myocytes. Activation by the α subunits occurs at lower concentrations than that by βγ but seems to be less efficient (13); the relative physiological importance of each pathway and the possible involvement of the arachidonic acid pathway (14) are unclear.

A channel similar or identical to the ACh-operated KG channel can be activated in the atrium by adenosine (15), ATP (16), and epinephrine (17), probably also via a G-protein pathway. Furthermore, in nerve cells various 7-helix receptors, such as serotonin (5HT) 5HT1A, δ-opioid, γ-aminobutyric acid type B ($GABA_B$), somatostatin receptors, etc., couple to similar K^+ channels, probably through direct activation by G proteins (18–22). The similarity of the channels and of the signaling pathways in atrium and some nerve-cell preparations was strengthened by the demonstration of the coupling of a neuronal 5HT1A receptor (5HT1A-R), transiently expressed in atrial myocytes, to the atrial KG channels (23).

By electrophysiological and pharmacological criteria, the atrial KG channel belongs to a family of inward rectifiers that conduct K^+ much better in the inward than the outward direction, are blocked by extracellular Na^+, Cs^+, and Ba^{2+}, and are believed to possess a single-file pore with several permeant and blocking ion binding sites (24). Many inward rectifiers are not activated by transmitters or voltage but seem to be constitutively active. Inward rectification of the atrial KG channel is due to a block of K^+ efflux by intracellular Mg^{2+} (25), but for some channels of this family inward rectification may not depend on the Mg^{2+} block (26, 27). The molecular structures of atrial and neuronal KG channels are unknown. Inwardly rectifying K^+ channels structurally similar to voltage-activated K^+ channels have been cloned from plant cells (28, 29). Recently, the primary structures of two mammalian inward-rectifier channels have been elucidated by molecular cloning of their cDNAs via expression in *Xenopus* oocytes: an ATP-regulated K^+ channel from kidney, ROMK1 (30), and an inward rectifier from a macrophage cell line, IRK1 (31). Both appear to belong to a superfamily of K^+ channels, with only two transmembrane domains per subunit and a pore region homologous to that of K^+, Ca^{2+}, and Na^+ voltage-dependent channels (see ref. 32). It has been hypothesized that the structure of G-protein-activated inwardly rectifying K^+ channels should be similar to that of ROMK1 and IRK1 (31). Cloning of the atrial KG channel and its expression in a heterologous system would be of importance not only to test this hypothesis but also to allow an as yet unexplored molecular approach to investigate the mechanisms of direct G-protein–ion-channel coupling. As a first step to cloning of the atrial KG channel, we have expressed it in *Xenopus* oocyte injected with atrial RNA and characterized the macroscopic current properties, including a preliminary characterization of G-protein coupling.

MATERIALS AND METHODS

Preparation of RNA and Oocytes. Total RNA was extracted from atria and ventricles of 19- to 21-day-old rats of both

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Abbreviations: ACh, acetylcholine; 5HT, 5-hydroxytryptamine (serotonin); G protein, guanine nucleotide binding protein; KG channel, G-protein-activated K^+ channel; 5HT1A-R, 5HT1A receptor; I_{5HT} and I_{hK}, inward currents induced by 5HT and high K^+, respectively; PTX, pertussis toxin; GDP[βS], guanosine 5′-[β-thio]diphosphate; $[K_{out}]$, external K^+ concentration.

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sexes using the Chomczynski–Sacchi procedure (33). Poly(A) RNA was separated on an oligo(dT)-cellulose column (type 3, Collaborative Biochemical Products, Bedford, MA). Ventricle poly(A) RNA was fractionated by centrifugation (18 h, 30,000 × *g*, 4°C) on a linear 5–25% (wt/vol) sucrose gradient. *Xenopus laevis* oocytes were prepared as described (34) and injected with poly(A) RNA (50–120 ng per oocyte), total RNA (120–200 ng per oocyte), or fractionated poly(A) RNA (35 ng per oocyte). In most cases, 5HT1A-R RNA (5–20 ng per oocyte) was coinjected with atrial or ventricle RNA. The final volume of the injected RNA solution was 50 nl. The oocytes were incubated for 3–7 days in NDE solution [ND96 (see below) containing 1.8 mM $CaCl_2$ and supplemented with 2.5 mM sodium pyruvate and gentamycin (50 μg/ml)]. Occasionally, either 2.5–5% (vol/vol) heat-inactivated horse serum or 0.5 mM theophilline was added to the NDE solution. Incubation of oocytes in PTX (List Biochemicals) was done in NDE solution without the addition of pyruvate, serum or theophilline. cDNAs for 5HT1A-R (see ref. 23) and G_{i2} α subunit (a gift from M. I. Simon, California Institute of Technology) in pBluescript were linearized, and RNA was synthesized *in vitro* as described (34).

Electrophysiological Recordings. Recordings were performed using the two-electrode voltage clamp method with the Dagan 8500 amplifier (Dagan Instruments, Minneapolis) as described (35). The oocytes were usually kept in ND96 solution (96 mM NaCl/2 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$/5 mM Hepes, pH 7.5). Most measurements were done in the high K^+ solution (hK, 96 mM KCl/2 mM NaCl/1 mM $MgCl_2$/1 mM $CaCl_2$/5 mM Hepes, pH 7.5). Solutions containing intermediate concentrations of K^+ were made by replacing K^+ with Na^+. Solution exchange and drugs application were done by superfusing the cell placed in a 0.5-ml chamber. Guanosine 5′-[β-thio]diphosphate (GDP[βS]; (trilithium salt; Sigma) was injected by pressure (35). Stimulation, data acquisition, and analysis were performed using PCLAMP software (Axon Instruments, Foster City, CA).

RESULTS

To express the KG channel, the oocytes were injected with atrial total or poly(A) RNA. To avoid the possibility that a low level of expression of the muscarinic receptor will make a well-expressed KG channel undetectable, atrial RNA was usually supplemented with 5HT1A-R mRNA; oocytes injected with this RNA mixture will be termed RNA-injected oocytes throughout the paper. When expressed in atrial myocytes, the 5HT1A-R efficiently coupled to the KG channel normally existing in these cells (23), and it was expected to do so in the oocytes.

Four to 5 days after RNA injection, addition of 10 μM ACh or 1–2 μM 5HT to the ND96 bath solution did not cause any significant change in membrane current. Therefore, the effects of ACh and 5HT were tested in an hK solution with 96 mM K^+ and 2 mM Na^+. In this solution, the K^+ equilibrium potential (E_K) is close to 0 mV, and this should enable inward K^+ current flow through inwardly rectifying K channels at negative holding potentials (−80 mV was routinely used in this study).

Changing ND96 to the hK solution was accompanied by the development of an inward current that reached a steady level within the 0.5–1 min (high K^+-induced inward current, I_{hK}; Fig. 1*A*). I_{hK} was also observed in native (not injected with any RNA) oocytes and in oocytes injected with 5HT1A-R RNA alone, but it was always larger in RNA-injected oocytes ($P < 0.001$, two-tailed t test; Table 1).

In RNA-injected oocytes, application of 5HT or ACh in hK solution induced an inward current (I_{5HT}) that subsided upon removal of the transmitter (Fig. 1 *A* and *B*). The response to

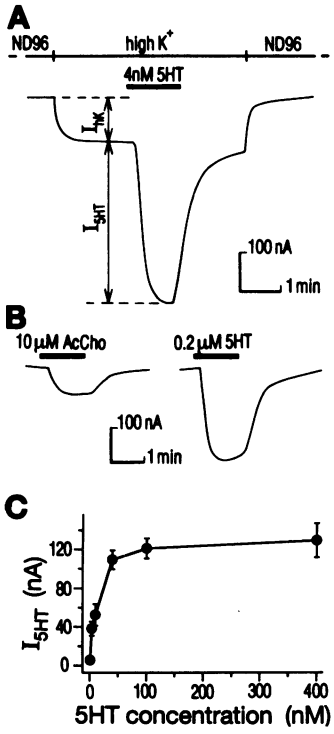

FIG. 1. Inward currents evoked by high K^+, 5HT, and ACh in RNA-injected oocytes. (*A*) I_{hK} and I_{5HT} in an oocyte injected with atrial RNA plus 5HT1A-R RNA. Holding potential in this and all following figures was −80 mV. (*B*) Inward currents evoked by ACh (AcCho) and 5HT in a single oocyte in hK solution. (*C*) Dependence of I_{5HT} amplitude on the 5HT concentration in oocytes from one frog. In each oocyte, the response to one 5HT concentration was tested. Data represent the mean ± SEM in four to six cells at each concentration.

ACh was usually smaller than to 5HT when measured in the oocytes of the same frog (Fig. 1*B*). Thus, in oocytes of one frog, I_{5HT} was 1102 ± 84 nA (n = 6), whereas the ACh response was 382 ± 45 nA (n = 6). I_{5HT} tended to decrease on repeated applications of 5HT, and this could be overcome by increasing the intervals between applications to 10 min or more, suggesting the presence of a desensitization process. I_{5HT} and an increased (in comparison with native oocytes) I_{hK} were also observed in oocytes injected with ventricle poly(A) RNA plus 5HT1A-R RNA, but the I_{5HT} was about 20 times smaller than with atrial poly(A) RNA (data not shown). 5HT had no effect in oocytes injected with atrial RNA without the 5HT1A-R RNA (n = 4) or with 5HT1A-R RNA alone or in native oocytes (Table 1).

The 5HT dose–response curve showed saturation at ≈100 nM and a half-maximal response at about 15 nM (Fig. 1*C*), which is characteristic of the 5HT1 receptor class (36). A similar current was evoked by a selective 5HT1A agonist, 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin; data not shown].

The current–voltage relationship characteristic of the oocyte membrane was studied by applying voltage steps from

Table 1. Inward currents evoked by high K^+ and by 5HT

Injected RNA	I_{hK}, nA	I_{5HT}, nA
None (native oocytes)	72 ± 6 (34)	0 (18)
5HT1A-R	54 ± 4 (11)	0 (12)
Atrial + 5HT1A-R	123 ± 8 (55)	290 ± 43 (55)

Entries are inward currents (mean ± SEM) measured at −80 mV in the hK solution. Numbers in parentheses are n. The 5HT concentration ranged from 100 nM to 2 μM.

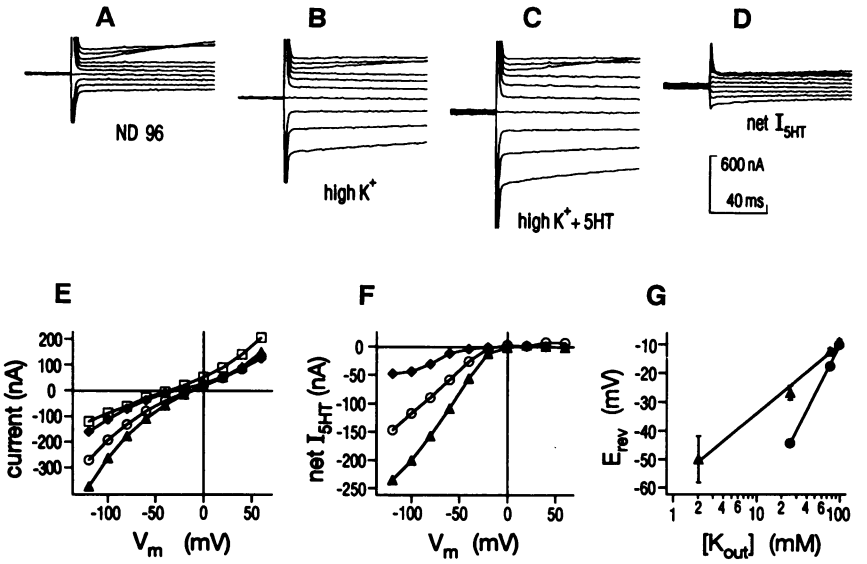

FIG. 2. I_{hK} and I_{5HT} are inwardly rectifying K^+ currents. (*A–D*) Currents evoked by voltage steps from the holding potential of −80 mV to voltages between −140 and 40 mV in 20-mV steps in ND96 (*A*), hK (*B*), and hK in the presence of 5HT (*C*). Net I_{5HT} (*D*) was obtained by digital subtraction of *B* from *C*. (*E*) Current–voltage relations of the total membrane current in a representative oocyte. □, ND96 (2 mM [K_{out}]); ◆, 25 mM [K^+_{out}]; ○, 75 mM [K_{out}]; ▲, hK (96 mM [K_{out}]). (*F*) Current–voltage relation of the net I_{5HT} in the same oocyte as in *E*. ◆, 25 mM [K_{out}]; ○, 75 mM [K_{out}]; ▲, 96 mM [K_{out}]. (*G*) Dependence of the reversal potentials of total membrane current (▲) and of I_{5HT} (●) on [K_{out}]. The straight lines represent least squares fits to data (mean ± SEM, n = 3 for each point).

a holding potential of −80 mV. In normal ND96, in the range of −140 to −20 mV, only voltage- and time-independent "leak" currents were observed (Fig. 2*A*), and the current–voltage curve was linear (Fig. 2*E*). Above −20 mV, a slowly developing outward current was observed (Fig. 2 *A–C*); this is known to be due to opening of a Cl^- channel activated by Ca^{2+} entry through voltage-dependent Ca^{2+} channels (37). The Ca^{2+}-activated Cl^- current was also seen in the hK solution; in addition, the total membrane current evoked by steps to −120 and up to −20 mV was larger than in ND96 (Fig. 2 *B* and *E*), whereas above 0 mV there was little or no change. This suggested that most or all of I_{hK} elicited at −80 mV by the exchange of ND96 to hK solution was due to a K^+ current flowing through a constitutively active inward-rectifier K^+ channel(s). This current showed some time-dependent inactivation at −140 mV (Fig. 2*B*) and at more negative potentials (data not shown); this inactivation phenomenon was not studied further. In the presence of 5HT, the membrane currents between −140 and −20 mV were further increased (Fig. 2*C*). Net 5HT-evoked currents, obtained by digital subtraction of total membrane currents in the absence of 5HT from currents in its presence (Fig. 2*D*), showed clear inward rectification; the 5HT-activated channels conducted little or no current above E_K at different external K^+ concentrations [K_{out}] (Fig. 2*F*). The extrapolated reversal potential of I_{5HT} showed an almost perfect selectivity of the 5HT-activated channel to K^+, changing by ≈58 mV per 10-fold change in [K_{out}] (Fig. 2*G*). The reversal potential of the total membrane current in the absence of 5HT also depended on [K_{out}] (Fig. 2*E*) but changed only by 24 mV per 10-fold change in [K_{out}] (Fig. 2*G*). This does not necessarily imply poor ion selectivity of the constitutively active inward rectifier but may reflect the relatively high contribution of Cl^- and Na^+ to the resting membrane conductance (38).

Block by external Ba^{2+} is one of the characteristic features of inward rectifiers (24). In normal ND96 solution, Ba^{2+} (5 μM to 3 mM) did not cause any significant changes in resting current or conductance in native or RNA-injected oocytes at the holding potential of −80 mV. In the hK solution, Ba^{2+} inhibited both I_{hK} and I_{5HT} (Fig. 3), and this was accompanied by a decrease in membrane conductance (data not shown). Ba^{2+} at 300 μM blocked ≈20% of I_{hK} but almost completely abolished I_{5HT} (Fig. 3*B*). The IC_{50} value for the Ba^{2+} block of I_{5HT} was ≈15 μM, whereas the IC_{50} value for I_{hK} block was >3 mM (Fig. 3*D*). It is noteworthy that, although the sensitivity of I_{hK} to the Ba^{2+} block was similar in native and RNA-injected oocytes, the latter did appear to have a small component of I_{hK} inhibited by low doses of Ba^{2+} (Fig. 3*D*). This raises the possibility that the atrial I_{hK} is more sensitive to the Ba^{2+} block than the oocyte I_{hK} or that a fraction of the

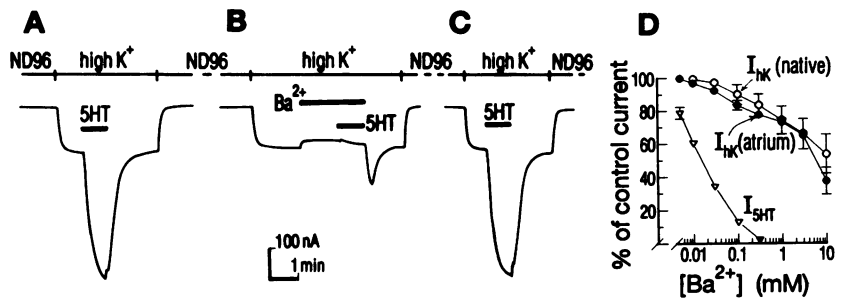

FIG. 3. Ba^{2+} block of I_{hK} and I_{5HT}. (*A–C*) Records taken from the same oocyte at 10-min intervals. Between the records, the cell was bathed in ND96. The 5HT concentration was 4 nM. Note that in *B* 300 μM Ba^{2+} reduces I_{hK} and almost completely blocks I_{5HT}. Ba^{2+} and 5HT were washed out simultaneously, and this resulted in an inward current "tail." (*D*) Dose dependence of Ba^{2+} inhibition of I_{hK} in native oocytes (○), I_{hK} in RNA-injected oocytes (●), and I_{5HT} in RNA-injected oocytes (▽). Data are the mean ± SEM (n = 3 to 7 for each point).

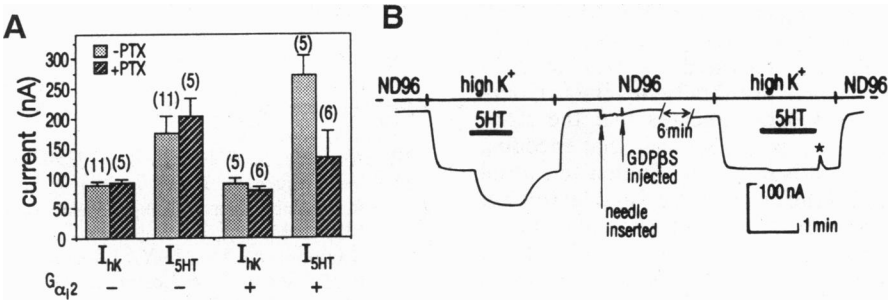

FIG. 4. I_{5HT} is mediated by activation of a G protein. (*A*) The effect of PTX treatment (500 ng/ml, 20–26 h) on I_{hK} and I_{5HT}. The cells were injected with total atrial RNA (120 ng per oocyte), 5HT1A-R RNA (11 ng per oocyte), and, where indicated, with $G_{i2}\alpha$ RNA (11 ng per oocyte). (*B*) GDP[βS] injection inhibits I_{5HT} but not I_{hK} in an oocyte injected with atrial plus 5HT1A-R RNAs. The 5HT concentration was 0.4 μM. A small outward current deflection (denoted by ⋆) upon removal of 5HT was caused by an inadvertent perfusion of ND96 for a few seconds.

highly Ba^{2+}-sensitive channels underlying I_{5HT} could be active in the absence of agonist. Note also that there was an inward current "tail" observed when Ba^{2+} and 5HT were washed out simultaneously (Fig. 3*B*), presumably because the rate-limiting step in deactivation of the channel proceeds more slowly than the unblock from Ba^{2+}.

To estimate the size of RNA encoding the expressed inward rectifiers, ventricle poly(A) RNA (available in larger amounts) was fractionated on a sucrose gradient. The size distribution of the fractions was measured by RNA gel blots probed with ^{32}P-labeled poly(T) (39). The RNA encoding I_{5HT} was found mainly in two size fractions between 2.5 and 5.5 kb. The peak expression of ventricle I_{hK} was in lower-size fractions of 1.5–3 kb (data not shown).

In the atrium, the muscarinic receptor is coupled to the KG channel via a PTX-sensitive G protein (8). Surprisingly, in RNA-injected oocytes, I_{5HT} was not affected by treatment with PTX; neither was I_{hK} (Fig. 4*A*). To test whether the 5HT1A-R couples to the K^+ channel via a G protein, the oocytes were injected with the nonhydrolyzable analog of GDP, GDP[βS], which is known to inhibit the activity of PTX-sensitive and PTX-insensitive G proteins (40), at 400–800 pmol per oocyte. In four cells, GDP[βS] injection had no effect on I_{hK} (115 ± 8% of control) but strongly inhibited I_{5HT} to 4 ± 1% of control (Fig. 4*B*). Thus, it appears that the coupling between the 5HT1A-R and the KG channel occurs via an oocyte's endogenous PTX-insensitive G protein.

We examined whether an overexpressed PTX-sensitive G-protein α subunit (e.g., $G_{i2}\alpha$) could compete with the native PTX-insensitive α subunit for the expressed 5HT1A-R, thus restoring the PTX sensitivity of the KG channel activation. As shown in Fig. 4*A*, in oocytes injected with atrial RNA plus complementary RNAs encoding 5HT1A-R and $G_{i2}\alpha$, PTX inhibited I_{5HT} by ≈50% ($P < 0.01$, two-tailed t test), whereas I_{hK} was unaffected.

DISCUSSION

The present results demonstrate that the atrial inward rectifier K^+ (KG) channel, which in the native tissue is activated by ACh via a PTX-sensitive G protein, is expressed in oocytes injected with atrial RNA. Current through the channel can be activated by ACh or, if RNA encoding a neuronal 5HT1A-R is coinjected with atrial RNA, by 5HT. Activation of the channel probably occurs via a muscarinic ACh receptor synthesized after the atrial RNA injection, rather than via the oocyte's endogenous muscarinic receptor. The latter couples to phospholipase C, and its activation induces very characteristic large transient Cl^- current responses caused by Ca^{2+} release from intracellular stores (41). Fortunately, the majority of oocyte batches lose this response after defolliculation (42), and this response was not observed in the present study. Because the ACh-evoked currents were small in most cases, we concentrated on the study of the 5HT response; the latter was undoubtedly mediated by the introduced 5HT1A-R, as 5HT was ineffective in oocytes not injected with 5HT1A-R RNA, and the response displayed the expected pharmacological properties.

The evidence presented here indicates that, in oocytes injected with atrial and 5HT1A-R RNAs, activation of the 5HT1A-R leads to opening of a K^+ channel that bears the following distinctive features of an anomalous rectifier, similar to those of the atrial KG. (*i*) It conducts inward but not outward K^+ current; (*ii*) it is blocked by low concentrations of Ba^{2+}; (*iii*) the gating of the channel does not depend solely on voltage but on E_K. The expression of this channel must truly be directed by atrial RNA, because (*i*) no hormone or transmitter-activated current of this kind is observed in native oocytes and (*ii*) expression of the 5HT1A-R alone does not cause the appearance of such a response. Based on ventricle RNA fractionation data, the RNA encoding the 5HT-activated channel is in a broad size range between 2.5 and 5.5 kb. This is similar to or somewhat smaller than the reported 4- to 5-kb mRNA size of some constitutively active inward rectifiers expressed in *Xenopus* oocytes (43, 44) and of the cloned IRK1 (5.5 kb; ref. 31) and ROMK1 (4 kb; ref. 30) channels. The properties of I_{5HT} directed by ventricle and atrial RNA are very similar, and it is reasonable to assume that they are encoded by the same RNA species.

Opening of the inward rectifier by 5HT is mediated by activation of a G protein, as expected for the KG channel, because (*i*) 5HT1A-R belongs to the family of 7-helix receptors, all of which act via G proteins (40) and (*ii*) I_{5HT} was inhibited by intracellular injection of GDP[βS]. However, the G protein participating in this pathway was PTX-insensitive, possibly an endogenous oocyte G protein. It is not clear why in the oocyte the channel activation pathway involves a PTX-insensitive G protein. The atrial KG channel normally couples to G_i (9), there are at least two subspecies of G_i in the oocyte (45), some G_i may be expressed from atrial RNA, and also, in the hippocampus, the 5HT1A-R opens a K^+ channel by activating a PTX-sensitive G protein (21). One possibility is that a vast excess of this undefined PTX-insensitive G protein overrides the others in the competition for coupling to the 5HT1A-R. Whatever the reason for this unexpected coupling, our results show that the PTX sensitivity of the KG channel activation can be partially restored by overexpression of the α subunit of G_i. Since the actual identity of the α subunit does not seem to be important for activation of the expressed KG channel, these results imply that the $\beta\gamma$ subunit complex doublet may be the activator of the channel (cf. refs. 10 and 11).

Atrial and ventricle RNAs also induce an enhanced activity of an additional inward rectifier that is active in the absence of any specific stimulation (referred to herein as I_{hK}). I_{hK} in atrial RNA-injected oocytes is about twice as large as in

native oocytes or oocytes injected with 5HT1A-R RNA alone. This current does not appear to represent the "basal" activity of the same channel activated by 5HT or ACh because it has a much lower sensitivity to Ba^{2+} block. Moreover, the fractionation data indicates that the RNA directing the expression of I_{hK} is smaller than that encoding the KG channel. However, it is not clear whether this atrial (or ventricle) RNA encodes the channel itself or a factor that enhances the expression or the activity of a native channel. Further studies, such as expression cloning, will help to identify the messages encoding the two inward rectifiers whose expression is reported here.

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1. Sakmann, B., Noma, A. & Trautwein, W. (1983) *Nature (London)* **303,** 250–253.
2. Ijima, T., Irisawa, H. & Kameyama, M. (1985) *J. Physiol. (London)* **359,** 485–501.
3. Pfaffinger, P. G., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. (1985) *Nature (London)* **317,** 536–538.
4. Breitweiser, G. E. & Szabo, G. (1985) *Nature (London)* **317,** 538–540.
5. Kurachi, Y., Nakajima, T. & Sugimoto, T. (1986) *Am. J. Physiol.* **251,** H681–H684.
6. Yatani, A., Codina, J., Brown, A. M. & Birnbaumer, L. (1987) *Science* **235,** 207–211.
7. 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.
8. Kurachi, Y., Tung, R. T., Ito, H. & Nakajima, T. (1992) *Prog. Neurobiol.* **39,** 229–246.
9. Brown, A. M. & Birnbaumer, L. (1990) *Annu. Rev. Physiol.* **52,** 197–213.
10. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. & Clapham, D. E. (1987) *Nature (London)* **325,** 321–326.
11. Kurachi, Y., Ito, H., Sugimoto, T., Katada, T. & Ui, M. (1989) *Pflügers Arch.* **413,** 325–327.
12. Codina, J., Yatani, A., Grenet, D., Brown, A. M. & Birnbaumer, L. (1987) *Science* **236,** 442–445.
13. Ito, H., Tung, R. T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ui, M. & Kurachi, Y. (1992) *J. Gen. Physiol.* **99,** 961–983.
14. Kim, D., Lewis, D. L., Graziadei, L., Neer, E. J., Bar-Sagi, D. & Clapham, D. E. (1989) *Nature (London)* **337,** 557–560.
15. Kurachi, Y., Nakajima, T. & Sugimoto, T. (1986) *Pflügers Arch.* **407,** 264–276.
16. Friel, D. D. & Bean, B. P. (1990) *Pflügers Arch.* **415,** 651–657.
17. Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I. & Ui, M. (1989) *Pflügers Arch.* **414,** 102–104.
18. Codina, J., Grenet, D., Yatani, A., Birnbaumer, L. & Brown, A. M. (1987) *FEBS Lett.* **216,** 104–106.
19. North, R. A., Williams, J. T., Suprenant, A. & Christie, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5487–5491.
20. Andrade, R., Malenka, R. C. & Nicoll, R. A. (1986) *Science* **234,** 1261–1265.
21. Andrade, R. & Nicoll, R. A. (1987) *J. Physiol.* **394,** 99–124.
22. VanDongen, A. M. J., Codina, J., Olate, J., Mattera, R., Joho, R., Birnbaumer, L. & Brown, A. M. (1988) *Science* **242,** 1433–1437.
23. 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.
24. Hille, B. (1992) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA), 2nd Ed.
25. Horie, M. & Irisawa, H. (1987) *Am. J. Physiol.* **253,** H210–H214.
26. Ciani, S., Krasne, S., Myazaki, S. & Hagiwara, S. (1978) *J. Membr. Biol.* **44,** 103–134.
27. Silver, M. R. & DeCoursey, T. E. (1990) *J. Gen. Physiol.* **96,** 109–133.
28. Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.-M., Gaymard, F. & Grignon, C. (1992) *Science* **256,** 663–665.
29. Anderson, J. A., Huprikar, S. S., Kochian, L. V., Lucas, W. J. & Gaber, R. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3736–3740.
30. 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.
31. Kubo, Y., Baldwin, T. J., Jan, Y. N. & Jan, L. Y. (1993) *Nature (London)* **362,** 127–132.
32. Aldrich, R. (1993) *Nature (London)* **362,** 107–108.
33. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
34. Dascal, N. & Lotan, I. (1992) in *Methods in Molecular Biology, Vol. 13: Protocols in Molecular Neurobiology*, eds. Longstaff, A. & Revest, P. (Humana, Totowa, NJ).
35. 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.
36. Hoyer, D. & Schoeffer, P. (1991) *J. Recept. Res.* **11,** 197–214.
37. Barish, M. E. (1983) *J. Physiol. (London)* **342,** 309–325.
38. Dascal, N., Landau, E. M. & Lass, Y. (1984) *J. Physiol. (London)* **352,** 551–574.
39. Lubbert, H., Snutch, T. P., Dascal, N., Lester, H. A. & Davidson, N. (1987) *J. Neurosci.* **7,** 1159–1165.
40. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
41. Dascal, N. (1987) *CRC Crit. Rev. Biochem.* **22,** 317–387.
42. Miledi, R. & Woodward, R. M. (1989) *J. Physiol.* **416,** 601–621.
43. Lewis, D. L., Ikeda, S. R., Aryee, D. & Joho, R. H. (1991) *FEBS Lett.* **290,** 17–21.
44. Perier, F., Coulter, K. L., Radeke, C. M. & Vanderberg, C. A. (1992) *J. Neurochem.* **59,** 1971–1974.
45. Olate, J., Martinez, S., Purcell, P., Jorguera, H., Codina, J., Birnbaumer, L. & Allende, J. E. (1990) *FEBS Lett.* **268,** 27–31.