Korean J Biol Sci 2: 471-476, 1998

Expression and Characterization of G Protein-activated Inward Rectifier K^+ Channels in *Xenopus* Oocytes

Han-Seop Kim, Changho Lee[1], and Churl K. Min*

Department of Biological Science, College of Natural Sciences, Ajou University, Suwon 442-749, Korea;
[1]Department of Pharmacology, College of Medicine, Hanyang University, Seoul 133-792, Korea

Key Words:
Xenopus oocytes
G protein-activated inward rectifier
Voltage clamp
Pertussis toxin sensitivity

The G protein-activated inwardly rectifying K^+ channel (GIRK1) was coexpressed in *Xenopus* oocytes along with the 5-HT_{1A} receptor, a 7-helix receptor known to be coupled to K^+ channels in many neural tissues. Thus, the activation of the 5-HT_{1A} receptor by its agonist leads to the opening of GIRK1. The GIRK1 current was measured using the two electrode voltage clamp technique with bath application of 5-HT in the presence of various external potassium concentrations $[K^+]_o$. GIRK1 showed a strong inward rectification since only hyperpolarizing voltages evoked inward currents. K^+ was the major ion carrier as evidenced by about 44 mV voltage shift corresponding to a 10-fold external $[K^+]$ change. 5-HT induced a concentration-dependent inward K^+ current ($EC_{50} \cong 10.7$ nM) which was blocked by Ba^{2+}. Pertussis toxin (PTX) pre-treatment reduced the K^+ current by as much as about 70%, suggesting that PTX-sensitive G protein (G_i or G_o type) are involved in the 5-HT_{1A} receptor-GIRK1 coupling in *Xenopus* oocytes.

G protein-activated inwardly rectifying K^+ (GIRK) channel is the potassium channel whose opening is regulated by neurotransmitters, and thus determine the neuronal membrane excitability by selectively permitting the flux of K^+ ions near the resting membrane potential of the cell (North, 1989). Neurotransmitters such as dopamine, opioids, somatostatin, acetylcholine, 5-HT (serotonin), adenosine, and γ-aminobutyric acid (GABA) type B exert their inhibitory actions in part by activating inwardly rectifying K^+ channels (Nicoll et al., 1990; Hille., 1992b)

Since the first inward rectifier K^+ channel was described in skeletal muscles and egg-cell membranes (see Doupnik et al., 1995a for review), they are now found in many cell types. In atrial cells, acetylcholine released by stimulation of the vagal nerve causes the opening of a GIRK channel (I_{KACh}) via the activation of the m_2 muscarinic receptor, inducing hyperpolarization and thus slowing the cardiac frequency. A GIRK channel, termed either GIRK1 (Kubo et al., 1993) or K^+ channel G protein-activated (KGA) (Dascal et al., 1993) encodes a G protein-activated inward rectifier K^+ channel found in both brain and cardiac tissues (Dascal et al., 1993; Kubo et al., 1993; Ashford et al., 1994; De Paoli et al., 1994; Karschin et al., 1994; Kobayashi et al., 1995). In the atrial myocyte, GIRK1 seems to play a crucial role in regulating the heart beat via increasing the inward K^+ influx. In the brain, on the other hand, this channel may regulate the firing rate, membrane potential, and neurotransmitter responses. GIRK1 is one of the best examples of a direct membrane-delimited signalling pathway that involves a G protein, i.e. the ligand-activated G protein binds directly to the cytoplasmic side of the channel, thus induces the opening or closing of the channel (Dascal et al., 1993)

5-HT, which plays an important role in the modulation of mood, nociception, motor behavior, endocrine secretion, cardiovascular function and appetite, are known to exert its actions via a multiplicity of binding sites. These sites have been classified as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1c}, 5-HT_{1D}, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 receptors (Millan et al., 1992, Ruat et al., 1993). Most of these receptors belong to the G protein-coupled receptor family, and their mechanism of action involves modulating levels of second messengers such as cAMP, IP_3, and Ca^{+2}. Among them, 5-HT_{1A} receptor-mediated inhibition of adenylyl cyclase was sensitive to pertussis toxin (PTX) treatment, thus implicating a direct coupling of 5-HT_{1A} receptors to the G_i protein. Moreover, electrophysiological studies have demonstrated that the 5-HT_{1A} receptor mediates stimulation of K^+ conductances in various cell types including neurons (Andrade et al., 1987; Andrade and Nicoll, 1987a; Andrade and Nicoll, 1987b; Colino et al., 1987). Similarly, many neurons have PTX-senstitive GIRKs, and these channels are activated either by m_2 muscarinic, D_2 dopamine, histamine, opioid, somatostatin, and α_2 adrenergic receptors to produce inhibitory postsynaptic potential (North, 1989). The physiological relevance of the coupling of the 5-HT_{1A} receptor to various transduction pathways in the brain (i.e. adenylyl

* To whom correspondence should be addressed.
Tel: 82-331-219-2621, Fax: 82-331-219-1615
E-mail: minc@madang.ajou.ac.kr

cyclase stimulation, PI turnover, Ca^{2+} conductance, *etc*) remains, however, to be elucidated (Claustre et al., 1988; Mestikaway et al., 1991) although GIRK is known to decrease membrane excitability by hyperpolarizing the membrane potential, slowing membrane depolarization, and shortening the action potential.

To understand the function of GIRK1, we have coexpressed GIRK1 and the human 5-HT_{1A} receptor in *Xenopus* oocytes, and ionic conductance of GIRK1 was elicited by the activation of the 5-HT_{1A} receptor via endogenous oocyte G proteins. The properties of GIRK1 were examined in many respects such as voltage-current relationship of conductances, PTX sensitivity, ion selectivity and blockade by Ba^{2+}, and 5-HT dose-response relations. Our results indicated that the coupling between the 5-HT_{1A} receptor and GIRK1 is mediated by the PTX-sensitive G protein (G_i or G_o type) and that the main ion carrier for GIRK1 is K^+ and thus Ba^{2+}-sensitive.

Materials and Methods

In vitro synthesis of cRNAs

Rat GIRK1 cDNA (Dascal et al., 1993) and human 5-HT_{1A} receptor cDNA (Kobilka et al., 1987) were previously cloned and kindly provided by Dr. Lester at Caltech. The synthesis of cRNA was conducted using an Ambion *in vitro* transcription kit with T_7 RNA polymerase (Ambion, USA).

cRNA injection and electrophysiology

Female *Xenopus laevis* frogs were anesthetized by immersion in 0.2% MS-222 (Sigma) and stage V or VI oocytes were surgically removed. Isolation of oocytes and cRNA injection were carried out as described elsewhere (Quick and Lester, 1994). An oocyte was co-injected with 1-10 ng of GIRK1 cRNA and 5-15 ng of 5-HT_{1A} receptor cRNA, and maintained at 20℃ in an incubation medium of $ND96^{++}$ (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 1.8 mM $CaCl_2$, 2.5 mM Na-pyruvate, 50 μg/ml gentamicin, pH 7.4-7.6). Electrophysiological recordings were performed 2.5-3 d of postinjection using the two electrode voltage clamp configuration (Smith et al., 1980) with a Geneclamp 500 amplifier (Axon Instruments) interfaced to an IBM PC via Digidata 1200 (Axon Instrument). While the current signals were continuously recorded on a strip chart recorder, stimulation, data acquisition, and data analysis were performed using the pCLAMP6.0 (Axon Instruments) and Origin4.0 (Microcal Software Inc.). Currents of 5-HT dose-response were fitted to the logistical equation:

$$\frac{(I_{max} - I_{min})}{1+([5\text{-HT}]/EC_{50})^P} + I_{min} = I_{5HT}$$

where I_{max} represents the inward current evoked by the highest concentration of 5-HT, I_{min} represents the inward current in the absence of 5-HT, EC_{50} represents the concentration of 5-HT at which the size of I_{5HT} is a half of $I_{max} - I_{min}$, and P represents the Hill coefficient.

An oocyte coexpressing the 5-HT_{1A} receptor and GIRK1 was placed in a recording chamber consistently perfused either with Ca^{+2}-free ND96 or Ik (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4-7.6) or high K^+ (hk) solution that was identical to ND96 except that NaCl was replaced with 96 mM KCl, and then switched to various drug-solved hk solutions (test solutions). Current-voltage (*I-V*) characteristics were obtained by a voltage ramp protocol (between -80 mV and either +40 or +60 mV). All experiments were performed at room temperature.

Drugs

5-HT and PTX were from Sigma or GibcoBRL. 1-10 mM stocks of 5-HT were made up in H_2O and stored as frozen aliquots at -20℃ until use. The stock solution of PTX was made up in a concentrated stock solution of 1.0 μg/μl in H_2O and then diluted to the concentrations indicated before use with the high K^+ recording solution. All other chemicals were from Sigma.

Results

In voltage clamped *Xenopus* oocytes coexpressing GIRK1 and the 5-HT_{1A} receptor, 1 μM of 5-HT activated an inward current (I_{5HT}) in the presence of high K^+ (96 mM). Inward currents were measured in hk solution because in 96 mM of external K^+, the K^+ equilibrium potential (E_K) is close to 0 mV, and this causes the K^+ current to flow inwardly. As shown in Fig. 1, changing the ND96 solution to the hk solution was accompanied by the development of an inward current (I_{hk}) reflecting basal activity of inward rectifier K^+ channels and other K^+ channels within 0.5-1.0 min. Application of 5-HT in the hk solution evoked a secondary inward current (I_{5HT}) within 10 sec. In the continued presence of 5-HT, the I_{5HT} decreased slowly reflecting a desensitization process, and the removal of 5-HT and changing to ND96 returned the current to the basal level. At -80 mV of the holding potential, the average size of I_{hk} and I_{5HT} were -150 nA and -600 nA, respectively (n=5). In a control oocyte in which neither cRNA was injected, the hk solution evoked the I_{hk} of reduced size (-50 nA), but no I_{5HT} was apparent in the presence of 5-HT (Fig. 1 inset).

Fig. 2(A) showed a typical current trace of GIRK1 in the presence of increasing concentrations of 5-HT at -80 mV. The activation of GIRK1 (both I_{hk} and I_{5HT}) was conducted by application of 5-HT in the high K^+ solution which was varied from 0.032 nM to 100 nM. In order to minimize the desensitization of the 5-HT_{1A} receptor due to repeated application of 5-HT, the

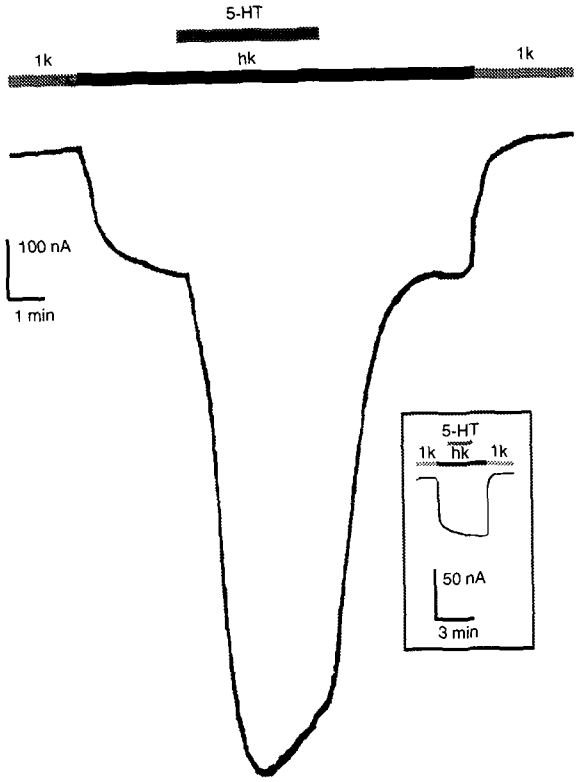

Fig. 1. Inward currents evoked in a *Xenopus* oocyte coexpressing the 5-HT_{1A} receptor and GIRK1 by the 5-HT_{1A} receptor agonist 5-HT. The holding potential was -80 mV. The oocyte was first perfused with ND96 (or Ik with the K^+ concentration of 2 mM), and then switched to 96 mM K^+-containing hk solution, which evoked a small inward current (I_{hk}, -150 nA). 5-HT (1 μM) application evoked a larger inward current (I_{5HT}, -600 nA) which decreased slightly in the continued presence of 5-HT due to the 5-HT_{1A} receptor desensitization. Switching back to Ik solution returned the current to the the basal level.

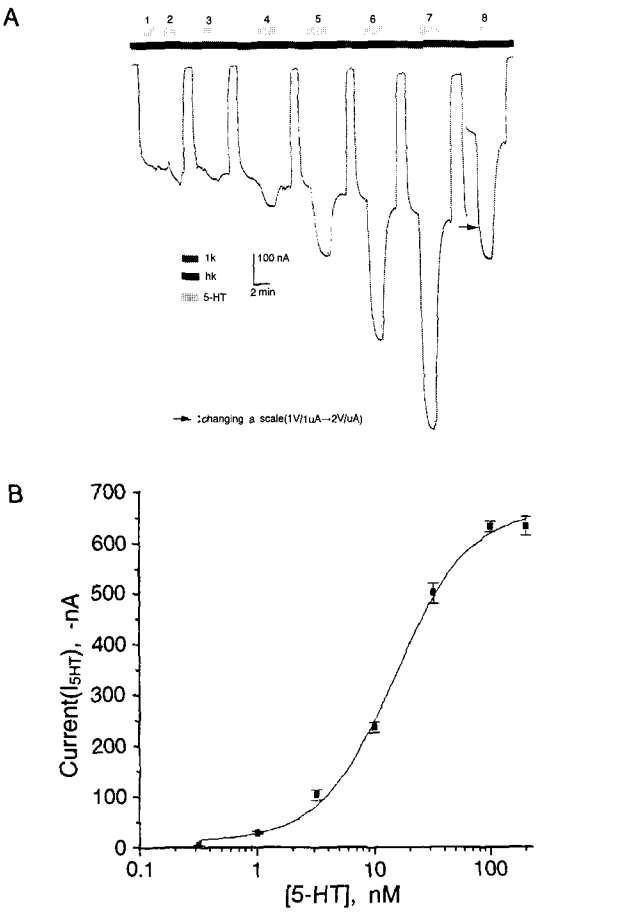

Fig. 2. The dose-response relationship of 5-HT-evoked K^+ conductance (I_{5HT}) for an oocyte coexpressing the 5-HT_{1A} receptor and GIRK1. A, Voltage clamp currents at a holding potential of -80 mV were traced in the presence of increasing concentrations of 5-HT (1: 0.032 nM; 2: 0.1 nM; 3: 0.32 nM; 4: 1.0 nM; 5: 3.2 nM; 6: 10 nM; 7: 32 nM; 8: 100 nM). 5-HT was bath-applied and intervaled by a wash out with hk and Ik solution. B, 5-HT dose-response relations from 3-5 separate oocytes. The solid curve is a least-squares fit of the data points to the logistical equation: $(I_{max} - I_{min})/\{1 + ([5\text{-HT}]/EC_{50})^P\} + I_{min} = I_{5HT}$, where the EC_{50} was calculated to be 10.7 nM with P of 0.92.

interval of the washout between consecutive 5-HT applications was 3-5 min. However, at higher concentrations (200 nM or 320 nM 5-HT) 5-HT-induced desensitization persisted even after 5 min of wash out (data not shown). The amplitude of I_{5HT} was plotted as a function of the 5-HT concentration in Fig. 2B. The solid line was computer-generated according to the logistical equation with EC_{50} of 10.7 nM and P of 0.92 (see materials and methods). I_{5HT} desensitization during the cumulative dose-response experiment may distort the curve at high concentrations of 5-HT, but the EC_{50} of 10.7 nM is relevant to the high affinity binding site of the 5-HT_{1A} receptor previously described (Frazer et al., 1990). I_{5HT} seemed to be saturated at about 100 nM 5-HT.

Previous studies showed that in *Xenopus* oocytes, I_{5HT} is dependent on the receptor-activated G protein and the coupling between the $5HT_{1A}$ receptor and GIRK1 is mediated by an endogenous G protein as revealed by the sensitivity to guanosine 5′-[β-thio] triphosphates ($GTP_\gamma S$, Dascal et al., 1993; Kubo et al., 1993). Thus, PTX treatment (1.0-3.0 μg/ml for 24-48 hr incubation as described elsewhere (Quick et al., 1994)) was performed to investigate which G protein(s) are involved in the coupling. PTX sensitivity is one of the ways to distinguish G proteins involved in a cellular signalling pathway. G_i or G_o type G protein is ADP-ribosylated, thus inactivated by PTX (Taylor et al., 1991). The PTX treatment reduced the I_{5HT} to about 10-70%, depending upon the batch of oocytes (Fig. 3). Thus it seemed that PTX-sensitive G proteins are involved in the coupling of the 5-HT_{1A} receptor to GIRK1 in *Xenopus* oocytes.

The properties of the I_{5HT} were further examined using a voltage ramp from -80 mV to +40 mV so that varying membrane potentials were evoked in the presence of increasing external K^+. The voltage-current (*I-V*) characteristics of the I_{5HT} in different external K^+ concentrations showed (1) a strong inward rectification and (2) a reversal potential (V_{rev}) near the predicted E_K of ~0 mV at 96 mM of the external K^+ (Fig. 4A). The lack of significant outward I_{5HT} may be attributed to

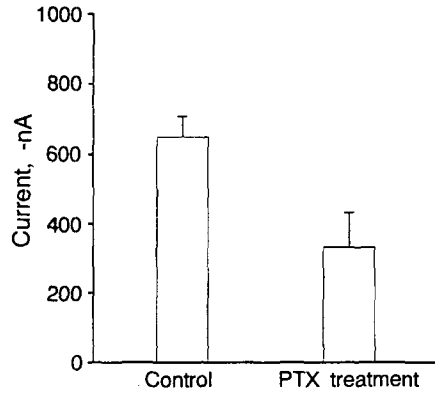

Fig. 3. The effect of PTX treatment on I_{5HT} in oocytes coexpressing the 5-HT_{1A} receptor and GIRK1. Approximately 1 d after the cRNA injection, oocytes were placed in a medium containing 1-3 μg/ml PTX for 24-48 hr before measurement. The data are mean ± SEM for 3-5 separate oocytes.

high internal levels of Mg^{2+} that blocks outward currents of the inwardly rectifying K^+ (K_{ir} currents) (Kubo et al., 1993). The reversal potential of the I_{5HT} shifted by about 44 mV per 10-fold change in external K^+ concentrations (Fig. 4B), suggesting a selectivity for K^+. The predicted value for the reversal potential shift from the Nernst equation is 58 mV if K^+ is the only charge carrier. This discrepancy indicates that other ions are also permeable through GIRK1, most likely Na^+. Block by external Ba^{2+} is one of the characteristics of K^+ channels including the inward rectifier (Hille, 1992a). Both I_{5HT} and I_{hk} were reduced by Ba^{2+} in a concentration-dependent manner (Fig. 5). However, the extent to which Ba^{2+} blocks the I_{hk} or the I_{5HT} was different. The I_{5HT} was more sensitive to the Ba^{2+} blockade than the I_{hk}. Ba^{2+} at 100 μM blocked ~50% of the I_{hk}, but blocked more than 90% of the I_{5HT}. The IC_{50} value for the Ba^{2+} block of the I_{5HT} was ~13 μM, whereas the IC_{50} value for the I_{hk} block was ~90 μM. This may be due to the fact that GIRK1 is capable of being in two different conformations, one with less K^+ selectivity and less voltage-dependence in gating (I_{hk}) and the other with more K^+ selectivity and voltage and/or G protein-dependence in gating (I_{5HT}). The former is described as the basal activity of GIRK1 since it is evoked even without the coexpressed 5-HT_{1A} receptor.

Discussion

We have successfully expressed GIRK1 in *Xenopus* oocytes. The activation of GIRK1 is mediated by the coexpressed 7-helix receptor, the 5-HT_{1A} receptor. Therefore, a functional coupling between the GIRK1 and 5-HT_{1A} receptor via activated G proteins is established. 5-HT_{1A} receptor agonist 5-HT evoked K_{ir} through GIRK1. K_{ir} is composed of two components: the I_{hk} component is evoked mainly due to the net inward driving force generated by the high external K^+; the I_{5HT} component is evoked upon applying 5-HT, and

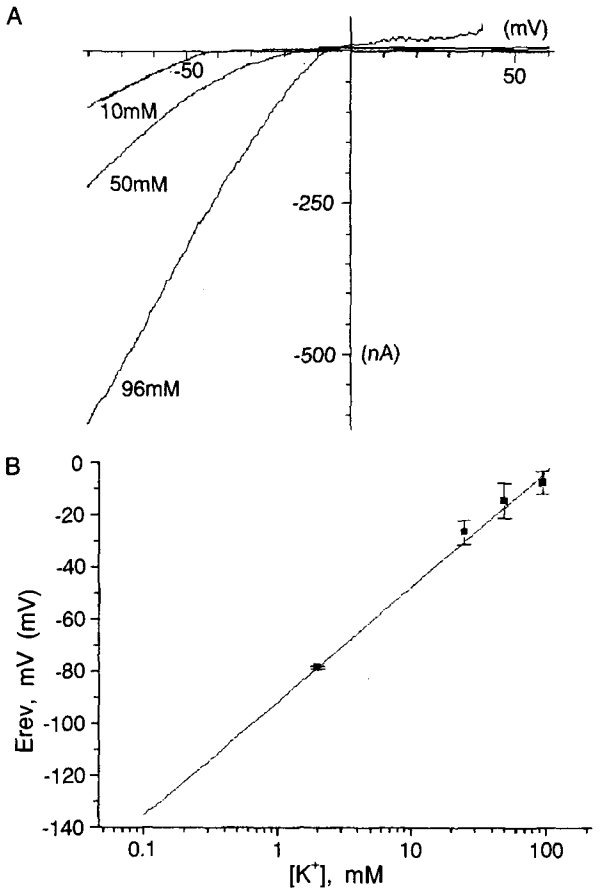

Fig. 4. Current-voltage (I-V) relations of I_{5HT} in the presence of various external K^+ concentrations. A, The membrane potential of an oocyte was ramped from -80 mV to +40 or +60 mV, and the I_{5HT} were evoked by 1.0 μM 5-HT in either 10 mM, 50 mM, or 96 mM K^+-containing solution. B, Dependance of the reversal potential (E_{rev}) on the external K^+ ion concentration. The E_{rev} for I_{5HT} was obtained from the interception to the X-axis in Fig. 4(A). The data points were mean ± SEM for one to three oocytes, and the solid line was a least-squares fit of the data points to a straight line. E_{rev} (44 mV) agreed well with the predicted E_K values calculated from the Nernst equation assuming an intracellular K^+ concentration of 90 mM.

thus this component is the 5-HT_{1A} receptor-activated component. The I_{hk} is the basal activity with or without the 5-HT_{1A} receptor whereas the I_{5HT} is evoked upon activation of the coexpressed 5-HT_{1A} receptor. The 7-helix receptors known to be coupled to GIRK1 includes the muscarinic m_2 receptor (Doupnik et al., 1995b), β_2-adrenergic receptor (Lim et al., 1995), and the $GABA_B$ receptor (Sleginger et al., 1997). In this study, the 5-HT_{1A} receptor was shown to be coupled to the GIRK1. Activation of GIRK1 induces an inward K^+ current, and therefore this channel may regulate the firing rate, membrane potential, and neurotransmitter responses. Therefore GIRK1 is an example where multiple arrays of 7-helix receptors exert their effects convergently. The finding that GIRK1 displays basal activity (I_{hk}) with or without the coexpressed 7-helix 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

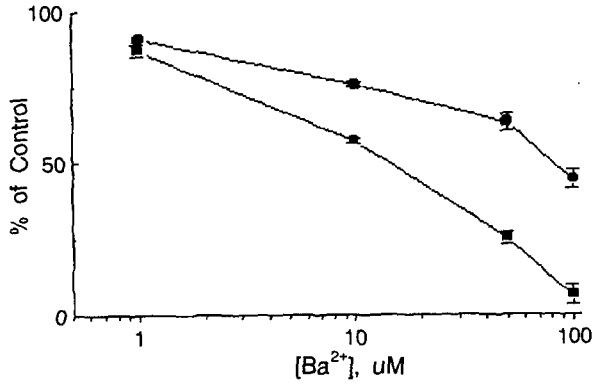

Fig. 5. Ba^{2+} *inhibition of the GIRK1 current:* (I_{hk})(●) and I_{5HT} (■). An oocyte was voltage-clamped at -80 mV, and I_{hk} and I_{5HT} were evoked in the presence of 96 mM K^+ and 96 mM + 5-HT, respectively. Ba^{2+}-containing hk or hk + 5-HT was then perfused to block the currents. Normalized values against the size of the current in the absence of Ba^{2+} were plotted against Ba^{2+} concentrations. The data are mean ± SEM (n=4-6).

activated receptor (Hille, 1992b).

The results of our study is consistent with the idea that the gating of GIRK1 results from G protein-channel coupling, rather than second messenger-mediated processes such as phosphorylation. The data that supports this conclusion is as follows. First, the time delay between the 5-HT application and the evoking of I_{5HT} is within 10 sec, which is more consistent with the direct contact of the G protein with GIRK1. In *Xenopus* oocytes, a second messenger-mediated channel opening (CFTR coupled to the β_2-adrenergic receptor for example) occurred in the time range of minutes (Uezono et al., 1993). Second, the 5-HT_{1A} receptor has been known to be negatively coupled to adenylyl cyclase activity, i.e. the receptor activation leads to cAMP reduction (Yocca et al., 1992). Decreasing the intracellular cAMP concentration by 5-HT application may not activate an ion channel unless the lack of cAMP is a mediator for channel gating. Additional evidence for the conclusion described above is the fact that many 7-helix receptors, either adenylyl cyclase-coupled or phospholipase-coupled, are capable of gating GIRK1.

The easiest way to distinguish G protein species that are involved in the G protein-GIRK1 coupling is to measure the toxin-sensitivity. PTX inactivates the G_o/G_i type G protein by ADP-ribosylation of G_α. Our results indicated that the PTX-sensitive G_α subunit was involved in the 5-HT_{1A} receptor-GIRK1 coupling since the I_{5HT} was reduced by as much as 70% at 1-3 μg/ml of PTX. GIRK1 activation via m_2-muscarinic receptors was also PTX-sensitive (Dascal et al., 1993). However, it is worth pointing out here that a recent finding indicates that the $G_{\beta\gamma}$ dimer, rather than the G_α subunit, appears to be responsible for the GIRK1-7-helix receptor coupling (Wickman et al., 1994). This could clarify why the K_{ir} currents are able to be elicited by a variety of 7-helix receptors such as the m_2 muscarinic receptor, β_2-adrenergic receptor, and $GABA_B$ receptor in various tissues.

We also described the voltage and K^+ dependence of GIRK1. As in native atrial myocytes, GIRK1 showed a strong inward rectification, and from the K^+ substitution and the Ba^{2+} inhibition experiments, it was clear that GIRK1 had higher selectivity for K^+ over Na^+. It is of interest to note that the GIRK1 current has two components, I_{hk} and I_{5HT}. The I_{hk} is evoked by the high K^+ driving force, thus called the basal activity of GIRK1 whereas the I_{5HT} is evoked by the activation of the 5-HT_{1A} receptor. Further, the I_{5HT} was more sensitive to the Ba^{+2} blockade than the I_{hk}. The discrepancy between the extent of blocking of two components of GIRK1 by Ba^{2+} deserves some comment. Even in uninjected control oocytes, the I_{hk} is induced with the size of one third of that from GIRK1 cRNA-injected oocytes (see Fig. 1), reflecting that the I_{hk} of GIRK1 is slightly contaminated by nonspecific K^+ currents. However, it is highly likely that the I_{hk} and the I_{5HT} represent two different conformations or states of GIRK1. By assuming that Ba^{+2} behaves only as a channel blocker, it would be reasonable to predict that they have different Ba^{+2} affinities.

Acknowledgements

We thank H-S Kang and Y-S Yoon for the preparation of oocytes. This work was supported by the 1995 genetic engineering research granted by MOE to Churl K. Min.

References

Andrade R, Matenka RC, and Nicoll R (1987) A G protein couples serotonin and $GABA_B$ receptor to the same channels in the hippocampus. *Science* 234: 1261-1265.

Andrade R and Nicoll R (1987a) Novel anxiolytics discriminate between postsynaptic serotonin receptors of the rat hippocampus. *Naunyn-Schmiedeberg Arch Pharmacol* 336: 5-10.

Andrade R and Nicoll, R (1987b) Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded *in vitro*. *J Physiol* 349: 99- 125.

Ashford ML, Bond CT, Blair TA, and Adelman JP (1994) Cloning and functional expression of a rat heart K_{ATP} channel. *Nature* 348: 125-313.

Claustre Y, Bénavieds J, and Scatton B (1988) 5-HT_{1A} receptors agonists inhibit carbarchol-induced stimulation of phosphoinositide turnover in the rat hippocampus. *Eur J Pharmacol* 149: 149-153.

Colino A and Halliwell JV (1987) Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* 328: 73-77.

Dascal N, Schreibmayer W, Lim NF, Wang WZ, Chavkin C, Dimagno L, Labarca C, Kiehher BL, Gaveriauxruff C, Trollinger D, Lester HA, and Davidson N (1993) Atrial G protein-activated K^+ channel: expression cloning and molecular properties. *Proc Natl Acad Sci USA* 90: 10235-10239.

De Paoli AM, Bell GI, and Stoffel M (1994) G protein-activated inwardly rectifying potassium channel (GIRK1/KGA) mRNA in adult rat heart and brain by *in situ* hybridization histochemistry. *Mol Cell Neurosci* 5: 515-522.

Doupnik CA, Davidson N, and Lester HA (1995a) The inward rectifier potassium channel family. *Curr Opin Neurobiol* 5: 268-277.

Doupnik CA, Lim NF, Kofuji P, Davidson N, and Lester HA

(1995b) Intrinsic gating properties of a cloned G protein-activated inward rectifier K^+ channel. *J Gen Physiol* 106: 1-23.

Frazer A, Maayami S, and Wolfe BB (1990) Subtypes of receptors for serotonin. *Annu Rev Pharmacol Toxicol* 30: 307-348.

Hille B (1992a) Ionic Channels of Excitable Membranes, 2nd ed. Sinauer, New York.

Hille B (1992b) G protein-coupled mechanisms and nervous signaling. *Neuron* 9: 187-195

Karschin C, Schreibmayer W, Dascal N, Lester H, Davidson N, and Karschin A (1994) Distribution and localization of a G protein-coupled inwardly rectifying K^+ channel in the rat. *FEBS Lett* 348: 139-144.

Kobayashi T, Ikeda K, Ichikawa T, Abe S, Tagashi S, and Kumanishi T (1995) Molecular cloning of a mouse G-protein-activated K^+ channel (mGIRK1) and distinct distributions of three GIRK (GIRK1, 2, and 3) mRNAs in mouse brain. *Biochem Biophys Res Commun* 208: 1166-1173.

Kobilka BK, Frielle T, Collins S, Yang-Feng T, Kobilka TS, Francke U, Lefkowitz RJ, and Caron MG (1987) An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature* 329: 75-79.

Kubo Y, Reuveny E, Slesinger PA, Jan YN, and Jan LY (1993) Primary structure and functional expression of a rat G-protein coupled muscarinic potassium channel. *Nature* 364: 802-806.

Lim NF, Dascal N, Labarca C, Davidson N, and Lester HA (1995) A G protein-gated K^+ channel is activated via β_2-adrenergic receptors and $G_{\beta\gamma}$ subunits in *Xenopus* oocytes. *J Gen Physiol* 105: 421-439.

Mestikawy S, Fargin A, Raymond JR, Gozlan H, and Hnatowich M (1991) The 5-HT_{1A} receptor: an overview of recent advances. *Neurochem Res* 16: 1-10.

Millan MJ, Rivert JM, Canton H, Lejeune F, Bervoets K, Brocco M, Gobert A, Lefebvere De Ladonchamps B, Le Marouille-girardon S, Verriele L, Laubie M, and Lavielle M (1992) S14671: a naphtylpiperazine 5-hydroxytryptamine$_{1A}$ agonist of execeptional potency and high efficacy possessing antagonist activity at 5-$HT_{C/2}$ receptors. *J Pharmacol Exp Therap* 262: 451-467.

Nicoll RA, Malenka RC, and Kauer JA (1990) Functional comparision of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev* 70: 513-363.

North RA (1989) Drug receptors and the inhibition of nerve cells. *Br J Pharmacol* 98: 13-28.

Quick MW, Simon MI, Davidson N, Lester HA, and Aragay AM (1994) Receptors expressed in *Xenopus* oocytes. *J Biol Chem* 269: 30164-30172.

Quick MW and Lester HA (1994) Methods for expression of excitability proteins in *Xenopus* oocytes. In: Narahashi T (ed), Ion Channels of Excitable Cells, Academic Press, San Diego, pp 261-279.

Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang J-M, and Schwartz J-C (1993) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation. *Proc Natl Acad Sci USA* 90: 8547-8551.

Sleginger PA, Stoffel M, Jan YN, and Jan LY (1997) Defective γ-aminobutyric acid type B receptor-activated inwardly rectifying K^+ currents in cerebellar granule cells isolated from weaver and GIRK2 null mutant mice. *Proc Natl Acad Sci USA* 94: 12210-12217.

Smith TG, Barker JL, Smith BM, and Colburn TL (1980) Voltage clamping with microelectrodes. *J Neurosci Meth* 3: 105-128.

Taylor SJ, Chae HZ, Rhee SG, and Exton JH (1991) Activation of the β1 isozyme of phospholipase C by α subunits of the Gq class of G proteins. *Nature* 350: 516-518.

Uezono Y, Bradley J, Min C, McCarty NA, Quick M, Riordan JR, Chavkin C, Zinn K, Lester HA, and Davidson N (1993) Receptors that couple to 2 classes of G proetins increase cAMP and activate CFTR expressed in *Xenopus* oocytes. *Recep Chann* 1: 233-241.

Wickman KD, Inlguez-Lluhl JA, Davenport PA, Taussig R, Krapivinsky GB, Linder ME, Gilman AG, and Clapham DE (1994) Recombinant G-protein $\beta\gamma$-subunits activate the muscarinic-gated atrial potassium channel. *Nature* 368: 255-257.

Yocca FD, Iben L, and Meller E (1992) Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine$_{1A}$ receptors negatively coupled to adenylyl cyclase activity in rat hippocampal membranes. *J Mol Pharmacol* 41: 1066-1072.

[Received August 3, 1998; accepted August 28, 1998]