Metabotropic Glutamate Receptors Activate G-Protein-Coupled Inwardly Rectifying Potassium Channels in *Xenopus* Oocytes

Julie A. Saugstad,1 Thomas P. Segerson,1,2 and Gary L. Westbrook1,3

1Vollum Institute for Advanced Biomedical Research, and Departments of 2Medicine and 3Neurology, Oregon Health Sciences University, Portland, Oregon 97201

Receptor-mediated activation of a G-protein-coupled inwardly rectifying potassium channel (GIRK) is a common mechanism for synaptic modulation in the CNS. However, evidence for metabotropic glutamate receptor (mGluR) activation of GIRK is virtually nonexistent, despite the widespread and overlapping distribution of these proteins. We examined this apparent paradox by coexpressing mGluRs 1a, 2, and 7 with the GIRK subunits Kir3.1 and Kir3.4 in *Xenopus* oocytes. Functional expression of GIRK was confirmed by coexpression with the D2 dopamine receptor that is known to activate GIRK in neurons. Agonist activation of each of the three mGluRs evoked inward potassium currents in symmetrical KCl solutions. The current amplitudes evoked by mGluR1a, mGluR2, and D2 were comparable, whereas mGluR7 currents were somewhat smaller. mGluR1a-evoked GIRK currents were not blocked in BAPTA-treated oocytes, demonstrating that GIRK activation was distinct from phospholipase C-mediated activation of the endogenous calcium-dependent chloride current (*I*_\(_{\text{CaCl}}\)). Pertussis toxin (PTX) treatment significantly reduced both the mGluR and D2 receptor-evoked GIRK currents. In oocytes in which mGluR2 and D2 were coexpressed, activation of mGluR2 occurred additional D2 receptor current, indicating that mGluR2 and D2 receptor coupling to GIRK involves a common G-protein. The efficient coupling of mGluRs to GIRK in oocytes suggests either that mGluR activation of GIRK has been overlooked in neurons or possibly that mGluRs are excluded from GIRK-containing microdomains.

Key words: metabotropic glutamate receptor; G-protein-coupled inwardly rectifying potassium channel; synaptic modulation; *Xenopus* oocyte; microdomains; glutamate receptors; synaptic inhibition

Metabotropic glutamate receptors (mGluRs) regulate neuronal excitability in the CNS by modulating several classes of ion channels, including voltage-dependent potassium channels, voltage-dependent calcium channels, nonselective cation channels, and ligand-gated ion channels (for review, see Saugstad et al., 1995a). The mGluR family consists of eight receptors that are subdivided into three groups on the basis of amino acid homology, agonist profile, and signal transduction pathway. Group I includes mGluRs 1 and 5, group II includes mGluRs 2 and 3, and group III includes mGluRs 4, 6, 7, and 8 (Pin and Duvoisin, 1995). Immunohistochemical studies have localized mGluRs to pre- and postsynaptic sites, and many neurons express multiple subtypes (Martin et al., 1992; Baude et al., 1993; Nomura et al., 1994; Ohishi et al., 1994; Romano et al., 1995; Bradley et al., 1996). Thus, the compartmentalization of receptors and channels as well as the signal transduction pathway may determine the effect of mGluR activation on synaptic transmission. The specific matching of mGluR subtypes with effectors under physiological conditions is still far from clear. For example, activation of each of the three subgroups of mGluRs can inhibit transmitter release (Pin and Bockaert, 1995). In the presynaptic terminal, multiple effectors seem to be involved, including voltage-gated calcium channels (Lester and Jahr, 1990; Swartz and Bean, 1992; Trombley and Westbrook, 1992; Sahara and Westbrook, 1993; Swartz et al., 1993; Rothe et al., 1994) as well as calcium-independent (cadmium-insensitive) mechanisms (Gereau and Conn, 1995; Scanziani et al., 1995; Schoppa, personal communication).

Many G-protein-coupled receptors also can activate potassium channels (for review, see Clapham, 1994). In many cases, these receptors mediate postsynaptic inhibition, but opening of potassium channels may also be a mechanism of presynaptic inhibition. A G-protein-coupled inwardly rectifying potassium channel (GIRK) subunit targeted by G-protein-coupled receptors recently has been cloned (Dascal et al., 1993a; Kubo et al., 1993). Although the physiologically identified actions of GIRK have been dendritic, the channels are localized by immunohistochemistry to nerve terminals as well as to dendrites (Ponce et al., 1996). The above evidence would seem to suggest that mGluRs should activate GIRK. However, despite extensive pharmacological studies of glutamate receptors (Mayer and Westbrook, 1987), the only reports of direct glutamate activation of potassium conductances are from retinal bipolar cells (Kondo and Toyoda, 1980; Navv and Copenhagen, 1990; Hirano and McLeish, 1991) and molluscs (Bolshakov et al., 1993).

To determine whether mGluRs can activate inwardly rectifying potassium channels, we coexpressed two inward rectifiers (Kir3.1 and Kir3.4; for review, see Doupnik et al., 1995) with mGluRs and the D2 dopamine receptor in *Xenopus* oocytes. Our results demonstrate that representative members of each mGluR group can activate GIRK in a manner similar to a known activator of GIRK in neurons, the D2 receptor (Lacey et al., 1987; Lledo et al., 1992). These results imply that mGluR–GIRK interactions may have
been overlooked. Alternatively, mGluRs or GIRK may be targeted to separate membrane compartments.

**MATERIALS AND METHODS**

*Reagents and cDNA clones.* Pharmacological reagents were purchased from Sigma (glutamate, DL-AP4, dopamine; St. Louis, MO), Tocris Cookson (L-AP4; Bristol, UK), List (PTX; Campbell, CA) and Molecular Probes (BAPTA-AM; Eugene, OR). Glutamate and AP4 were prepared as 100 mM stocks in equivalent NaOH and were stored frozen. Dopamine was prepared fresh in distilled water as needed. RNA transcription kits were purchased from Stratagene (La Jolla, CA) and RNA polymerases from Life Technologies (Gaithersburg, MD). The cDNA clones were generously shared by the following investigators: rat mGluR1a, Dr. E. Mulvihill (Zymogenetics, Seattle, WA); rat mGluR2, Dr. S. Nakanishi (Kyoto University, Japan); rat Kir3.1, Dr. H. Lester (California Institute of Technology, Pasadena, CA); Kir3.4, Dr. J. Adelman (Vollum Institute, Portland, OR); human D2 (prD2 short), Dr. D. Grandy (Vollum Institute, Portland, OR); rat 5HT2, Dr. P. Seeburg (ZMBH, University of Heidelberg, Germany). The rat mGluR7 and mGluR5 cDNAs were isolated as described (Saugstad et al., 1994), and cRNAs were prepared by standard in *vitro* transcription reactions as described (Melton et al., 1984).

Expression of cRNAs in Xenopus oocytes. Stage V–VI oocytes were harvested from mature anesthetized *Xenopus laevis* (Xenopus One, Ann Arbor, MI) and enzymatically defolliculated as described previously (Dascal et al., 1986; Saugstad et al., 1995b). Oocytes were maintained at 14°C in ND96 containing (in mM): 96 NaCl, 2 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, and 5 HEPES, pH 7.5, supplemented with 2.5 mM Na pyruvate, 0.5 mM theophylline (Sigma), and 50 μM gentamycin (Life Technologies). Oocytes were incubated in 500 ng/ml PTX in ND96 solution for 48 hr before recording. For the BAPTA experiments, the oocytes were incubated in 0.5 mM BAPTA-AM in Ca$^{2+}$-free OR2 containing (in mM): 82.5 NaCl, 2 KCl, 1 MgCl$_2$, and 5 HEPES for 30 min before recording.

*Electrophysiology.* Electrophysiological recordings were performed 24–72 hr after injection. Patch pipettes with tip diameters of ~1.2 μm were used as electrodes and filled with 3 M KCl. Oocytes were voltage-clamped at ~80 mV in two-electrode voltage-clamp mode (Axoclamp 2A, Axon Instruments, Foster City, CA). Currents were filtered at 10 kHz and acquired at 1.6 Hz with MacLab 3.3 (AD Instruments, Milford, MA). The oocytes were placed in a 2 ml chamber and bathed continuously in ND96 at ~35 mV. Solutions were changed by using a solenoid valve controller; the exchange time was 15–30 sec. The protocol for recording of GIRK currents was as follows. Oocytes were bathed initially with ND96 and then switched to a high potassium solution (hK) containing (in mM): 96 KCl, 2 NaCl, 1.8 CaCl$_2$, 1 MgCl$_2$, and 5 HEPES, pH 7.5, to determine the amplitude of the basal potassium current. When the basal current had reached equilibrium, agonists diluted in hK were applied. The hK-induced current was subtracted from the total current to obtain the agonist-induced current. For comparison of GIRK and endogenous calcium-dependent chloride ($I_{C_{AC}}$) current amplitudes, $I_{C_{AC}}$ was measured in ND96 at $V_h$ = –60 mV. Current amplitudes were measured off-line and analyzed with Student’s t test, as appropriate. Current amplitudes are expressed as mean ± SE; a p value of <0.05 was considered significant unless otherwise indicated.

**RESULTS**

**mGluR2 activates GIRK in oocytes**

Because GIRK activation results in very small outward currents in normal extracellular solution, we reversed the driving force for potassium by raising extracellular K$^+$ to 96 mM (hK solution). In oocytes expressing GIRK, hK produced a basal inward current at a holding potential of ~80 mV, representing the tonic level of GIRK activation (Dascal et al., 1993b). With injection of 50 ng of GIRK (Kir3.1 plus Kir3.4), the basal inward current saturated the amplifier; thus GIRK cRNAs were diluted 1:400 for all experiments, producing basal currents of 860 ± 203 nA (n = 5). In oocytes coinjected with mGluR2 and GIRK cRNAs, an additional agonist-induced inward current was evoked by 100 μM glutamate (998 ± 68 nA, n = 16; Fig. 1). We injected oocytes with D2 and GIRK cRNAs to compare the activation of mGluRs in the oocyte expression system with a receptor known to activate GIRK in neurons and oocytes (Lacey et al., 1987; Seabrook et al., 1995). In these oocytes, dopamine (1 μM) evoked currents (402 ± 66 nA, n = 22). Both mGluR2- or D2-evoked currents were significantly different from the hK-induced currents (Fig. 3). During agonist applications, some desensitization was observed (Fig. 1, left), as has been reported with $G_i$ inhibition of the GIRK channel (Schreibmayer et al., 1996).

GIRK was predominantly responsible for the inward current in hK solutions because uninjected oocytes, or oocytes injected with mGluR2 cRNA in the absence of GIRK, produced only small hK currents (32 ± 6.5 nA, n = 10; 10.8 ± 5.5 nA, n = 4, respectively). Likewise, the agonist-evoked current was attributable to the activation of GIRK, because glutamate application did not evoke currents in oocytes injected either with water (1.0 ± 0.8 nA, n = 9) or mGluR2 cRNA (8.2 ± 5.1 nA, n = 4). Oocytes injected only with GIRK also had no significant glutamate-evoked current (5.2 ± 5.2 nA, n = 5).

**Group I and group III mGluRs also can activate GIRK**

To test whether Group I and III mGluRs can also couple to GIRK, oocytes were injected with GIRK cRNA in combination with mGluR1a or mGluR7 cRNAs. Unlike mGluR2, the Group I receptor mGluR1a couples to phospholipase C in oocytes, leading to activation of an endogenous $I_{C_{AC}}$ in ND96 or hK solutions. The mGluR1a-evoked $I_{C_{AC}}$ is inward at $V_h$ = –80 mV and transient and rapidly desensitizes on repeated agonist application such that full recovery requires 10–15 min (Saugstad et al., 1995b). In oocytes expressing mGluR1a and GIRK, the first application of glutamate (100 μM) evoked a transient current (1442 ± 247.6 nA, n = 8), but subsequent applications of glutamate evoked a nondesensitizing current (405 ± 86.1 nA, n = 8; Fig. 2A). The nondesensitizing...
current was attributable to activation of GIRK, because oocytes expressing mGluR1a alone evoked a large $I_{\text{CaCl}}$ on the first application of glutamate (2137 ± 470 nA, n = 3), but subsequent applications produced no additional current (0 nA, n = 3). To confirm that the mGluR1a-induced GIRK currents were distinct from $I_{\text{CaCl}}$, oocytes coexpressing mGluR1a and GIRK were incubated in 0.5 mM BAPTA-AM for 30 min before recording (Alder et al., 1992). The first application of glutamate to BAPTA-treated oocytes produced significantly smaller currents (692.0 ± 97.3 nA, n = 6), but the currents showed little desensitization, as expected, when $I_{\text{CaCl}}$ was blocked whereas GIRK was not. Subsequent applications of glutamate evoked GIRK currents of equivalent size (535.3 ± 116.6 nA, n = 6; Fig. 2A). The hK current after activation of mGluR1a was often smaller than the initial hK current. This may reflect protein kinase C modulation of GIRK channels as has been reported for IRK1 (Jones, 1996).

It was somewhat surprising that mGluR1a, which predominantly couples to $G_{\text{q/11}}$, coupled to GIRK in our experiments. Thus, we examined whether this was unique to mGluR1a and whether it reflected receptor overexpression. To determine whether mGluR1a activation of GIRK was unique, we coexpressed a $G_{\text{q/11}}$-coupled serotonin receptor (5HT2; Pritchett et al., 1988) with or without GIRK. Currents evoked by serotonin (1 μM) were similar to those evoked by mGluR1a activation with the two-pulse protocol of Figure 2A. In the absence of GIRK, the first application of serotonin (1 μM) evoked large, transient $I_{\text{CaCl}}$ currents (2255.2 ± 420.4 nA, n = 5), but a second application at a 30 sec interval produced little or no current (54.6 ± 21.3 nA, n = 5). However, in the presence of GIRK, serotonin induced large responses to the first (1996.7 ± 481.9 nA, n = 7) and second (533.9 ± 115.4 nA, n = 7) applications. The nondesensitizing second response was significant only in the GIRK-injected oocytes, indicating that other receptors that can couple to $G_{\text{q/11}}$ may also activate GIRK in oocytes.

To test whether mGluR1a activation of GIRK was attributable to receptor overexpression, we compared receptor-activated $I_{\text{CaCl}}$ and GIRK current amplitudes in sister oocytes as a function of the amount of mGluR1a cRNA injected. GIRK currents were measured in oocytes that had been preincubated with BAPTA-AM (100 μM) to avoid contamination with $I_{\text{CaCl}}$. In the presence of a constant amount of GIRK cRNA, serial dilutions of mGluR1a cRNA (1, 0.1, 0.01, and 0.001 ng/nl) resulted in parallel decreases in the amplitudes of the two responses. The response amplitudes, normalized to currents at 1 ng/nl, included the following: 1.0 ± 0.34, 0.70 ± 0.40, 0.22 ± 0.09, and 0.08 ± 0.004 for $I_{\text{CaCl}}$, and 1.0 ± 0.11, 0.83 ± 0.24, 0.37 ± 0.07, and 0.11 ± 0.02 for GIRK. Each group had three to seven oocytes. These results show that coupling to PLC and GIRK occurs at similar levels of mGluR1a expression, suggesting that GIRK coupling is not an artifact of receptor overexpression.

The Group III receptor mGluR7 is widely expressed in brain and has been suggested to be involved in presynaptic inhibition (Okamoto et al., 1994; Saugstad et al., 1994; Kinzie et al., 1995; Bradley et al., 1996). Using a saturating concentration of the Group III selective agonist DL-AP4 (2 mM), we found that no currents were present when mGluR7 was expressed alone, consistent with previous results (Saugstad et al., 1994). However in oocytes expressing mGluR7 and GIRK, application of 2 mM DL-AP4 evoked currents that were significantly larger than basal activation of GIRK (131 nA, n = 14; Fig. 2C). The results for the mGluR- and D2-evoked GIRK currents are shown in Figure 3. Basal current activations were similar for all receptor groups, suggesting that none of these receptors caused significant constitutive activation of GIRK channels. The relative amplitudes of GIRK currents varied between receptors, with mGluR7 being the least “effective,” although the levels of receptor expression potentially could account for these differences. For example, the cDNA construct used for mGluR7 cRNA has a large 5' untranslated region. When this region was deleted, the expression of mGluR7 was enhanced as assessed by GIRK currents or Western blots of mGluR7 cRNA-injected oocyte membranes probed with an mGluR7 antibody (J. A. Saugstad, unpublished observations).
Thus, our evidence suggests that members of each of the three families of mGluRs can couple to GIRK with qualitatively similar efficacy.

The coupling mechanism of mGluRs to GIRK

In atrial myocytes, coupling to GIRK seems to involve βγ subunits derived from G_i heterotrimeric (Logothetis et al., 1987; Hille, 1992). To assess which G-protein families are involved in coupling the mGluRs to the activation of GIRK, oocytes expressing mGluRs and GIRK were incubated in 500 ng/ml PTX for 48 hr before recording. The normalized current amplitudes of the untreated oocytes and the PTX-treated oocytes are shown in Figure 4. For the four receptors tested, GIRK currents were reduced significantly by PTX. The inhibition seemed to be slightly more pronounced for mGluR7 (80.8% reduction, n = 4) and mGluR2 (80.6%, n = 17) as compared with D2 (56.9%, n = 4) and mGluR1a (61.6%, n = 4). In contrast, the mGluR1a-activated I_{Ca11} mediated by G_{q/11} was not reduced significantly by PTX (n = 4) consistent with previous reports (Houamed et al., 1991; Masu et al., 1991). These results suggest that mGluRs couple to GIRK via G_βγ, consistent with the coupling mechanism of other neurotransmitter receptors in neurons that are known to activate GIRK (Lledo et al., 1992).

The coupling of mGluRs to GIRK in oocytes stands in contrast to the lack of apparent coupling in neurons. To further assess whether mGluRs stimulate the same pool of oocyte G-proteins as a known GIRK activator, we coexpressed the D2 receptor and mGluR2 along with GIRK. We chose mGluR2, because it inhibits adenylate cyclase in heterologous expression systems (Tanabe et al., 1992) presumably by coupling to G_i, and the effects of mGluR2 in neurons are also PTX-sensitive (Ikeda et al., 1995). GIRK was activated by saturating concentrations of glutamate (100 μM) or dopamine (1 μM). As shown for the oocyte in Figure 5A, the maximal current evoked by dopamine did not occlude the GIRK current evoked by a subsequent application of glutamate in the presence of dopamine (left panel). However, when glutamate was applied first (Fig. 5A, middle panel), subsequent application of dopamine produced no significant additional current (n = 7). D2 responses were evoked immediately after washout of both agonists (Fig. 5A, right panel). The peak current amplitudes in the presence of glutamate and dopamine were not significantly different in the three protocols. In contrast, dopamine application did evoke additional GIRK current after submaximal activation of mGluR2 with low glutamate concentrations (2 μM, Fig. 5B, right panel; n = 3). These results suggest either that mGluR2 and D2 use the same pool of G-proteins or that mGluR2 produces heterologous desensitization of D2 receptors. The latter seems unlikely given the fast recovery of D2 responses, the lack of kinase activation or calcium transients induced by mGluR2 in oocytes (Tanabe et al., 1992), and the lack of apparent heterologous desensitization of mGluR2 by D2. However, we cannot exclude that α subunits generated by mGluR2, but not by D2, reduce the concentration of free βγ subunits necessary for GIRK activation.

The interaction between mGluR2 and D2 responses was unaffected by changes in mGluR2 or GIRK cRNA. A 10-fold decrease in mGluR2 cRNA significantly decreased mGluR2 responses (581.4 ± 53.8 nA, n = 12 to 448.5 ± 38.9 nA, n = 6), but D2 responses were still occluded by mGluR2 activation. Likewise, a fourfold increase in GIRK cRNA significantly increased the maximal currents (429.0 ± 39.9 nA, n = 5 to 655.6 ± 42.5 nA, n = 5) but produced the same occlusion pattern as Figure 5A. These results demonstrate that the expression of mGluR2 or GIRK was not limiting, suggesting either that D2 is specifically inhibited by mGluR receptor activation or that the two receptors use a common pool of oocyte G-proteins.

**DISCUSSION**

Our results provide the first evidence that metabotropic glutamate receptors can directly activate G-protein-coupled, inwardly rectifying potassium channels. A member of each of the three groups of mGluRs activated GIRK by using a PTX-sensitive G-protein. A comparison of GIRK currents activated by the D2 receptor and mGluR2 also suggests that mGluRs can activate GIRK in a
manner similar to a known activator of GIRK in neurons. These results raise the question of whether mGluR-mediated GIRK responses in neurons have been overlooked or whether mGluRs and GIRK are directed to separate cellular compartments.

Expression of mGluRs in Xenopus oocytes

Electrophysiological studies in Xenopus oocytes have provided an efficient expression system for studies of the Group I, phospholipase C-coupled metabotropic glutamate receptors, and led to the initial cloning of mGluR1 (Houamed et al., 1991; Masu et al., 1991). However, no current is evoked when Group II or III receptor cRNA is injected into oocytes, as is characteristic of G-protein-coupled receptors that inhibit adenylyl cyclase. Our experiments provide clear evidence that members of each of the three groups of mGluRs can couple to inwardly rectifying potassium channels. The ratio of agonist-activated current to basal GIRK current seemed to be less for mGluR7 than for mGluR1a, mGluR2, and the D2 receptor. Although mGluR7 has a lower EC50 for glutamate than any other mGluR subtype when assayed in transfected cells, saturating concentrations of agonist were used for each subtype; therefore, the receptors should have been occupied fully. It remains possible that mGluR7 is less efficient in coupling to GIRK. However, a quantitative assessment of efficiency would require accurate knowledge of the number of expressed receptors. mGluR7 also may be unique, because a closely related Group III clone, rat mGluR8, robustly activates GIRK in oocytes (Saugstad, unpublished observation). Besides the implications for signal transduction, the coupling of mGluRs to GIRK in oocytes may provide a much-needed expression system for this family of receptors.

mGluR and PTX-sensitive G-protein interactions

An important issue is the extent to which the G-protein involved in mGluR coupling to GIRK in oocytes is predictive of the situation in neurons. Oocytes contain mRNA for Gαs, G11,3, and Gαo (Onate et al., 1992), and, in some instances, coupling in oocytes matches that found in neurons. For example, mGluR1a most prominently activates phospholipase C in oocytes and neurons (Sladecek et al., 1985; Houamed et al., 1991; Masu et al., 1991), presumably by coupling to Gα11 (Birnbaumer, 1992). D2 receptors couple to Gβγ in D2-expressing Ltk− cells (Bates et al., 1991). In addition, Lledo et al. (1992) showed that D2 receptors increased potassium currents through a Gi-coupled pathway but reduced calcium currents through a Gαi-coupled pathway in pituitary cells. Thus, it seems likely that D2 receptors use Gi for coupling to GIRK in oocytes. As mGluR and D2 activation of GIRK were equally PTX-sensitive, and mGluR2 occluded D2 receptor-mediated activation of GIRK, our results suggest that at least mGluR2 also couples to GIRK via Gi. Consistent with this, mGluR2 and mGluR7 inhibit forskolin-stimulated cAMP production in transfected cells, suggesting coupling to Gα (Tanabe et al., 1992; Okamoto et al., 1994; Saugstad et al., 1994).

For mGluR1a, the Gα11-mediated Ca-dependent chloride current was separated easily from the GIRK response on the basis of PTX sensitivity, sensitivity to calcium chelators, and rapid desensitization of current evoked by the Gα11 pathway. Thus, mGluR1a seems to be capable of activating GαGαα at least in oocytes. There is also evidence for mGluR1a use of G-proteins other than Gα11 from studies on transfected cells. Aramori and Nakamichi (1992) suggested that a PTX-sensitive G-protein normally mediates an inhibitory effect on cAMP accumulation that is masked by the simultaneous coupling of mGluR1a to Gs. The coupling of mGluR1a via GαGαα also does not seem to be the result of receptor overexpression, because I calcium and GIRK had similar cRNA dose–response relationships. However, other Gααα-coupled receptors also can activate GIRK in oocytes; therefore, mGluR1a is not unique in this respect.

Several groups have used antisense gene inhibition to examine the components involved in receptor-mediated responses in oocytes (Quick et al., 1994; de la Peña et al., 1995; Schreibmayer et al., 1996). We attempted to define rigorously the specific G-protein involved in GIRK coupling by injecting antisense oligonucleotides to inhibit the expression of Xenopus Gαα or Gααα. However, this approach was not interpretable in our hands, because both agonist-induced current and hK currents were reduced in parallel at higher concentrations of antisense (1.6 pm to 80 pm), suggesting that the decrease in GIRK current amplitudes was a result of antisense toxicity.

Implications for mGluR activation of GIRK in neurons

Except for presynaptic inhibition, responses attributable to mGluRs and ionotropic glutamate receptors are primarily excita-
tory. Activation of GIRK by mGluRs should cause neuronal inhibition via membrane hyperpolarization, yet the evidence for such responses is very limited. Glutamate activation of potassium channels simply could have been overlooked in previous studies because of masking by depolarizing inward currents or by potassium channel blockers. Glutamate-mediated hyperpolarizations have been observed, but these responses were attributable either to sodium/potassium ATPase activity (Ransom et al., 1975; Johnson et al., 1992) or to the activation of calcium-dependent potassium channels (Nicol and Alger, 1981; Zorumski et al., 1989; Shirasaki et al., 1994). mGluR–GIRK coupling might also be limited to presynaptic terminals that are inaccessible to direct physiological measurements. Thus, our results warrant an increased awareness of potential mGluR–GIRK coupling.

Several explanations for the lack of obvious mGluR–GIRK coupling in neurons can be excluded. Receptor activation of specific β subunits does not seem to be required, because several βγ combinations are equally effective in activating GIRK subunits (Wickman et al., 1994). Individual neurons also seem to have the necessary components. For example, CA1 hippocampal pyramidal neurons have prominent GABA_A-mediated hyperpolarizations (Andrade et al., 1986) and express several mGluRs (Pin and Duvoisin, 1995) but lack obvious mGluR-mediated GIRK responses.

A more likely possibility is that the coupling of receptors to GIRK occurs in restricted “microdomains,” e.g., by βγ subunit association with cytoskeletal proteins (Neubig, 1994) or by the lateral mobility of βγ subunits in the membrane. Such microdomains might break down in oocytes in the presence of overexpression of one or more of the components. However, the coupling of receptors to GIRK would not seem to be highly ordered, because different G-protein-coupled receptors can activate the same pool of GIRK channels in neurons (North and Williams, 1985; Andrade et al., 1986). What might be different about mGluRs? Structurally, the mGluRs are divergent from other G-protein-coupled receptors. For example, G-protein coupling involves the second intracellular loop rather than the third cytoplasmic loop, as seen in monoamine receptors (Pin et al., 1994). Thus, it seems plausible that mGluR-specific targeting or synaptic localization (Bauge et al., 1993) is also distinct from other G-protein-coupled receptors.

REFERENCES


Lacey MG, Mercure NB, North RA (1987) Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J Physiol (Lond) 392:397–416.


