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Kainate Receptors Exhibit Differential Sensitivities to (*S*)-5-Iodowillardiine

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ABSTRACT

Characterization of the role of kainate receptors in excitatory synaptic transmission has been hampered by a lack of subtype-selective pharmacological agents. (*S*)-5-Iodowillardiine (IW), an analog of willardiine [(*S*)-1-(2-amino-2-carboxyethyl)pyrimidine-2,4-dione], a heterocyclic amino acid found in *Acacia* and *Mimosa* seeds, was previously shown to be highly potent on native kainate receptors in dorsal root ganglion neurons. We examined the responses evoked by IW from recombinant homomeric and heteromeric kainate receptors expressed in human embryonic kidney 293 cells. IW potently elicited currents from glutamate receptor 5 (GluR5)-expressing cells, but showed no activity on homomeric GluR6 or GluR7 receptors. Co-expression of these receptor subunits with KA-2 subunits produced receptors that were weakly sensitive to IW. GluR5/KA-2 receptors had a higher EC_{50} value than homomeric GluR5 and exhibited a much faster recovery from desensitization. Finally, we found that the IW selectivity for GluR5 compared with GluR6 was determined by amino acid 721, which was previously shown to control α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate sensitivity of these kainate receptor subunits. The pharmacological selectivity and commercial availability of IW suggests that this compound may be of use in characterizing the molecular constituents of native kainate receptor responses.

To understand the complexity of glutamatergic synaptic transmission, it will be necessary to distinguish between different receptors within the AMPA and kainate subfamilies. Most neurons and glia express a variety of ionotropic glutamate receptor subtypes, including members of both non-NMDA glutamate receptor families. AMPA and kainate receptors respond to many of the same agonists, and there are few pharmacological tools that allow discrimination between mixed receptor populations within individual cells. Differentiation of AMPA and kainate receptor responses has been achieved using moderately selective agonists as well as potentiation of responses with selective allosteric modulators, such as cyclothiazide and concanavalin A (Partin *et al.*, 1993). Recently, elimination of AMPA receptor currents with the selective and potent noncompetitive AMPA receptor antagonist GYKI 53655 [1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine] facilitated detection and characterization of neuronal kainate receptor responses (Paternain *et al.*, 1995; Wilding and Huettner, 1997). Using GYKI 53655, a number of groups have demonstrated that kainate receptors are involved in both pre- and postsynaptic neurotransmission in the central nervous system (Castillo *et al.*, 1997; Clarke *et al.*, 1997; Rodriguez-Moreno *et al.*, 1997; Vignes and Collingridge, 1997). Gene knockout experiments in mice have now identified GluR6 as at least one of the kainate receptor subunits involved in postsynaptic hippocampal neurotransmission (Mulle *et al.*, 1998).

Although different AMPA receptor subunits and heteromeric receptors combinations give qualitatively similar responses to a range of agonists, kainate receptor responses are somewhat more heterogeneous. Recently, an agonist, ATPA, and two antagonists, LY293558 [(3*S*,4a*R*,6*R*,8a*R*)-6-{2-[1(2)*H*-tetrazol-5-yl]ethyl}decahydroisoquinoline-3-carboxylic acid] and LY294486, [(3*SR*,4a*R*,6*SR*,8a*RS*)-6-((((1*H*-tetrazol-5-yl-)methyl)oxy)methyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid] were shown to be selective for GluR5 over other kainate receptor subunits; additionally, ATPA and LY294486 had only low affinity for AMPA receptors (Clarke *et al.*, 1997). The demonstration that GluR5-containing receptors can modulate inhibitory synaptic transmission in the hippocampus (Clarke *et al.*, 1997) relied on the specificity of these compounds and underscores the need for additional selective and readily available drugs.

5-Substituted willardiine compounds have been shown to

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ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; DRG, dorsal root ganglion; ATPA, (*R*,*S*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid; IW, (*S*)-5-iodowillardiine; HEK, human embryonic kidney; willardiine, (*S*)-1-(2-amino-2-carboxyethyl)pyrimidine-2,4-dione; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMDA, *N*-methyl-D-aspartate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GluR, glutamate receptor.

activate neuronal AMPA and kainate receptors (Patneau *et al.*, 1992; Wong *et al.*, 1994). IW was found to be highly selective for DRG kainate receptors, which may be composed predominantly of GluR5 subunits (Partin *et al.*, 1993). Additionally, in equilibrium binding studies with human recombinant AMPA and kainate receptors, IW was recently shown to have a higher affinity for human GluR5 compared with other receptor subunits (Jane *et al.*, 1997). In this report, we characterize the physiological responses evoked by IW from both homomeric and heteromeric rat kainate receptors expressed in HEK 293 cells. We found that IW was selective for GluR5 homomeric receptors, because it did not evoke currents from homomeric GluR6 or GluR7 receptors. Heteromeric receptors containing GluR6 or GluR7 co-expressed with KA-2 subunits were weakly activated by IW, which is similar to their sensitivity to AMPA. Additionally, we have identified amino acid 721 as a residue critical for IW activation of GluR5 and GluR6 receptors.

Materials and Methods

HEK 293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum. One day before transfection, cells were split to low density on glass coverslips coated with 100 μg/ml poly-D-lysine and collagen. Transfection of receptor cDNAs was by standard calcium-phosphate precipitation with 0.5–1 μg of cDNA for 5–12 hr at 37° and 5% CO_2. We used the unedited (glutamine-containing) GluR5 and GluR6 cDNAs. All receptor subunits were co-transfected with a CD8 antigen-containing plasmid (0.2 μg/coverslip). Electrophysiological recordings were made 1–3 days after transfection. To facilitate identification of transfected cells, coverslips were incubated with polystyrene beads coated with anti-CD8 antibody (Dynal, Lake Success, NY). Patch clamp recordings were made using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Patch electrodes were thick-walled borosilicate glass (Warner Instruments, Hamden, CT) and had a final resistance of 2–4 MΩ after fire-polishing. The internal solution was composed of 110 mM CsF, 30 mM CsCl, 4 mM NaCl, 0.5 mM $CaCl_2$, 10 mM HEPES, and 5 mM EGTA (adjusted to pH 7.3 with CsOH). The external bath solution contained 150 mM NaCl, 2.8 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 10 mM HEPES (pH was adjusted to 7.3 with NaOH). Drugs were applied through three-barrel glass tubing (Vitro Dynamics, Rockaway, NJ), which had been pulled to a internal barrel diameter of ~100 μm and mounted on a piezo-ceramic bimorph. The piezo bimorph was driven by voltage pulses from pClamp v. 6.03 software (Axon Instruments) fed through a stimulation-isolation unit (S-100; Winston Electronic, Millbrae, CA). To allow resolution of fast-desensitizing currents, cells were lifted off the coverslip after a

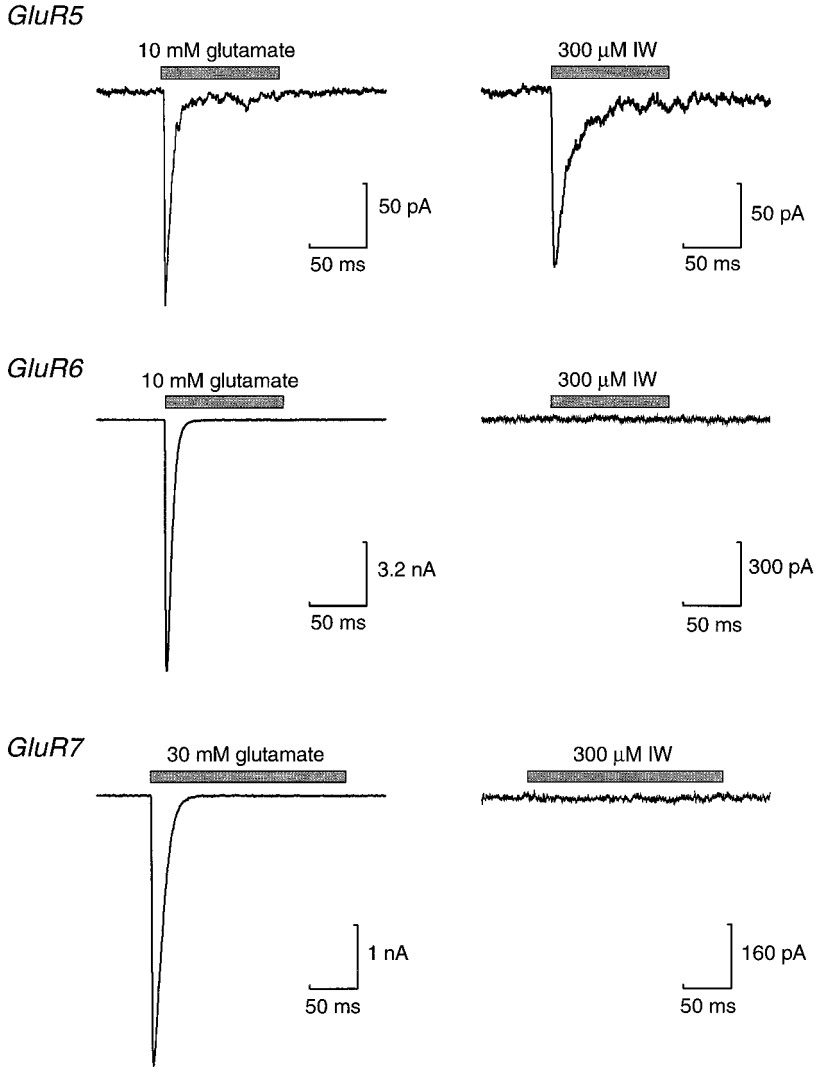

Fig. 1. Application of 10 or 30 mM glutamate (*left column*) or 300 μM IW (*right column*) to whole-cell, patch-clamped HEK 293 cells expressing homomeric GluR5 (*top*), GluR6 (*center*), or GluR7 (*bottom*) receptors. Drugs were applied for 100 msec to cells lifted from the coverslip. The holding potential was −70 mV in each case. For each receptor type, the glutamate and IW responses shown are from the same cell.

whole-cell patch was obtained. Data were acquired directly to a computer and were analyzed off-line using pClamp software. Exponential decays were fitted with the Chebyshev or Simplex least-squares algorithms in Clampfit. Dose-response curves were fitted to the Hill equation using Origin software (Microcal Software, Northampton, MA). L-Glutamate was purchased from Sigma Biochemicals (St. Louis, MO); IW was from Tocris (Ballwin, MO).

All receptor cDNA clones used in these experiments were from rat and were contained in cytomegalovirus-promoter vectors; GluR5a cDNA was kindly supplied by Peter Seeburg. Site mutants were constructed as described in Swanson *et al.* (1997). The human CD8 cDNA was generously provided by B. Seed (Massachusetts General Hospital, Boston, MA).

For radioligand binding assays, membranes were prepared as described in Swanson *et al.* (1997). Samples were incubated in 10 mM HEPES, pH 8.0, containing [^{3}H]kainate (58 Ci/mmol; New England Nuclear, Wilmington, DE) in a final volume of 0.5 ml for 1 hr at 0°. Nonspecific binding was defined as that not displaced by 100 μM kainate. For competition studies, 10 nM [^{3}H]kainate was used. Bound and unbound radioligand were separated by vacuum filtration onto GF/C or GF/B filters (Whatman, Maidestone, UK), presoaked for 1 hr in 0.1% polyethyleneimine (RBI, Natick, MA), followed by two 4-ml washes in ice-cold HEPES. All assays were performed in triplicate. Results from competition curves were fitted to the Hill equation.

Results

To determine the activity of IW on homomeric kainate receptors, we expressed GluR5, GluR6, or GluR7 subunits in HEK 293 cells and examined their current responses to 100-msec applications of IW using whole-cell patch clamp. As shown in Fig. 1, we found that IW was selective for GluR5 receptors. At a concentration of 1 mM, IW elicited currents with a mean amplitude of 255 ± 58 pA from cells expressing GluR5 receptors (n = 8). GluR5 IW currents desensitized with a τ_{des} = 8.9 ± 1.6 msec (n = 6) and exhibited very little steady state current (5.1% of the peak current). We could not detect IW currents (300 μM to 1 mM) from either GluR6- or GluR7-expressing cells (n = 5 for each, Fig. 1). We verified the presence of functional GluR6 and GluR7 receptors in the HEK 293 cells by evoking currents with 10 (GluR6) or 30 (GluR7) mM glutamate; these currents had amplitudes and desensitization rates similar to those reported previously (GluR6: mean peak amplitude = 3.6 ± 2.3 nA, τ_{des} = 3.8 ± 0.2 msec; GluR7: mean peak amplitude = 1.8 ± 0.8 nA, τ_{des} = 7.6 ± 0.53 msec) (Heckmann *et al.*, 1996; Schiffer *et al.*, 1997; Swanson *et al.*, 1997; Traynelis and Wahl, 1997). In GluR5-expressing cells, currents evoked by 10 mM glutamate desensitized with a τ_{des} = 4.4 ± 0.5 msec (n = 22), consistent

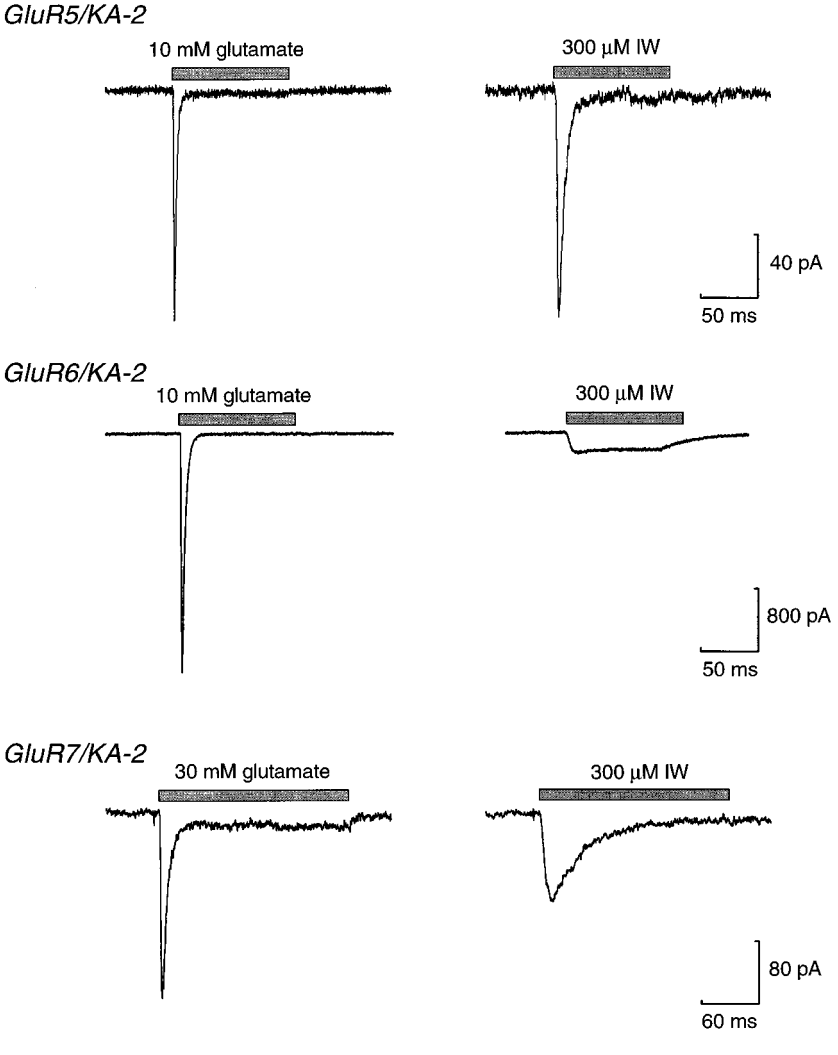

Fig. 2. Application of 10 or 30 mM glutamate (*left column*) or 300 μM IW (*right column*) to whole-cell patch-clamped HEK 293 cells expressing heteromeric GluR5/KA-2 (*top*), GluR6/KA-2 (*center*), or GluR7/KA-2 (*bottom*) receptors. Drugs were applied for 100 msec to cells lifted from the coverslip. The holding potential was −70 mV in each case. For each receptor type, the glutamate and IW responses shown are from the same cell.

with previous reports (Sommer *et al.*, 1992; Swanson *et al.*, 1997).

Co-expression of low affinity (GluR5, -6, and -7) and high affinity (KA-1 and KA-2) kainate receptor subunits produces heteromeric channels with distinct functional properties as compared with their homomeric counterparts. To compare the IW sensitivity of heteromeric and homomeric kainate receptors, we co-expressed the KA-2 subunit with the low affinity kainate receptor subunits (Fig. 2). IW currents from GluR5/KA-2-expressing cells had a mean peak amplitude of 207 ± 52 pA (n = 7), similar to that from homomeric GluR5; however, the desensitization rate was significantly faster for GluR5/KA-2 receptors (2.6 ± 0.2 msec versus 8.9 msec, $p <$ 0.05, unpaired t test) (Fig. 2). Similarly, GluR5/KA-2 glutamate currents desensitized 3-fold faster than GluR5 currents (1.4 ± 0.2 msec versus 4.4 msec, $p < 0.05$). Heteromeric GluR6/KA-2 showed a small, weakly desensitizing response to IW similar to that seen with AMPA on this receptor. At a concentration of 1 mM, IW currents did not desensitize to an appreciable degree (Fig. 2); however, at 3 mM, IW currents desensitized from the peak current by 36 ± 3% (n = 3; data not shown). Glutamate responses in GluR6/KA-2 cells desensitized rapidly, with a time constant of 2.3 ± 0.2 msec (n = 9). GluR7/KA-2-expressing cells also showed responses to IW; however, the relative amplitudes when compared with the control glutamate currents were variable, suggesting that a mixture of homomeric and heteromeric channels were present in the cells. GluR7/KA-2 glutamate currents desen-

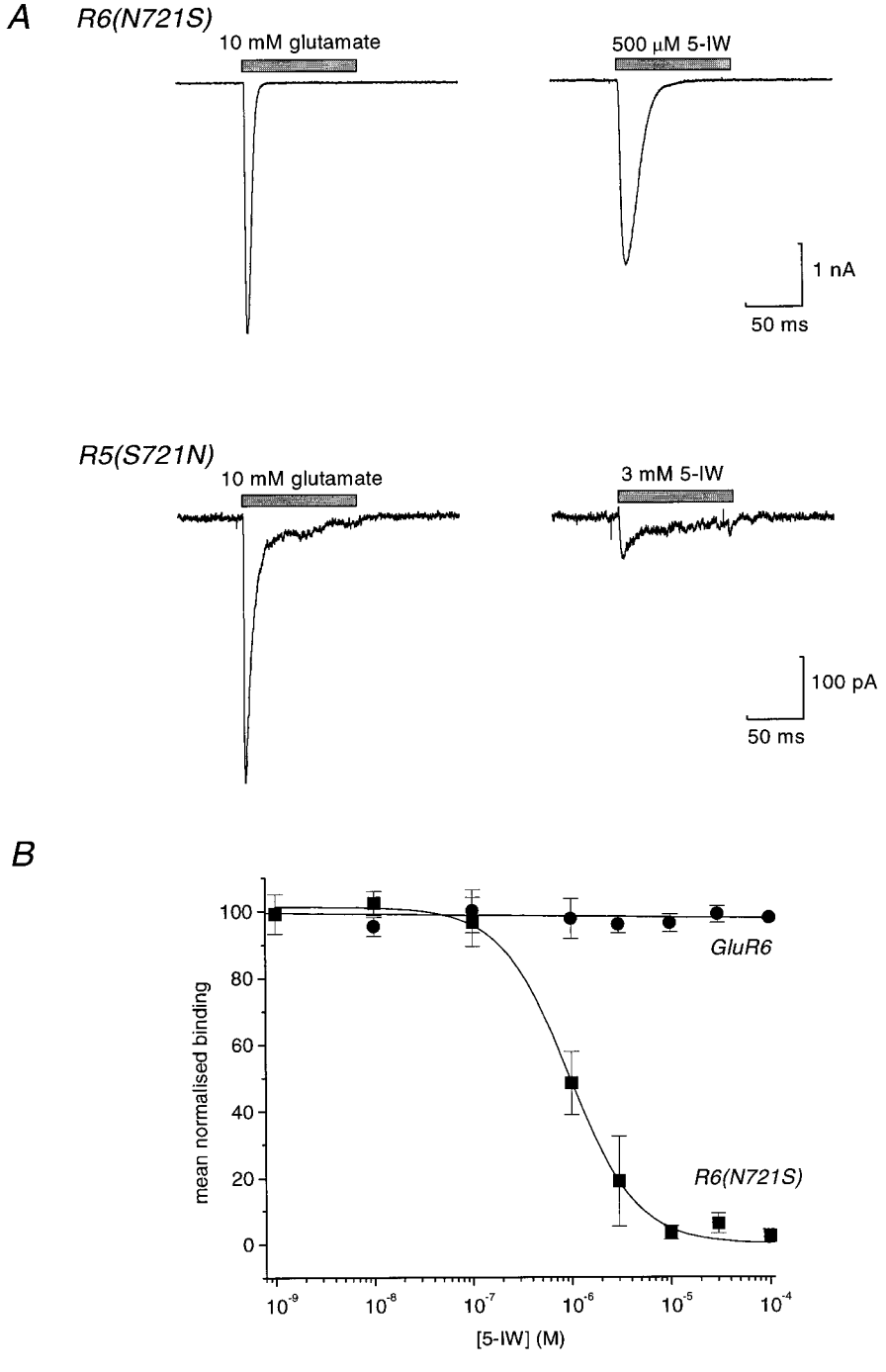

Fig. 3. A, Application of 10 mM glutamate (*left column*) or 500 μM or 3 mM IW (*right column*) to whole-cell, patch-clamped HEK 293 cells expressing R6(N721S) or R5(S721N) mutant receptors. Drugs were applied for 100 msec to cells lifted from the coverslip. The holding potential was −70 mV in each case. For each receptor type, the glutamate and IW responses shown in the figure were from the same cell. B, Mean IW displacement curves for GluR6 and R6(N721S) receptors expressed in HEK 293 cells. In the displacement experiments the concentration of [^{3}H]kainate was 5 nM for GluR6 and 10 or 15 nM for R6(N721S). For R6(N721S), the data from three independent displacement assays were fitted to the Hill equation. The K_d for [^{3}H]kainate on R6(N721S) was previously measured as 62 nM (Swanson *et al.*, 1997); this value was used to calculate a mean K_i value of 0.90 ± 0.17 μM for IW.

sitized with a τ_{des} of 6.6 ± 1.0 msec, consistent with our previous report (Schiffer *et al.*, 1997). These data demonstrate that the KA-2 subunit confers IW sensitivity to GluR6 and GluR7 receptors, and alters the kinetic properties of receptors formed from GluR5 subunits.

The selectivity that IW demonstrates for GluR5, and the low sensitivity of GluR6/KA-2 and GluR7/KA-2 heteromeric receptors, is qualitatively similar to responses seen with AMPA on these receptors. We demonstrated previously that the differential sensitivity to AMPA between GluR5 and GluR6 was conferred by the amino acid at position 721, which is located in an extracellular region of the protein, between membrane domains three and four (Swanson *et al.*, 1997). This residue is a serine in GluR5 and an asparagine in GluR6 and GluR7. We tested whether the same residue was responsible for conferring IW sensitivity to homomeric kainate receptors by expressing two mutants, R6(N721S) and R5(S721N) (Fig. 3A). In sharp contrast to GluR6, cells expressing the mutant receptor R6(N721S) gave robust, rapidly desensitizing responses to IW (Fig. 3A). At a concentration of 1 mM, IW currents desensitized with a τ_{des} = 5.9 ± 1.2 msec, compared with 3.3 ± 0.3 for 10 mM glutamate currents in the same cells. Conversely, R5(S721N) receptors gave much reduced, but detectable, responses to IW. Currents activated by 3 mM IW were only 17 ± 4% of peak glutamate currents in the same cells (n = 4); in contrast, in GluR5-expressing cells, this concentration of IW gave larger peak responses than currents evoked by 10 mM glutamate (Fig. 4). These data demonstrate that the residue at 721 largely determines the kainate receptor sensitivity to IW; this interpretation is further supported by the insensitivity of homomeric GluR7 receptors, which contain an asparagine at the analogous site.

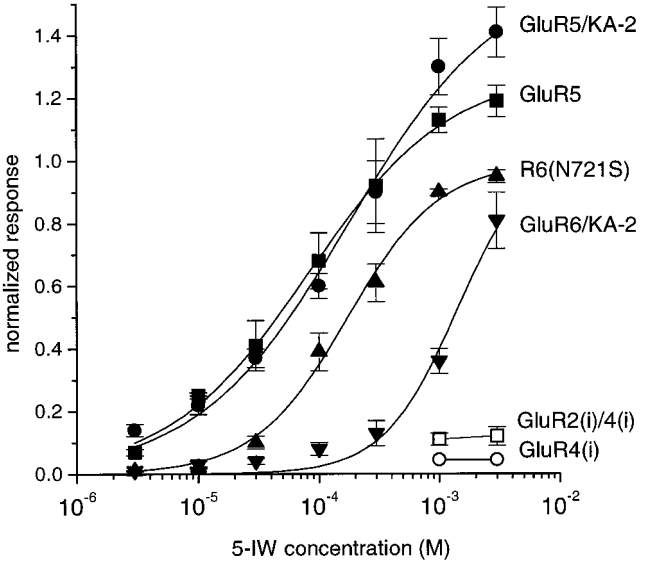

Fig. 4. Dose-response relationships for the peak responses to IW normalized to the amplitude of a preceding application of 10 mM glutamate. Each *data point* on the concentration-response assays represents the mean normalized response in three to five individual cells; *error bars*, mean ± standard error. The holding potential was −70 mV for all measurements. Data were fitted to the Hill equation (with the exception of GluR4). Values obtained from the fits were: GluR5 (■), EC_{50} = 83 μM, n_H = 0.74; GluR5/KA-2 (●), EC_{50} = 176 μM, n_H = 0.69; R6(N721S) (▲), EC_{50} = 173 μM, n_H = 1.12; GluR6/KA-2 (▼), EC_{50} = 1.4 mM, n_H = 1.3; GluR4(flip) (○), not determined; GluR2(flip)/GluR4(flip) (□), not determined.

The R6(N721S) mutation increased the affinity for AMPA in ligand binding experiments (Swanson *et al.*, 1997). To determine if replacement of N721 with a serine residue in GluR6 caused a shift in binding affinity for IW similar to that seen with AMPA, we compared the displacement of [^{3}H]kainate by IW for GluR6 and the mutant R6(N721S) in equilibrium binding assays. As shown in Fig. 3B, IW has no detectable affinity for GluR6 receptors; displacement of [^{3}H]kainate was <10% at 100 μM. In contrast, IW exhibited an K_i of 0.90 ± 0.17 μM on R6(N721S) receptors, which supports the idea that residue 721 is a shared component of the IW and AMPA binding sites on these kainate receptors.

The peak EC_{50} values of IW for recombinant kainate receptors were determined by analysis of dose-response data (Fig. 4). For GluR5, GluR5/KA-2, GluR6/KA-2, and R6(N721S) receptors, the peak amplitudes of the IW currents were normalized to preceding applications of glutamate, and the data were fitted to the Hill equation. Peak currents for the GluR5-containing receptors gave EC_{50} values of 83 μM (GluR5) and 176 μM (GluR5/KA-2). Interestingly, for both these receptors high concentrations of IW elicited larger currents than glutamate and the Hill slopes of the fitted curves were shallow. For GluR5 the predicted maximal normalized response was 1.28, suggesting that at 3 mM the receptors were 93% saturated. In contrast, GluR5/KA-2 responses to IW were not saturated even at a concentration of 3 mM, at which concentration the IW peak currents were on average 1.4-fold the amplitude of the control responses to glutamate. The Hill slopes for GluR5 and GluR5/KA-2 were 0.74 and 0.69, respectively. R6(N721S) and GluR6/KA-2 receptor dose-response curves gave EC_{50} values of 173 μM and 1.4 mM, respectively. The GluR6/KA-2 EC_{50} value was estimated from a fit of its nonsaturated curve with the maximal normalized current set to 1.0. These data suggest that the KA-2 subunit co-assembles with the GluR5 and GluR6 subunits to produce a relatively low affinity site for IW in the heteromeric receptor complexes—that is, a site with enough affinity to endow sensitivity to IW to GluR6, but one that reduces the IW potency for GluR5. To compare these potencies for kainate receptors with that of representative AMPA receptor subunits, we expressed homomeric GluR4 and heteromeric GluR2/GluR4 receptors (both subunits contained the "flip" splice cassette) and measured the peak responses at concentrations of 1 and 3 mM IW. As is shown in Fig. 4, both concentrations of IW evoked very small currents from GluR4 receptors (4% of the peak response to 10 mM glutamate). Responses from GluR2/GluR4 cells were also of low amplitude compared with GluR5, but were significantly higher at both 1 and 3 mM compared with homomeric GluR4 ($p < 0.05$). These data suggest that heterogeneity in the sensitivity to IW between AMPA receptors may exist; this interpretation is supported by differing equilibrium binding affinities observed for IW on human GluR1, GluR2, and GluR4 receptors (Jane *et al.*, 1997). Indeed, in this previous study, GluR4 had the lowest affinity among the three AMPA receptor subunits assayed (Jane *et al.*, 1997).

IW currents were shown to recover from desensitization in DRG neurons with a time course of minutes (Wong *et al.*, 1994). Because DRG neurons express both GluR5 and KA-2 mRNA (Partin *et al.*, 1993), we examined the recovery from desensitization for GluR5 and GluR5/KA-2 receptors to determine if either receptor had a similarly slow time course of

recovery. For these experiments, IW was applied at increasing time intervals after an initial control application; the peak amplitudes of the subsequent test applications were normalized to the initial application. As shown in the representative traces in Fig. 5A, GluR5 receptors exhibited only partial recovery of the peak current amplitude after a 30-sec interval between applications. In contrast, GluR5/KA-2 receptors were fully recovered after the 30-sec wash period. The striking difference between the recovery kinetics of these receptors was quantified by fitting their time courses of recovery with a single exponential component (Fig. 5B). GluR5 currents recovered with time constant of about 2.5 min; in contrast, recovery of GluR5/KA-2 receptors was significantly faster and was fitted to a τ_{rec} of 12 sec. These data demonstrate that the KA-2 subunit influences multiple aspects of the desensitization kinetics of GluR5 kainate receptors.

Discussion

In this report we have demonstrated that IW can be used to distinguish different combinations of recombinant kainate receptors on the basis of pharmacological and kinetic properties. Pharmacological differentiation of AMPA and kainate receptors has recently allowed the detection and character-

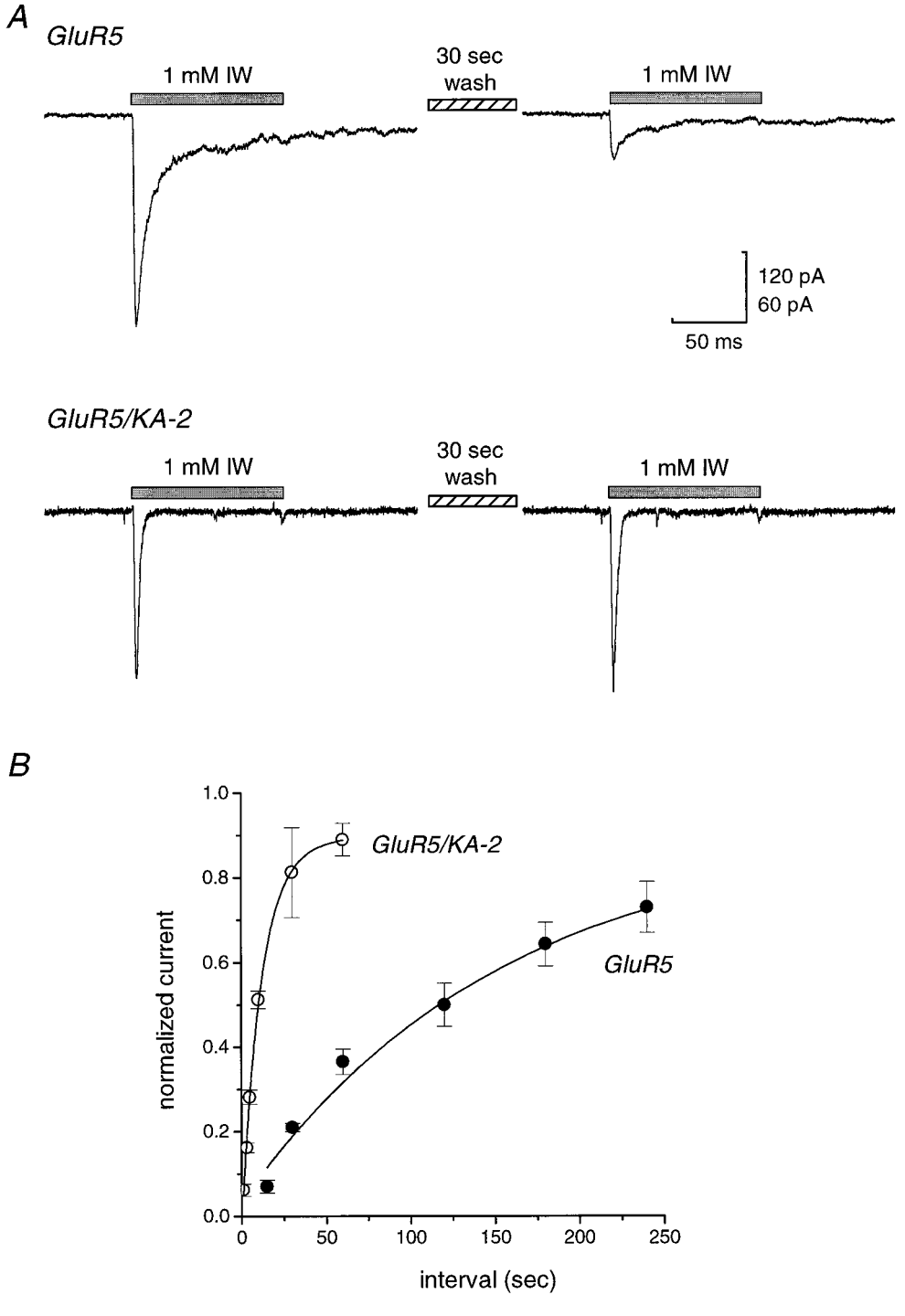

Fig. 5. A, Application of 1 mM IW to whole-cell, patch-clamp HEK 293 cells at 30-sec intervals. Note that GluR5/KA-2 responses are completely recovered after 30 sec, whereas GluR5 receptors remain substantially desensitized. Drugs were applied for 100 msec to cells lifted from the coverslip. The holding potential was −70 mV in each case. B, Recovery from desensitization relationship for GluR5 and GluR5/KA-2 responses to IW. Responses at increasing time intervals were normalized to the initial response amplitude. It is likely that some rundown occurs with the longer GluR5 recovery intervals. Data were fitted with a single exponential component to obtain time constants of ~2.5 min (GluR5, ●) and 12 sec (GluR5/KA-2, ○).

ization of native kainate receptors involved in hippocampal synaptic transmission (Chittajallu *et al.*, 1996; Castillo *et al.*, 1997; Clarke *et al.*, 1997; Rodriguez-Moreno *et al.*, 1997; Vignes and Collingridge, 1997). Further separation of native responses arising from GluR5- or GluR6-containing receptors has been achieved using an agonist, ATPA, and an antagonist, LY294486, that are selective for GluR5 receptors (Clarke *et al.*, 1997). With these compounds, Clarke *et al.* (1997) were able to demonstrate that GluR5-containing receptors modulated monosynaptic inhibitory neurotransmission in CA1 pyramidal cells by a presynaptic mechanism. Concurrently, Rodriguez-Moreno *et al.* (1997) provided additional compelling evidence that activation of presynaptic kainate receptors reduced inhibitory synaptic transmission in neuronal cultures, CA1 pyramidal neurons in hippocampal slices, and *in vivo* hippocampal field recordings. Because the number of readily available and selective non-NMDA receptors agonists is limited, the information provided in this report will prove useful for further attempts to define the role and molecular identity of kainate receptors in synaptic transmission.

IW was shown to have a high degree of selectivity for native kainate receptors compared with AMPA receptors based on its action on DRG and cultured hippocampal neurons (Patneau *et al.*, 1992; Wong *et al.*, 1994). After treatment with concanavalin A to reduce desensitization, IW evoked currents with an EC_{50} value of 0.14 μM in DRG neurons (Wong *et al.*, 1994). This potency was roughly 130-fold higher than that for hippocampal AMPA receptors (Wong *et al.*, 1994). This difference in the steady state EC_{50} values between AMPA and kainate receptors seems also to be reflected in the peak EC_{50} values, because we found that recombinant homomeric GluR4 and heteromeric GluR2/GluR4 receptors showed little activation by IW at concentrations of up to 3 mM. As is suggested by recent binding studies with recombinant AMPA receptor subunits (Jane *et al.*, 1997), it is possible that other subunits or splice forms of AMPA receptors may be more sensitive to IW than the flip isoform of GluR4 tested in this study. The recently described GluR5-selective agonist ATPA has a higher EC_{50} than IW for activating steady state DRG kainate currents (0.6 versus 0.14 μM) and shows little activity at recombinant AMPA receptors (Clarke *et al.*, 1997). The pharmacological similarities between IW and ATPA suggest that IW may also be useful for selective activation of GluR5-containing receptors in native cells.

The kinetics of IW-induced currents mediated by recombinant kainate receptors support the idea that homomeric GluR5 subunits compose the majority of the kainate receptor population in postnatal DRG neurons. Among non-NMDA receptors, GluR5 was shown to be the predominant subunit mRNA expressed in DRG neurons (Partin *et al.*, 1993), and kainate receptor currents in DRG neurons matched well with the whole-cell and single-channel properties of homomeric GluR5 receptors (Huettner, 1990; Sommer *et al.*, 1992; Partin *et al.*, 1993; Swanson *et al.*, 1996). IW recovers from desensitization with a particularly slow time course in DRG neurons (35 sec and 4.4 min) (Wong *et al.*, 1994), which correlates well with the ~2-min recovery we observed with homomeric GluR5 receptors and contrasts with the faster recovery of GluR5/KA-2 receptors (Fig. 5). Although the EC_{50} of IW for activation of steady state currents after treatment of DRG neurons with concavalin A was nearly 3 orders of magnitude lower than that for peak responses for recombinant GluR5 or GluR5/KA-2 (0.14 μM versus 83 and 176 μM, respectively), this is consistent with the lower EC_{50} values generally observed for desensitized steady state currents as compared with peak responses.

In DRG neurons, an extensive structure-activity study of 5-substituted willardiines provided strong evidence for the presence of a hydrophobic site in the ligand binding pocket of DRG kainate receptors (Wong *et al.*, 1994). The interaction of IW with amino acid 721 in GluR6, and the similarity of its pharmacological behavior to that of AMPA, suggest that the hydrophobicity of this residue may be a determinant of the kainate receptor-selectivity of these agonists. In GluR5 a serine occupies position 721; mutation of this site to less hydrophobic asparagine substantially reduces sensitivity to both IW and AMPA (Fig. 3) (this report) (Swanson *et al.*, 1997). It is of interest that the selective GluR5 agonist ATPA differs from its analog AMPA by a 5-*t*-butyl group substituted for the 5-methyl group and therefore has a more hydrophobic moiety in a position generally equivalent to the 5-subsitution used in the willardiine study (Wong *et al.*, 1994). It is possible that the increased selectivity for GluR5 demonstrated by ATPA is caused by interaction with serine 721. Further site mutants with a panel of substituted residues will be necessary to conclusively demonstrate that the hydrophobicity of residue 721 does indeed determine the kainate receptor agonist selectivity.

In summary, these data suggest that IW may serve as an effective tool for selective activation of GluR5-containing glutamate receptor within a mixed population of AMPA or kainate receptor subtypes. Additionally, the demonstration that specificity of IW arises from its interaction with a single amino acid on the kainate receptor protein may prove useful for further development of subtype-selective compounds.

Acknowledgments

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