Effect of RNA editing and subunit co-assembly on single-channel properties of recombinant kainate receptors

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1. Patch-clamp methods have been used to examine single-channel properties of recombinant GluR5 and GluR6 kainate-preferring glutamate receptors which differ in a single amino acid residue as a result of RNA editing at the Q/R (glutamine/arginine) site. Subunits were expressed alone or in combination with the high-affinity kainate receptor subunit KA-2 in transfected human embryonic kidney (HEK-293) cells.

2. In outside-out patches, unedited homomeric GluR6(Q) receptors exhibited directly resolved domoate-activated single-channel conductances of 8, 15 and 25 pS. Variance analysis of GluR6(Q) responses gave a mean conductance of 5·4 pS, while the edited isoform GluR6(R) had an unusually low channel conductance (225 fS).

3. Homomeric channels composed of GluR5(Q) subunits exhibited three conductance states of 5, 9 and 14 pS characterized by prolonged burst activations in the presence of domoate. In contrast, the GluR5(R) subunit, which has not previously been reported to form functional homomeric receptors, had an extremely low conductance (< 200 fS).

4. Heteromeric GluR6(Q)/KA-2 kainate receptors gave single-channel events indistinguishable from homomeric GluR6(Q) channels. Conversely, openings produced by GluR5(Q)/KA-2 and GluR5(Q) receptors differed from each other in their kinetic properties. The primary effect of co-expression of KA-2 with GluR5(Q) was a dramatic shortening in channel burst length.

5. Spectral and variance analyses were used to estimate mean single-channel conductances of heteromeric edited receptor–channels; channel conductances were 950 fS for GluR5(R)/KA-2 receptors and 700 fS for GluR6(R)/KA-2 receptors. Both receptor types had significantly higher conductances than the respective homomeric channels, GluR5(R) and GluR6(R).

6. We conclude that Q/R site editing dramatically reduces single-channel conductance. Furthermore, we find similarity between the kainate receptor–channels described in sensory neurones and the recombinant GluR5(Q) homomeric channel. Characterization of recombinant single-channel properties could therefore aid identification of the native kainate receptors.

Recombinant non-NMDA glutamate receptors have been the subject of extensive functional and molecular characterization. This family of ligand-gated ion channels can be subdivided into AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate)-preferring (GluR1 to 4, or A to D) and kainate-preferring (GluR5 to 7, KA-1 and KA-2) receptors, based on similarities in their primary sequences and their biophysical and pharmacological characteristics (reviewed in Wisden & Seeburg, 1993; Hollmann & Heinemann, 1994). Kainate-preferring receptors mediate whole-cell currents that profoundly desensitize both in the presence of glutamate and kainate (Bettler *et al.* 1990; Egebjerg, Bettler, Hermans-Borgmeyer & Heinemann, 1991; Sommer, Burnashev, Verdoorn, Keinänen, Sakmann & Seeburg, 1992; Herb, Burnashev, Werner, Sakmann, Wisden & Seeburg, 1992). Functional information about recombinant non-NMDA receptors has given valuable clues about the possible subunits constituting native receptors, particularly when considered with molecular approaches such as single-cell polymerase chain reaction (PCR)

(Lambolez, Audinat, Bochet, Crépel & Rossier, 1992; Bochet *et al.* 1994; Jonas, Racca, Sakmann, Seeburg & Monyer, 1994; Ruano, Lambolez, Rossier, Paternain & Lerma, 1995). Studies of this sort have added support to the idea that AMPA-preferring receptors are involved in the initial fast component of excitatory postsynaptic currents in the central nervous system. In contrast, the contribution of kainate-preferring receptors to synaptic transmission remains to be elucidated, despite the fact that kainate receptor immunoreactivity can be localized to postsynaptic densities (Good, Huntley, Rogers, Heinemann & Morrison, 1993; Huntley *et al.* 1993; Petralia, Wang & Wenthold, 1994).

Analysis of recombinant non-NMDA receptors has revealed the existence of a relatively rare (in mammalian species) RNA-editing mechanism, which has been shown to affect channel properties (reviewed by Wisden & Seeburg, 1993; Hollmann & Heinemann, 1994; Lomeli *et al.* 1994). Editing occurs in precursor RNA and can result in the substitution of a single amino acid in the receptor protein (Sommer, Köhler, Sprengel & Seeburg, 1991; Higuchi, Single, Köhler, Sommer, Sprengel & Seeburg, 1993). The Q/R (glutamine/arginine) editing site has been proposed to form a portion of the channel pore, as channels which include the edited subunits have reduced divalent ion permeability and a linear, rather than inwardly rectifying, current–voltage relationship (Wisden & Seeburg, 1993; Hollmann & Heinemann, 1994; Burnashev, Zhou, Neher & Sakmann, 1995). These changes in the macroscopic channel currents associated with Q/R site editing suggested to us that accompanying differences in the single-channel properties may exist between edited isoforms.

The diversity of non-NMDA subunit isoforms, and the fact that many neurones express more than one type, has made it difficult to identify the subunits composing native receptors. At the single-channel level, characterization of distinct non-NMDA glutamate receptors on the basis of their conductance and kinetic behaviour has also proved formidable. Channels in central and peripheral neurones give rise to a wide range of channel conductance levels (Jahr & Stevens, 1987; Cull-Candy & Usowicz, 1987; Ascher & Nowak, 1988; Huettner, 1990), which are likely to reflect the presence of more than one channel species, as well as complex conductance properties associated with individual receptors (Wyllie, Traynelis & Cull-Candy, 1993; Wyllie & Cull-Candy, 1994). For these reasons, characterization of the single-channel properties of recombinant receptors could be useful in identifying the subunit constitution of individual native receptor–channels.

We have determined the single-channel properties of a group of recombinant non-NMDA glutamate receptors, namely homomeric channels composed of the kainate receptor subunits GluR5 and GluR6, and heteromeric channels containing these subunits in combination with KA-2 subunits. Since both the edited and unedited forms of GluR5 and -6 are present *in vivo* (Sommer *et al.* 1991; Köhler, Burnashev, Sakmann & Seeburg, 1993), it was of interest to examine the consequences of Q/R site editing at the level of the single channel.

A preliminary report of some of our results has been published (Swanson, Feldmeyer & Cull-Candy, 1994).

METHODS

Maintenance and transfection of HEK-293 cells

Human embryonic kidney (HEK-293) cells were cultured in Dulbecco's modified Eagle's medium (DMEM–F12; Gibco BRL) with 10% heat-inactivated fetal bovine serum, 50 μg ml^{-1} penicillin and 50 μg ml^{-1} streptomycin at 37 °C in a 95% O_2–5% CO_2 incubator. One day before transfection, cells were replated on 11 mm glass coverslips coated with 100 μg ml^{-1} poly-L-lysine and 50 μg ml^{-1} fibronectin (Sigma). Transfections were carried out using a standard calcium phosphate precipitation protocol (Chen & Okayama, 1987) with 0·5 μg plasmid DNA per well for 3–6 h at 37 °C. In cells co-transfected with two receptor subunits, 0·25 μg of each plasmid DNA was used per well. Transfected cells were washed twice with media and allowed to grow for 1–3 days before use. Glutamate receptor cDNAs were harboured in a cytomegalovirus promoter-containing vector. Both GluR6(R) and GluR6(Q) cDNAs were edited at the first transmembrane domain sites (i.e. GluR6(V, C, Q/R); Köhler *et al.* 1993).

Electrophysiology

Recordings were made at room temperature (22–24 °C) using standard patch-clamp techniques. Patch pipettes were pulled from thick-walled glass (GC150F-7·5, Clark Electromedical, Pangbourne, UK), coated with Sylgard resin (184, Dow Corning) and fire-polished to a final resistance of 10–15 MΩ. The pipette solution contained (mM): CsF, 110; CsCl, 30; NaCl, 4; $CaCl_2$, 0·5; Hepes, 10; EGTA, 5 (adjusted to pH 7·3 with CsOH). The external bathing solution contained (mM): NaCl, 150; KCl, 2·8; $CaCl_2$, 1·0; Na-Hepes, 10 (pH adjusted to 7·3 with NaOH). Whole-cell and outside-out patch recordings were made with either an Axopatch 200A (Axon Instruments) or an L/M-EPC7 (List) amplifier. Cells were visualized under Nomarski optics on an Axioskop microscope (Zeiss Instruments). Currents were recorded on either FM tape (Racal Store 4; bandwidth DC to 5 kHz) or digital audio tape (BioLogic DTR-1204 (Intracell, Royston, UK); bandwidth DC to 20 kHz, −3 dB). Drugs were applied by local perfusion of the recording chamber. Domoate, kainate, AMPA, glutamate and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were purchased from Tocris Cookson, Bristol, UK. Concanavalin A (ConA) was purchased from Sigma.

Data analysis

For variance analysis, currents during the slow (>10 s) wash-off of 5 μM domoate were acquired by AxoTape software (Axon Instruments) after low-pass filtering at 2 kHz and digitization at 5 kHz. Currents used for variance and mean measurements were edited to omit artifacts and analysed in 0·5–1·0 s sections using software kindly provided by Stephen Traynelis (Emory University, Atlanta, GA, USA). Mean conductances were derived according to $\gamma = \sigma^2/[\mu_I(V_c - V_{rev})]$, where γ is the mean channel conductance, σ^2 is the current variance, μ_I is the mean current, V_{rev} is the reversal potential and V_c is the command potential. To determine

reversal potentials, ramp current–voltage relationships were generated using pCLAMP software (Axon Instruments) on an IBM PC with a TL-1 DMA digital interface and were fitted with the sum of polynomial equations. V_{rev} was at, or near, 0 mV for all recordings, and this value was used for all estimations of conductances. All of our current–variance relationships were linear ($r^2 = 0{\cdot}90$–$0{\cdot}99$), rather than parabolic, indicating that the probability of channel opening was low during the steady-state response to domoate. This is consistent with previous observations that steady-state responses were small relative to peak currents (Herb, Burnashev, Werner, Sakmann, Wisden & Seeburg, 1992; Köhler *et al.* 1993).

For time course fitting of single-channel openings, records were filtered at 2 kHz (−3 dB, 8-pole Bessel), digitized at 20 kHz (CED 1401 interface) and individual currents fitted with the response function of the recording system to a step input (Colquhoun & Sigworth, 1983). All time course fitting manipulations were carried out using software kindly provided by David Colquhoun (University College London, UK). Mean unitary current amplitudes were determined from maximum likelihood fits of the sum of Gaussian components. Only openings longer than two filter rise times (332 μs) were included in the amplitude distributions to minimize the inclusion of false events (Colquhoun & Sigworth, 1983). Open-time distributions were determined from the time course fitting of channel openings. A time resolution for openings of 200 μs was imposed to reduce the false event rate. All sublevel amplitudes were included in kinetic analyses. Shut-time distributions were generated from the time course fitted data in order to calculate a critical gap length t_c. The first two or three exponential components (depending on whether the full distribution was fitted by four or five components) of the shut times were concentration independent. Bursts of openings were defined as activations separated by shut times shorter than t_c, which was calculated to include an equal number of gaps misclassified as within bursts and gaps misclassified as between bursts. A test range of t_c values was also used to generate burst lengths and had only a marginal effect on the resulting distributions and therefore did not alter our conclusions. The number of openings per burst was subsequently calculated in a similar fashion.

For spectral analysis of steady-state (stationary) current noise responses to domoate, records were high-pass filtered at 0·2 Hz, low-pass filtered at 2 kHz (−3 dB, Butterworth; Barr & Stroud) and digitized at 4 kHz (CED 1401plus interface). Digitized records were split into sections $1/f_{res}$ in length (with f_{res} being 2–4 Hz), edited to remove artifacts and analysed using SPECTAN software kindly provided by John Dempster (Strathclyde University, UK). Net single-sided spectra were calculated by subtraction of control (background noise) spectra from agonist spectra. Noise spectra were not corrected for the frequency response of the recording system.

RESULTS

Q/R site editing reduces single-channel conductance of recombinant kainate receptors

GluR6. Figure 1*A* shows steady-state current responses to 5 μM domoate, a high-affinity kainate receptor agonist, applied to outside-out patches from HEK cells containing homomeric GluR6(Q) and -6(R) channels (top two traces), and heteromeric GluR6(Q)/KA-2 and -6(R)/KA-2 channels (bottom two traces). Inward currents were generally much larger for patches containing the unedited GluR6(Q) subunits. Thus GluR6(Q) and GluR6(Q)/KA-2-containing patches gave currents up to 150 pA, whereas patches containing GluR6(R) or GluR6(R)/KA-2 subunits gave currents of <10 pA. GluR6(Q) receptors also gave steady-state currents in response to kainate (100 μM, <10 pA) and glutamate (300 μM, <5 pA). We verified the formation of heteromeric GluR6/KA-2 receptors by testing their response to 300 μM AMPA, which does not activate the homomeric GluR6 channels (see Herb *et al.* 1992).

To estimate the mean single-channel conductance for each receptor type, we measured the variance of the membrane noise during the slow reduction in the response upon wash-off of 5 μM domoate, and plotted this against the mean inward current. As is apparent from the responses in Fig. 1*A*, the increase in variance associated with agonist-evoked currents in GluR6(R) patches was barely detectable (note the difference in calibration between GluR6(Q) and GluR6(R)). The single-channel conductance, estimated from the slope of the variance *versus* mean current relationship in Fig. 1*B*, was 5·7 pS for GluR6(Q) but only 250 fS for GluR6(R) channels. The mean single channel conductance values obtained were $5{\cdot}4 \pm 0{\cdot}8$ pS ($n = 4$; mean ± S.E.M.) for GluR6(Q) channels, and 225 ± 11 fS ($n = 4$) for GluR6(R) channels. Analysis of whole-cell current noise gave comparable values (not shown), indicating that single-channel conductances were not markedly altered by patch excision.

For heteromeric GluR6(Q)/KA-2 channels, the single-channel conductance estimated from the variance *versus* mean current plots in Fig. 1*C*, was 5·0 pS. Our mean value was $7{\cdot}1 \pm 1{\cdot}7$ pS ($n = 5$); this was not significantly different from the conductance of the homomeric GluR6(Q) channel. On the other hand, the estimated conductance of the heteromeric GluR6(R)/KA-2 channel in Fig. 1*C* was 0·84 pS. The mean value obtained was $0{\cdot}70 \pm 0{\cdot}14$ pS ($n = 5$; Fig. 1*C*); this was still quite low but significantly higher (~3-fold) than for the homomeric GluR6(R) channel ($P < 0{\cdot}05$, Student's unpaired t test).

GluR5. To determine the effect of RNA editing on the mean conductances of GluR5 receptors, we initially analysed whole-cell responses to 10 μM domoate using variance analysis. Previous efforts have failed to detect agonist-evoked currents through edited GluR5(R) channels in either *Xenopus* or mammalian expression systems (despite the presence of kainate binding sites), suggesting that the subunit was unresponsive or unable to form functional channels (Sommer *et al.* 1992; Partin, Patneau, Winters, Mayer & Buonanno, 1993). To maximize the likelihood of detecting a GluR5(R) response, we recorded from large clusters of electrically coupled transfected HEK cells (syncytium of up to 10 cells). These were pretreated

with 2·5 μM ConA to suppress desensitization of the kainate receptors (see Huettner, 1990). Figure 2*A* shows a representative whole-cell response to 20 μM domoate. Responses tended to diminish with repeated application of agonist, which may result from a run-down of the current response, or a decrease in the size of the syncytium. It is not possible to make an accurate estimate of the single-channel conductance from this type of recording because of the inherent space-clamp problems associated with the large group of cells. However, we were able to estimate the mean channel conductance in individual cells that gave small (<5 pA) whole-cell currents. We obtained a single-channel conductance of <200 fS from steady-state variance analysis. These observations therefore indicate that homomeric GluR5(R) subunits are capable of forming functional channels, albeit with an extremely low mean conductance.

As reported previously, receptors produced by co-expression of GluR5(R) and KA-2 channel subunits give steady-state whole-cell currents in response to domoate (Fig. 2*B*; Herb *et al.* 1992). To determine the effect of Q/R site editing on the single-channel properties of heteromeric GluR5 kainate receptors, we compared the mean channel conductance and the time constants underlying current noise of GluR5(Q)/ KA-2 and GluR5(R)/KA-2 channel activations. As illustrated in Fig. 2*B* and *C*, power spectra were well fitted by the sum of two Lorentzian curves. The channel conductance estimated from the GluR5(Q)/KA-2 spectrum in Fig. 2*C* was 3·9 pS; the mean conductance value was 4·5 ± 0·5 pS ($n = 3$). The channel conductance

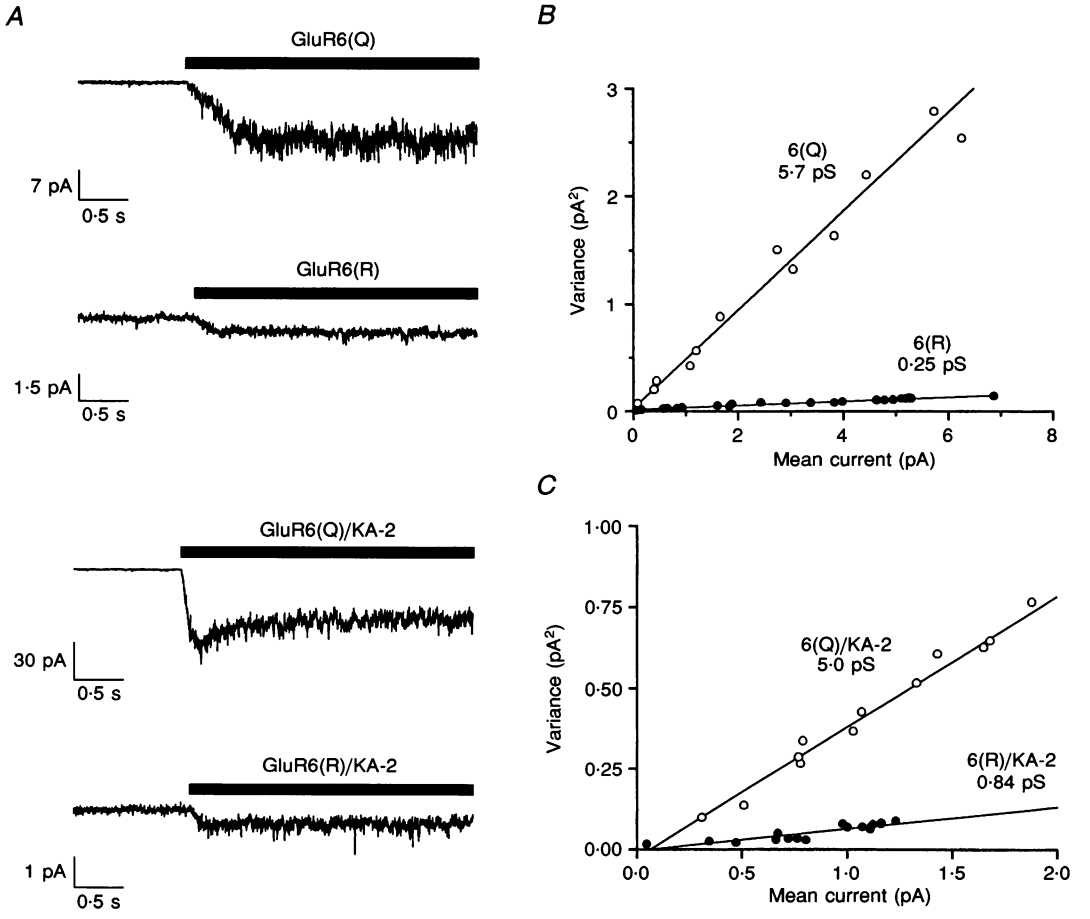

Figure 1. RNA editing reduces the mean conductance of GluR6 kainate receptors

A, representative patch current responses in outside-out membrane patches from HEK-293 cells expressing GluR-6(Q), -6(R), -6(Q)/KA-2 and -6(R)/KA-2 to bath application of 5 μM domoate (the holding potential, V_h, was −80 mV). Bars indicate domoate applications. *B* and *C* show representative variance to mean current plots calculated from fluctuation analysis of the wash-out phase of the patch current response to domoate. Regression lines were fitted to the data and intersected the axes near or at the origin. The mean conductance values (calculated from the slope of the linear regression) were: 0·25 pS for homomeric GluR6(R) (●) and 5·7 pS for GluR6(Q) (○) receptors (*B*) and 0·84 pS for heteromeric GluR6(R)/KA-2 (●) and 5·0 pS for GluR6(Q)/KA-2 (○) receptors (*C*).

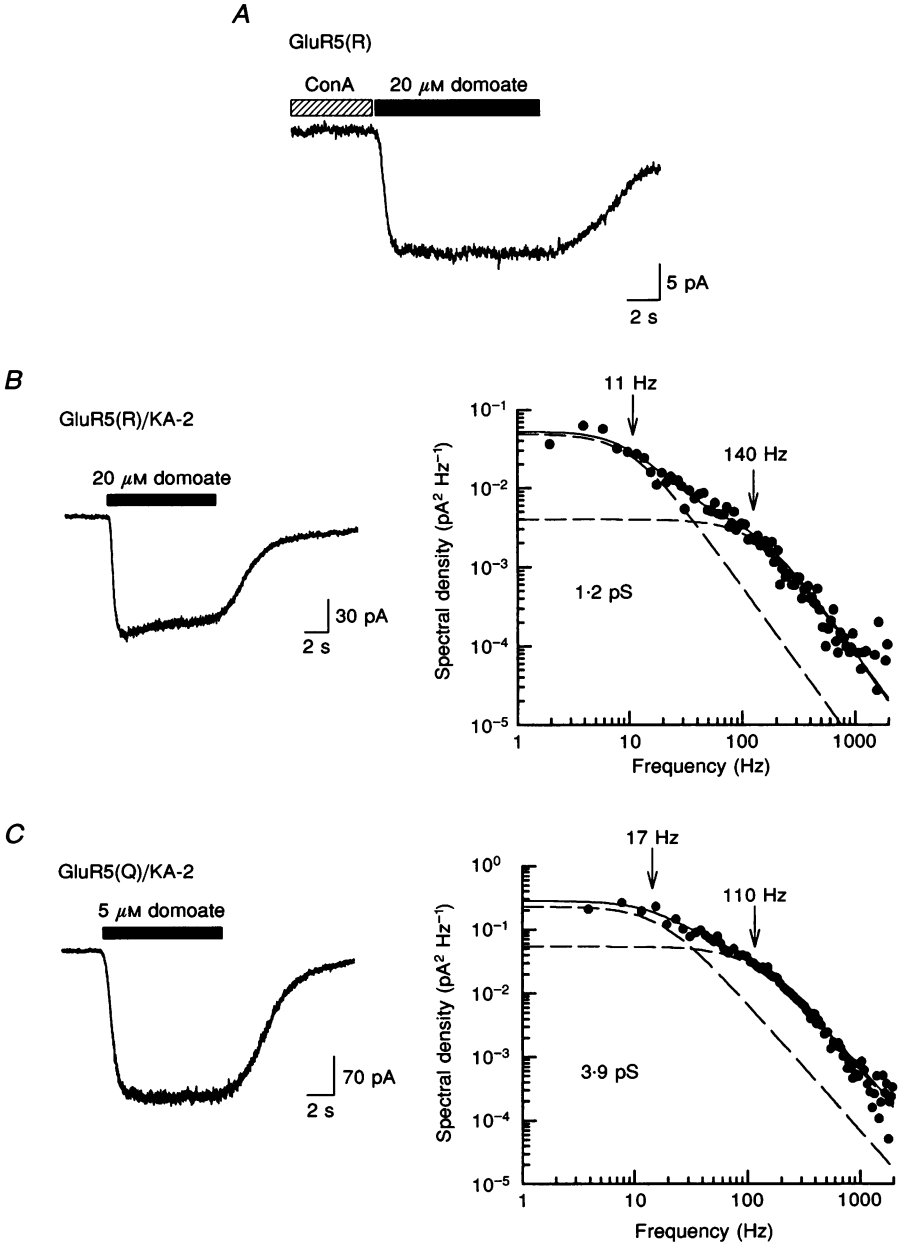

Figure 2. Edited homomeric and heteromeric GluR5 kainate receptors

A, GluR5(R) forms functional homomeric ion channels. Representative current response from an electrically coupled syncytium of HEK-293 cells expressing GluR5(R) to bath application of 20 μM domoate after treatment with 2·5 μM concanavalin A (ConA). V_h was −80 mV. *B*, representative GluR5(R)/KA-2 whole-cell current and spectrum. GluR5(R)/KA-2 gave a whole-cell response upon application of 20 μM domoate. V_h was −80 mV. The spectrum was fitted with the sum of two Lorentzian functions. The mean single-channel conductance and time constants were calculated from the Lorentzian fits as described in Methods. The spectrum shown gave a mean conductance of 1·2 pS. *C*, representative GluR5(Q)/KA-2 whole-cell current and spectrum. GluR5(Q)/KA-2 gave a whole-cell response upon application of 5 μM domoate. V_h was −80 mV. The spectrum was fitted with the sum of two Lorentzian functions. The mean single-channel conductance and time constants were calculated from the Lorentzian fits as described in Methods. The spectrum shown gave a mean conductance of 3·9 pS.

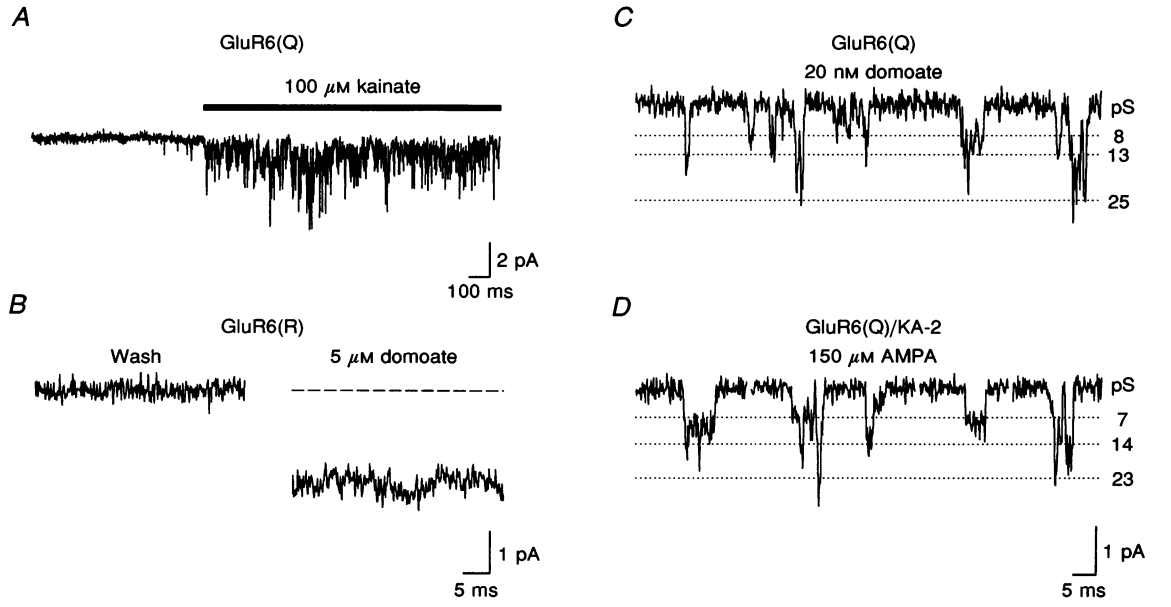

Figure 3. GluR6 single-channel events

A, a slow time scale recording of GluR6(Q) channel openings evoked by 100 μM kainate (patch held at −80 mV). *B*, GluR6(R)-containing patch in the absence (left) and presence (right) of 5 μM domoate. The dashed line indicates the position of the baseline current in the absence of domoate. Note the absence of resolvable events and the modest current noise increase. *C*, GluR6(Q) channel openings in the presence of 20 nM domoate. Dotted lines indicate the 8, 13, and 25 pS conductance levels fitted for this patch. *D*, GluR6(Q)/KA-2 channel openings in the presence of 150 μM AMPA. Dotted lines indicate the 7, 14, and 23 pS conductance levels fitted for this patch. This patch had an exceptionally large number of receptors; typical responses to AMPA were not as robust and tended to run down, thus making it difficult to obtain sufficient numbers of channel events to construct histograms.

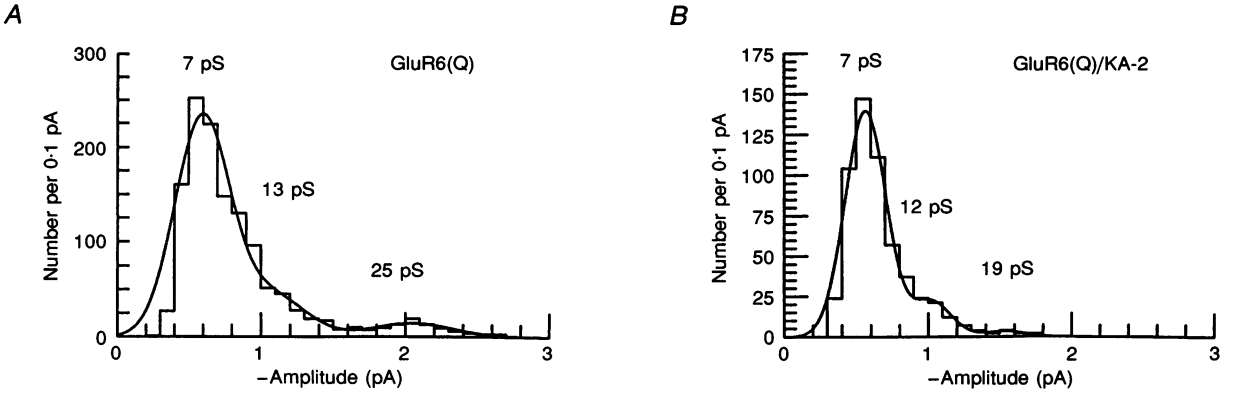

Figure 4. Amplitude histograms of GluR6(Q) and GluR6(Q)/KA-2 channels

A, GluR6(Q) channel amplitude distributions compiled from the time course fitted data. Resolution is set to two filter rise times (332 μs) to fully resolve single-channel amplitudes. Histogram was fitted with the sum of three Gaussian components; the peaks represent the mean current, from which the conductance of each level (7, 13 and 25 pS) was derived. *B*, GluR6(Q)/KA-2 channel distribution fitted with the sum of three Gaussian components (7, 12 and 19 pS). Single-channel currents were evoked by 10–50 nM domoate. Outside-out membrane patches were held at −80 mV.

estimated from the GluR5(R)/KA-2 spectrum in Fig. 2*B* was 1·2 pS; the mean value was 0·95 ± 0·22 pS ($n = 3$). Cut-off frequencies were 30 ± 11 and 169 ± 62 Hz for GluR5(Q)/KA-2 channel spectra, and 8 ± 2 and 100 ± 32 Hz for GluR5(R)/KA-2 spectra, yielding mean time constants of 6·0 ± 1·8 and 1·1 ± 0·4 ms for GluR5(Q)/KA-2, and 19·6 ± 3·5 and 1·7 ± 0·5 ms for GluR5(R)/KA-2.

Unedited kainate receptor–channels have multiple conductance levels

GluR6. We directly resolved the individual openings for homomeric and heteromeric receptor types. Figure 3*A* shows an example of a GluR6(Q) patch with a high level of channel activity in response to 100 μM kainate. On the other hand, GluR6(R) patches (Fig. 3*B*) gave a small noise increase (in response to 0·01–5 μM domoate) without resolvable single-channel events. Both GluR6(Q) and GluR6(Q)/KA-2 patches gave single-channel currents that were readily detected when the frequency of events was low (Fig. 3*C* and *D*).

Single-channel currents often exhibited rapid transitions between multiple conductance levels, indicating that homomeric and heteromeric kainate receptors can open to several discrete substates. Furthermore, as is apparent in Fig. 4, the conductance levels associated with GluR6(Q) and GluR6(Q)/KA-2 channels were similar, suggesting that the channel conductance was not markedly altered by the presence of the KA-2 subunit. In the examples shown these were 7, 13 and 25 pS for GluR6(Q) and 7, 12 and 19 pS for GluR6(Q)/KA-2. The majority of GluR6(Q) and GluR6(Q)/ KA-2 receptor activations appeared to produce brief bursts. In this respect, as well as in their conductance levels, they resembled high-conductance AMPA receptor–channels in cerebellar granule cells (Wyllie *et al.* 1993), rather than the GluR5-containing kainate receptor observed in sensory neurones (Huettner, 1990), or the recombinant GluR5(Q) channels described below (Fig. 5).

To allow a rigorous characterization of both the amplitude and duration of the individual GluR6(Q) and GluR6(Q)/ KA-2 channel openings we analysed the discrete events with time course fitting. Channel events occurring at low frequency were fitted with the step response function of the recording system and were then analysed after imposition of a minimal time resolution on the data set (see Methods). Amplitude histograms of GluR6(Q) channel currents activated by domoate (see for example Fig. 4*A*) were best fitted by three Gaussian components. The mean conductance values we obtained were 7·7 ± 0·3, 14·7 ± 1·1 and 24·7 ± 1 pS (relative areas, 81·2 ± 2·3, 14·5 ± 1·1 and 4·3 ± 1·2% of the fitted openings; $n = 4$). Similar multiple conductance levels were obtained in response to glutamate (data not shown). Openings to each amplitude level could occur independently, confirming that each represented a discrete conductance state (rather than, for example, the superimposed opening of multiple events).

GluR6(Q)/KA-2 channel openings were also fitted by three Gaussian components (see Fig. 4*B*). The mean conductance

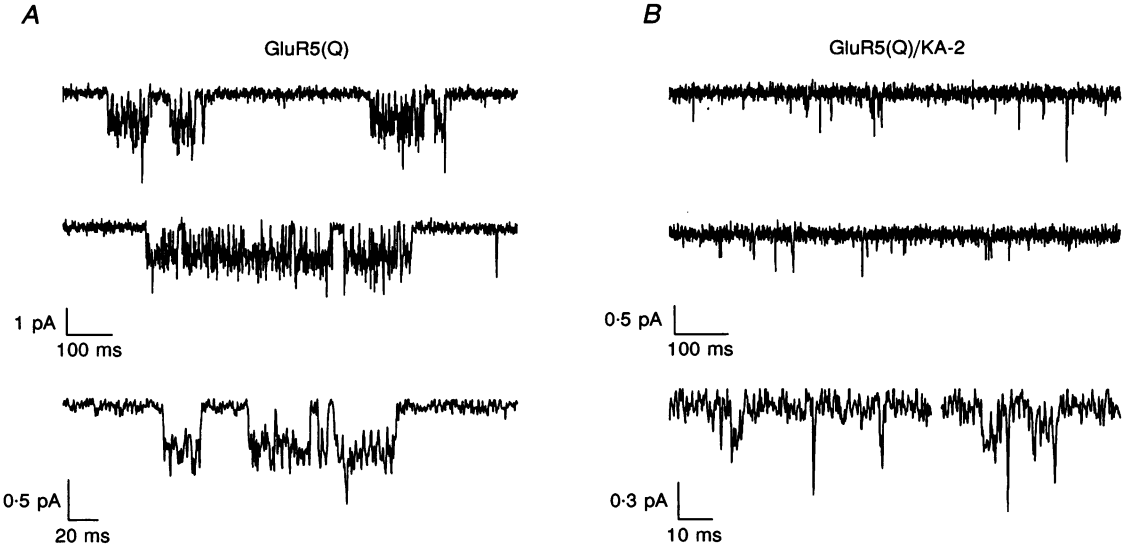

Figure 5. GluR5(Q) and GluR5(Q)/KA-2 single-channel events

A, GluR5(Q) single-channels events evoked by 200 nM domoate on a slow (top) and fast (bottom) time base. Note the brief dwell times at each subconductance level, as well as the large numbers of distinct amplitudes during a single burst activation. *B*, GluR5(Q)/KA-2 single-channel events evoked by 50 nM domoate on a slow (top) and fast (bottom) time base. Note the brief burst lengths and poorly resolved higher conductance levels. Recordings shown are from outside-out membrane patches excised from HEK-293 cells and were maintained at a holding potential of −80 mV.

values obtained were 7·4 ± 0·3, 12·8 ± 0·6 and 20·0 ± 1·3 pS (73·9 ± 6·1, 20·3 ± 3·7 and 5·8 ± 2·4%; $n = 4$). Again these values were not significantly different from those obtained with the homomeric receptor. The amplitudes of the AMPA-activated multiple conductance levels were comparable to those obtained with domoate (data not shown, but see Fig. 3*D*). Since the homomeric GluR6 channels are insensitive to AMPA (Herb *et al.* 1992) this observation is consistent with the idea that events in GluR6(Q)/KA-2 patches arose mainly from the activation of heteromeric receptors.

In patches where the background noise level was particularly low we were also able to detect a lower conductance level (<3 pS) following the activation of GluR6(Q) and GluR6(Q)/KA-2 receptors. These events were too small and brief to permit an accurate analysis. The presence of this and additional lower conductance states could explain the conductance estimates we obtained from variance *versus* mean current plots, which were somewhat lower than the weighted mean conductance that could be calculated from the amplitude histograms.

GluR5. As shown in Fig. 5*A*, homomeric receptors composed of GluR5(Q) subunits exhibited channel openings which occurred in characteristic bursts, separated by long shut intervals. The open-channel noise was high and seemed to arise from rapid switching between multiple conductance levels, rather than brief closures of the channel within an activation. However, because the primary conductance levels were relatively small, our ability to resolve brief closures was limited. As shown in Fig. 5*B*, domoate activated much briefer channel events in patches containing GluR5(Q)/KA-2 channels. No prolonged bursts were observed, and relatively few channel events appeared to reach a full amplitude. Furthermore, the application of agonist to the GluR5(Q)/KA-2 patches produced a clear increase in the baseline noise between the discrete channel events (see Fig. 5*B*, bottom trace), suggesting the presence of additional low unresolved conductances states for this receptor type. No resolvable single-channel events were detected in outside-out patches containing GluR5(R)/KA-2 channels.

In most records, visual inspection of the GluR5(Q) channel records suggested the existence of at least three discrete conductance levels for the homomeric channel. As shown in Fig. 6*A*, the sum of three Gaussian curves consistently provided the best fit to the amplitude histograms; in this example the values were 5, 9 and 15 pS. The three fitted Gaussian components gave mean conductance values of 5·1 ± 0·7, 8·6 ± 0·1 and 13·5 ± 1·3 pS (means ± S.E.M.; relative areas, 21 ± 4, 67 ± 16 and 12 ± 14%, respectively; $n = 4$ patches). The weighted mean conductance of GluR-5(Q) channels calculated from amplitude distributions was 9 pS. This value was significantly higher than that measured by variance analysis of the patch response (2·9 ± 0·5 pS; $n = 4$), suggesting that this receptor may also have conductance states below the level of resolution in outside-out patches. Amplitude histograms for GluR5(Q)/KA-2 activations were also best fitted with the sum of three Gaussian components. For the example shown in

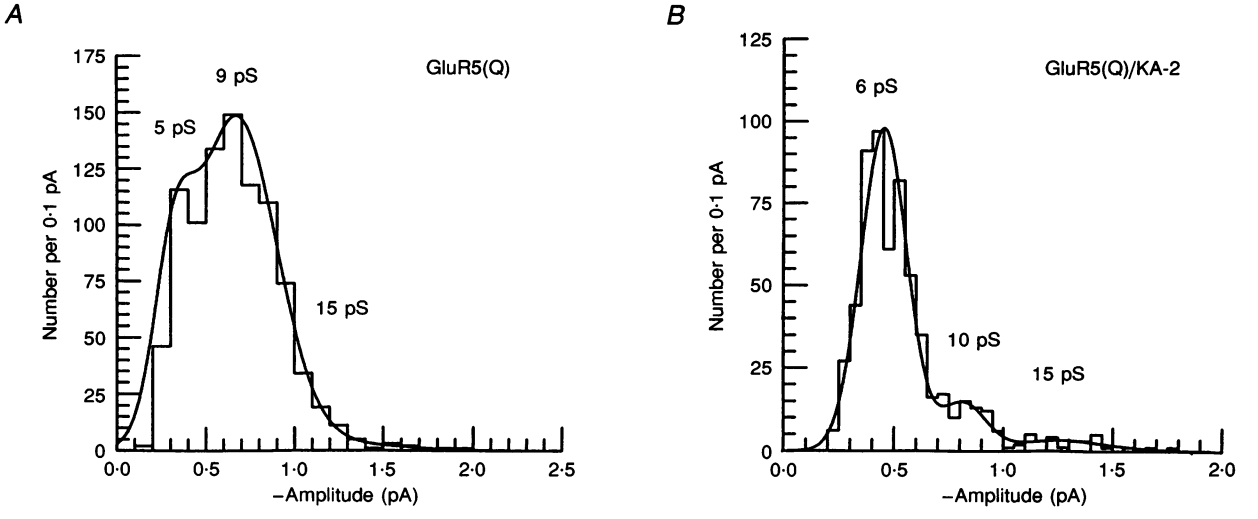

Figure 6. GluR5(Q) and GluR5(Q)/KA-2 amplitude histograms

A, GluR5(Q) channel amplitude distributions (5, 9 and 15 pS) compiled from the time course fitted data. *B*, GluR5(Q)/KA-2 channel amplitude distributions (6, 10 and 15 pS) compiled from the time course fitted data. The resolution is set to two filter rise times (332 μs) to fully resolve single-channel amplitudes. Histograms were fitted with the sum of three Gaussian components; the peaks represent the mean current from which the conductance of each level was derived. Conductance levels for these patches are shown on the histogram. See text for the mean values for all the patch recordings.

Fig. 6*B* the amplitudes were 6, 10 and 15 pS. Our mean conductance estimates for GluR5(Q)/KA-2 patches were 5·4 ± 0·2, 9·1 ± 1·6 and 16·9 ± 2·2 pS (relative areas, 77 ± 2, 18 ± 3 and 5 ± 3%, respectively; $n = 4$). These values were not significantly different from those of the homomeric GluR5(Q) channel. However, the relative proportion of the lowest conductance level (~5 pS) was considerably higher in the amplitude distributions obtained from heteromeric channels. This could, in part, reflect the faster kinetics of these channel activations.

Kinetic properties of kainate receptor–channels

GluR6. The kinetic properties of channel openings were analysed by constructing histograms of apparent open- (or dwell-) time distributions from the cursor-fitted data. Open-time histograms for GluR6(Q) and GluR6(Q)/KA-2 events were fitted by the sum of two exponential components (see Fig. 7*A* and *B*). The mean time constants obtained were 0·55 ± 0·14 and 2·30 ± 0·33 ms (76·2 and 23·8% of the open times, respectively; $n = 4$) for GluR6(Q) and 0·41 ± 0·15 and 2·05 ± 0·98 ms (87·5 and 12·5% of the open times, respectively; $n = 4$) for GluR6(Q)/KA-2. The open times were similar to the time constants derived from spectral analysis (not shown). These data suggest that, for the GluR6(Q) receptor channel, co-assembly with the KA-2 subunit does not significantly alter the kinetics of the domoate-activated channels.

GluR5. In contrast, the activations of the homomeric GluR5(Q) receptors were considerably longer than those produced by either GluR5(Q)/KA-2 or GluR6 receptors. For GluR5(Q), open-time distributions were best fitted by the sum of two exponential distributions (Fig. 8*A*). For GluR5(Q) patches the channels had mean apparent open times of 0·27 ± 0·05 and 0·61 ± 0·06 ms ($n = 4$). For GluR5(Q)/KA-2 patches (see Fig. 8*B*) the channel openings had a similar fast component ($\tau = 0{\cdot}26 \pm 0{\cdot}04$ ms) but no detectable slow component. These results indicate that co-assembly with KA-2 subunits shifts the mean open times of the GluR5 receptor to briefer durations.

The difference between activations of the two channel types was more strikingly reflected in the burst characteristics of the channels. To analyse the burst length we examined the shut-time distributions, which were typically fitted with four or five components (data not shown). Critical gap lengths were calculated from the first two to three exponential components, which appeared to be concentration independent. Burst-length distributions of GluR5(Q) and GluR5(Q)/KA-2 channel events are shown in Fig. 8*C* and *D*. Both distributions were fitted with the sum of exponential components. Typically, the GluR5(Q) patches yielded burst-length histograms with three components with mean values of 0·16 ± 0·04, 2·3 ± 1·0 and 31·0 ± 12·5 ms (relative areas, 53 ± 10, 28 ± 9 and 19 ± 2%, respectively; $n = 3$), clearly reflecting the long activations of this channel type. Within individual bursts, the probability of GluR5(Q) channels being open (burst P_{open}) was 0·78 ± 0·05. Higher-order clustering of channel burst activations was also observed, and was generally concentration dependent but highly variable. In contrast, GluR5(Q)/KA-2 patches gave openings with two burst-length components with mean values of 0·13 ± 0·03 and 1·80 ± 0·47 ms (areas, 62 and 38%, respectively; $n = 4$). These data therefore demonstrate that the KA-2 subunit primarily reduced the burst length of the GluR5(Q) channel.

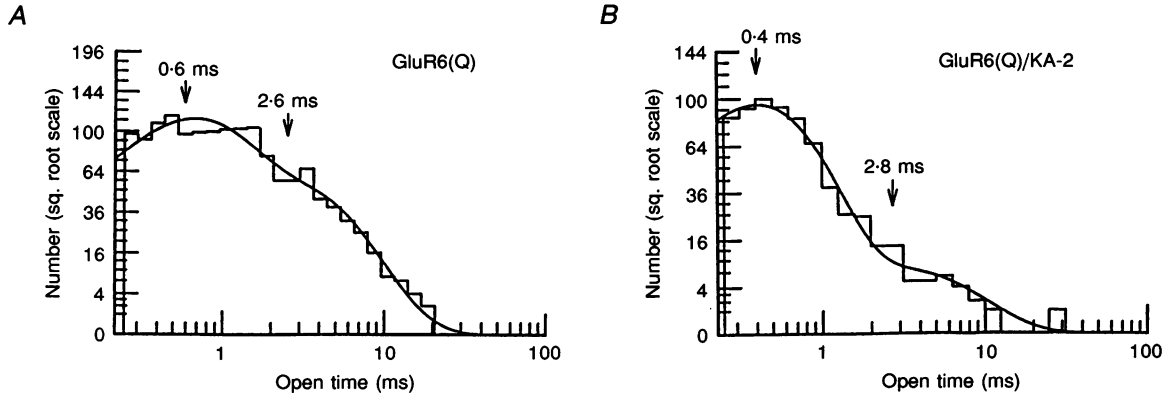

Figure 7. Open-time histograms of GluR6(Q) and GluR6(Q)/KA-2 channels

A, GluR6(Q) channel open-time distribution compiled from the time course fitted data. Resolution was set to 250 μs to minimize inclusion of false events. The histogram was fitted with the sum of two exponential components; the peaks represent the time constants of 0·6 and 2·6 ms. *B*, GluR6(Q)/KA-2 channel distributions fitted with the sum of two exponential components with time constants of 0·4 and 2·8 ms. Single-channel currents were evoked by 10–50 nM domoate. Outside-out membrane patches were held at −80 mV.

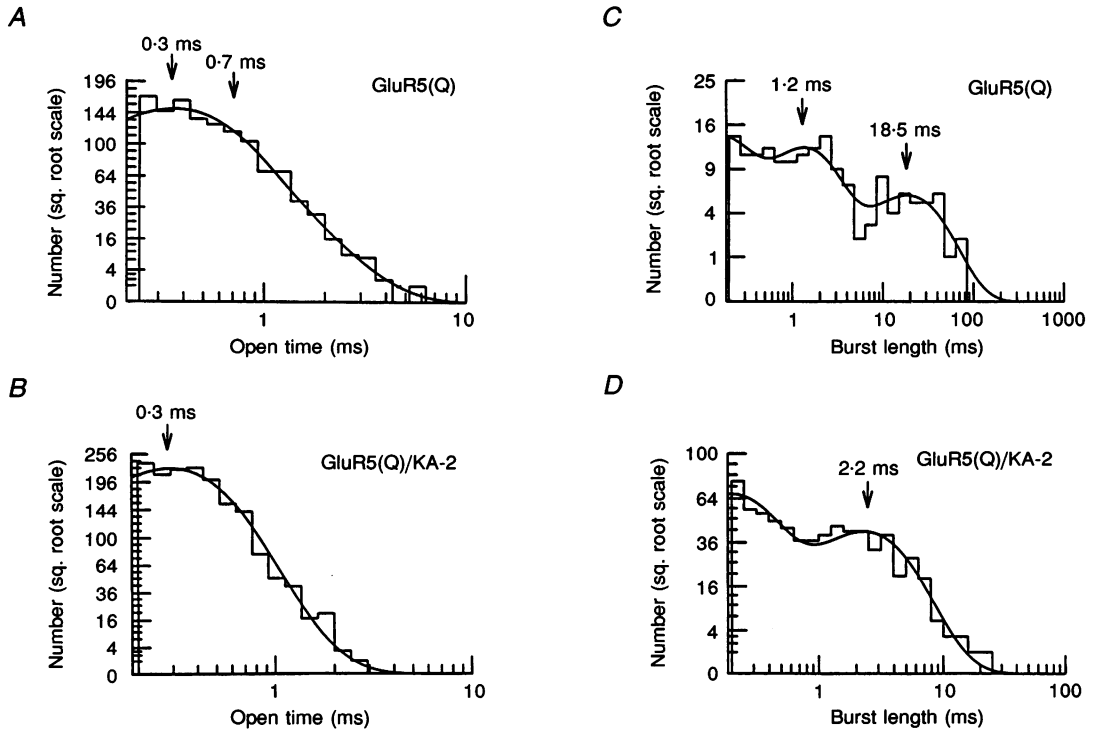

Figure 8. Kinetics of GluR5(Q) and GluR5(Q)/KA-2 single channels

A, GluR5(Q) channel open-time distribution compiled from the time course fitted data and fitted with the sum of two exponential components with time constants of 0·3 and 0·7 ms. *B*, GluR5(Q)/KA-2 channel open-time distribution fitted with a single exponential component (0·3 ms). *C*, distribution of burst lengths for GluR5(Q) openings, fitted with the sum of three exponentials. The two slowest time constants (1·2 and 18·5 ms) are given for the example shown. Mean time constants are given in the text. *D*, GluR5(Q)/KA-2 burst-length distribution fitted with the sum of two exponentials. Only the slowest time constant (2·2 ms) is given for the example shown (the shortest burst-time constant was below the resolution of the burst-length analysis). Analyses of burst structure were carried out as described in Methods and Results. Single-channel currents were evoked by 50–200 nM domoate. Outside-out membrane patches were held at −80 mV.

DISCUSSION

Subunit composition and single-channel properties

Our experiments demonstrate that the amino acid at the Q/R site directly influences the conductance properties of the kainate receptor channel. Thus the single amino acid change that results from RNA editing reduces the mean conductance of homomeric GluR6 channels by roughly 25-fold and of homomeric GluR5 channels by >10-fold. Taken together with the observations that editing at the Q/R site controls calcium permeability and rectification properties of kainate receptor currents (Hume, Dingledine & Heinemann, 1991; Burnashev, Monyer, Seeburg & Sakmann, 1992; Egebjerg & Heinemann 1993; Köhler *et al.* 1993; Burnashev *et al.* 1995), our results support the idea that the Q/R site participates in the formation of the channel pore.

One of our aims in characterizing single-channel properties of recombinant non-NMDA receptors was to obtain information which could aid the identification of native kainate receptors in neurones. Of the eight receptor types we have examined, each showed distinct channel properties. As summarized in Table 1, channels which contained an unedited GluR5 or GluR6 subunit gave directly resolved single-channel events with multiple conductance levels. Conversely, combinations with edited subunits resulted in channels with conductances that were too small to be directly resolved and merely produced a noise increase. The heteromeric and homomeric GluR6(Q)-containing receptors gave channel conductances that were similar to each other but could be distinguished pharmacologically, as only GluR6(Q)/KA-2 channels could be activated by AMPA (see Fig. 3 and Herb *et al.* 1992). Thus in conjunction with the pharmacological and molecular information, single-channel properties could prove useful in attempts to obtain a functional separation of the native non-NMDA receptor types. This has generally been hampered by the presence of mixed receptor populations with multiple conductance levels in central neurones (see Wyllie *et al.* 1993).

Table 1. Single-channel conductances of recombinant kainate receptors

Unedited	Edited
GluR6(Q) 8/15/25 pS (5·4 pS)	GluR6(R) 225 fS
GluR6(Q)/KA-2 7/13/20 pS (7·1 pS)	GluR6(R)/KA-2 700 fS
GluR5(Q) 5/9/14 pS (2·9 pS)	GluR5(R) < 200 fS
GluR5(Q)/KA-2 5/9/17 pS (4·5 pS)	GluR5(R)/KA-2 950 fS

Conductance levels determined from time course fitting of channels are given for the unedited kainate receptor–channels in the left-hand column. Mean conductances from noise analysis are given in parentheses for the purpose of comparison. In the right-hand column, the mean conductance value for edited kainate receptors are shown; these were derived from noise analysis as described in the text.

Contribution of KA-2 to channel function

Depending on the receptor isoform with which it was co-expressed, the KA-2 subunit was found to influence the channel conductance (for GluR5(R) and -6(R)) or kinetics (GluR5(Q)) of the low-affinity subunits. The KA-2 subunit, which has been characterized as non-functional when expressed alone (Herb *et al.* 1992), has glutamine at its Q/R site. The fact that KA-2 caused an appreciable change in the single-channel conductance only when co-expressed with the arginine-containing isoforms of GluR5 and -6 suggests that the number of arginines (which will depend on the stoichiometry of the channel) is a determinant of the channel conductance.

In the present study, we found that KA-2 subunits had a striking effect on the burst parameters of GluR5(Q) channels. In response to domoate, heteromeric GluR5(Q)/KA-2 channels showed very brief activations (generally less than a millisecond) whereas homomeric GluR5(Q) channels opened for extended periods of time. On the other hand, both homomeric and heteromeric GluR6(Q)-containing receptors exhibited brief channel activations (although somewhat longer than the GluR5(Q)/KA-2 channel openings). The contribution of the KA-2 subunit to microscopic channel kinetics was therefore subunit specific, suggesting that association of the low-affinity subunits with KA-2 may provide a selective mechanism for regulating the kinetic response of kainate receptors.

Comparison with native receptors

A native non-NMDA glutamate channel with an estimated conductance in the femtosiemens range, similar to that of the low-conductance homomeric GluR6(R) channels described here, has been described previously for cerebellar granule cells in culture (Cull-Candy & Ogden, 1985; Cull-Candy, Howe & Ogden, 1988). While it is uncertain whether the femtosiemens channel in granule cells is of the AMPA or kainate type, it is notable that glutamate channels of such exceptionally low conductance can occur in neurones. Furthermore, the homomeric GluR5(R) channel conductance may be even lower than the native femtosiemens channel or the GluR6(R) channel. The physiological significance (if they occur *in vivo*) of these extremely low-conductance homomeric channels is unclear; one possibility is that they could act as glutamate-binding proteins, rather than functional ion channels. As low-conductance channels have generally been difficult to study in neurones (due to the presence of high-conductance events arising from other glutamate receptor–channels), use of recombinant low-conductance channels could provide a valuable alternative.

There have been few patch-clamp studies of native kainate-preferring receptors, the first example being that of Huettner (1990) who described domoate-activated currents in a subset of freshly isolated dorsal root ganglion (DRG) neurones. These receptors show slowly desensitizing responses to domoate, typical of recombinant homomeric GluR5(Q) currents. The linear current–voltage (I–V) relationship of the response (Huettner, 1990) could be explained by wash-out of intracellular polyamines, which confer rectification on kainate receptors (for example, see Kamboj, Swanson & Cull-Candy, 1995). It was subsequently found that these small sensory neurones express high levels of mRNA for GluR5 and KA-2 and low levels for KA-1 (Sato, Kiyama, Park & Tohyama, 1993; Partin *et al.* 1993). Huettner (1990) described several features of DRG kainate receptors of particular relevance to our study. First, the estimated channel conductance obtained from domoate and kainate noise was 2–4 pS (after treatment with ConA to suppress desensitization). Second, the predominant single-channel conductance levels resolved in isolated patches were

4 and 8 pS. Third, the single-channel openings appeared to occur in long-lasting bursts. These properties match reasonably well those which we describe for homomeric GluR5(Q) single channels in the present study.

The prolonged burst length of domoate-activated GluR5(Q) channel events made them strikingly different from the other recombinant kainate receptor–channels. These events have a comparatively large amount of open-channel noise, which may arise from rapid switching between two primary conductance states (combined with a relatively low signal-to-noise ratio, due to the small conductance of the channels). We observed that the GluR5(Q) burst activations were grouped in clearly definable clusters, in which the probability of the channel being open was extremely variable. The physiological significance of this variability is still unclear, although similar channel behaviour in $GABA_A$ receptors of the superior cervical ganglion was suggested to result from kinetically distinct receptor populations or a complex interplay between activation and desensitization behaviour (Newland, Colquhoun & Cull-Candy, 1991). In the present study, the existence of different receptor populations seems unlikely, making a kinetic basis for the variability a more plausible explanation.

Kainate receptors and synaptic transmission

There is currently no direct electrophysiological evidence for an involvement of kainate receptors in synaptic transmission although in most cases their contribution to synaptic currents has not been examined in detail. Currents mediated by kainate receptors in central neurones have only been detected in culture (Lerma, Paternain, Naranjo & Mellstrom, 1993). This elusiveness could arise, in part, from the fact that kainate receptor responses to glutamate desensitize rapidly and may be electrically remote from the cell soma. If present at synapses, the unusually low conductance of the edited form of the GluR-6 kainate receptor would require that hundreds of channels be activated to generate a detectable response, even in small cells with high resolution of synaptic currents.

Do kainate receptor–channels exhibit any distinct characteristics that shed light on their possible roles *in vivo*? The potency of glutamate at kainate and AMPA receptors is not strikingly different; steady-state EC_{50} values are roughly 2- to 10-fold higher for kainate receptors when compared with AMPA receptors (see Hollmann & Heinemann, 1994). A more physiologically relevant peak (non-desensitized) glutamate EC_{50} for glutamate of 273 μM has been measured for GluR6 (Raymond, Blackstone & Huganir, 1993). Our apparent open times are comparable to the deactivation kinetics of AMPA receptors (~600 μs for GluR4(flop), which shows the fastest gating of all AMPA receptor subunits; see Mosbacher, Schoepfer, Monyer, Burnashev, Seeburg & Ruppersberg, 1994). However, the latency from agonist binding to first channel opening is unknown and could influence the deactivation kinetics.

One intriguing possibility is that kainate receptors may be involved in postsynaptic calcium entry. The Ca^{2+} permeability of the edited GluR6(R) kainate receptor subunit has been subject to some uncertainty, as separate reports have suggested it is more permeable (Köhler *et al.* 1993) or less permeable (Egebjerg & Heinemann, 1993) than unedited GluR6(Q) channels. Recent measurements of fractional calcium currents have demonstrated that the calcium flux through GluR6(R) channels, in physiological bathing solutions, is extremely low (Burnashev *et al.* 1995). Thus the net result of Q/R site editing on Ca^{2+} influx through kainate receptors appears similar to its effect on AMPA receptors (despite the apparent high calcium permeability of GluR6(R); see Burnashev *et al.* 1995). However, Ca^{2+}-permeable kainate receptors are likely to comprise a significant proportion of the total kainate receptor population (based on the relative proportion of unedited to edited GluR6 mRNAs; Köhler *et al.* 1993), while RNA for the AMPA receptor subunit GluR2 (which is present in the majority of neurones) is almost completely edited at birth (Sommer *et al.* 1991). It is therefore tempting to speculate that kainate receptors may be expressed preferentially at those synapses where an ability to adjust the Ca^{2+} permeability may be required.

RNA editing as a means of regulating channel properties

RNA editing is clearly important for channel function and appears to be tightly regulated. Thus the rate and extent of editing differs between subunits and also between editing sites on the same subunit. Similarly, editing of a given site may proceed to dissimilar extents in different cell populations (Sommer *et al.* 1991; Burnashev *et al.* 1992; Köhler *et al.* 1993; Bernard & Khrestchatisky, 1994; Paschen, Dux & Djuricic, 1994; Puchalski *et al.* 1994; Paschen & Djuricic, 1995). Furthermore, GluR5 mRNA assessed with single-cell PCR in hippocampal CA1 neurones in acute slices was completely unedited (Mackler & Eberwine, 1993). As GluR5 mRNA was found to be edited by 50–60% at the Q/R site in the hippocampus (Sommer *et al.* 1991; Köhler *et al.* 1993; Bernard & Khrestchatisky, 1994; Paschen *et al.* 1994), these results imply that restricted neuronal populations may not necessarily reflect more global editing patterns (which may thus be of limited value in predicting the subunit complement of individual cells). In contrast, it has been demonstrated recently that edited and unedited RNAs of GluR6 co-exist within the same cell in a population of cultured hippocampal neurones (Ruano *et al.* 1995), although the evidence for functional co-assembly of subunit isoforms was equivocal.

RNA editing has also been shown to be developmentally regulated. The majority of GluR5 and -6 mRNAs are reported to be unedited in embryonic rat brain, with the degree of editing increasing to 50–70% and 75–80%,

respectively, in the adult (with roughly the same relative proportions in major subdivisions of the brain; Sommer *et al.* 1991; Köhler *et al.* 1993; Bernard & Khrestchatisky, 1994; Paschen *et al.* 1994; Paschen & Djuricic, 1995). This contrasts with RNA for the GluR2 AMPA receptor subunit which is almost completely edited at birth (Sommer *et al.* 1991). Our results suggest that editing would change not only the relative ionic permeabilities of non-NMDA receptors, but also the mean current amplitude. Furthermore, our findings imply that in the mature CNS low-conductance kainate receptors will predominate. Developmental and cellular regulation of Q/R site editing in GluR5 and -6 would have profound effects on a number of aspects of the receptor response to glutamate and thus could act as an elegant mechanism for modifying or modulating the contribution of kainate receptors to neurotransmission.

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