Regulation of Kinetic Properties of GluR2 AMPA Receptor Channels by Alternative Splicing

Maki Koike,1,2 Shota Tsukada,1 Keisuke Tsuzuki,1,2 Hiromasa Kijima,3 and Seiji Ozawa1,2

1Department of Physiology, Gunma University School of Medicine, Maebashi, Gunma 371–8511, Japan, 2Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawaguchi, Saitama 332–0012, Japan, and 3Department of Physics, Graduate School of Science, Nagoya University, Nagoya 464–8602, Japan

The four subunits of the AMPA-type glutamate receptor (GluR1–GluR4 or GluRA–GluRD) exist in two distinct forms, flip and flop, generated by alternative splicing of a 115 bp region. The GluR2 subunit plays a key role in determining the functional properties of the AMPA receptor channel. In this study, we examined the differences in kinetic properties between the flip and flop splice variants of the GluR2 subunit expressed in Xenopus oocytes using fast agonist application techniques. Glutamate was applied to outside-out patches from oocytes with piezo-driven double-barreled application pipettes. Because homomeric receptor channels composed of the edited form of GluR2 (GluR2R) produce no appreciable current responses, we expressed the unedited form of GluR2 (GluR2Q) in oocytes, which produced large current responses sufficient for analysis of the kinetic properties. The time constant for desensitization during application of 1 mM glutamate was 5.89 ± 0.17 msec (n = 50) in flip and 1.18 ± 0.05 msec (n = 37) in flop. The deactivation time constant was 0.62 ± 0.06 msec (n = 10) in flip and 0.54 ± 0.05 msec (n = 10) in flop. The steady-state non-desensitizing current was 6.8 ± 0.4% (n = 53) of the peak current in flip, whereas it was almost negligible in flop, being only 1.1 ± 0.1% (n = 36). The slower desensitization kinetics and larger steady-state current responses in the flip variant were also observed in heteromeric receptors assembled from GluR2Q/GluR2R. Thus, desensitization occurred much more prominently in the flop variant in the recombinant GluR2 receptor channels.

Key words: AMPA receptor channel; GluR2; flip/flop splice variants; Q/R site; desensitization; deactivation

AMPA receptor channels (AMPARs) mediate fast excitatory neurotransmission in most of the synapses in the CNS. Four cDNA clones of AMPA receptor subunits GluR1–GluR4 (GluRA–GluRD) have been isolated by molecular cloning (Hollmann et al., 1989; Boulter et al., 1990; Keinänen et al., 1990). The GluR2 subunit plays a key role in determining the functional properties of AMPAR, such as rectification properties and Ca2+ permeability (Hollmann et al., 1991; Bochet et al., 1994; Jonas et al., 1994). Receptors with GluR2 exhibit either either linear or outwardly rectifying current–voltage (I–V) relationships and little Ca2+ permeability, whereas those without GluR2 show inward rectification and high Ca2+ permeability. The unique role of GluR2 has been traced to a single amino acid residue in the second hydrophobic region (M2). This residue is an arginine (R) in GluR2, whereas it is a glutamine (Q) in the other subunits. This site has been referred to as the Q/R site (Hume et al., 1991; Monyer et al., 1991). The GluR2R subunit expressed in Xenopus oocytes using fast agonist application techniques. Glutamate was applied to outside-out patches from oocytes with piezo-driven double-barreled application pipettes. Because homomeric receptor channels composed of the edited form of GluR2 (GluR2R) produce no appreciable current responses, we expressed the unedited form of GluR2 (GluR2Q) in oocytes, which produced large current responses sufficient for analysis of the kinetic properties. The time constant for desensitization during application of 1 mM glutamate was 5.89 ± 0.17 msec (n = 50) in flip and 1.18 ± 0.05 msec (n = 37) in flop. The deactivation time constant was 0.62 ± 0.06 msec (n = 10) in flip and 0.54 ± 0.05 msec (n = 10) in flop. The steady-state non-desensitizing current was 6.8 ± 0.4% (n = 53) of the peak current in flip, whereas it was almost negligible in flop, being only 1.1 ± 0.1% (n = 36). The slower desensitization kinetics and larger steady-state current responses in the flip variant were also observed in heteromeric receptors assembled from GluR2Q/GluR2R. Thus, desensitization occurred much more prominently in the flop variant in the recombinant GluR2 receptor channels.

Key words: AMPA receptor channel; GluR2; flip/flop splice variants; Q/R site; desensitization; deactivation

AMPA receptor channels (AMPARs) mediate fast excitatory neurotransmission in most of the synapses in the CNS. Four cDNA clones of AMPA receptor subunits GluR1–GluR4 (GluR-A–GluR-D) have been isolated by molecular cloning (Hollmann et al., 1989; Boulter et al., 1990; Keinänen et al., 1990). The GluR2 subunit exists in two different forms, flip and flop, generated by alternative splicing of a 115 bp region immediately preceding M4 (Sommer et al., 1991; Burnashev et al., 1992). The molecular diversity of AMPAR subunits is further increased by the presence of splicing variants. Each of GluR1–GluR4 exists in two different forms, flip and flop, generated by alternative splicing of a 115 bp region immediately preceding M4 (Sommer et al., 1990; Monyer et al., 1991). Using a fast perfusion technique in outside-out membrane patches of Xenopus oocytes expressing recombinant AMPA receptor subunits, Mosbacher et al. (1994) have shown that, among homomeric GluR1, GluR3, and GluR4 receptors, the GluR4 flop receptor shows the fastest desensitization time constant, 0.9 msec, and the GluR3 flip receptor the slowest, 4.8 msec, when 1 mM glutamate is applied rapidly. The desensitization kinetics of homomeric GluR2, however, have not been investigated, because the homomeric receptor composed of the edited form of GluR2 does not generate sufficiently large current responses to allow detailed analysis of the channel-gating kinetics. In this study, we examined differences in the kinetics of desensitization and deactivation between flip and flop in homomeric GluR2Q and heteromeric GluR2Q/GluR2R receptors expressed in Xenopus oocytes, from which appreciable current responses could be recorded in the outside-out patch configuration.

MATERIALS AND METHODS

Plasmids and mutagenesis. GluR2Q cDNA was produced by site-directed mutagenesis (Sculptor IVM; Amersham, Buckinghamshire, UK) that exchanged G at position 1820 of the GluR2 flip to A. This point mutation

Received Oct. 29, 1999; revised Jan. 3, 2000; accepted Jan. 5, 2000.

This work was supported by the Japan Science and Technology Corporation and Research Fellowships from the Japan Society for the Promotion of Science for Young Scientists.

Correspondence should be addressed to Maki Koike, Department of Physiology, Gunma University School of Medicine, 3-39-22 Showa-machi Maebashi, Gunma, 371–8511 Japan. E-mail: mkoike@sb.gunma-u.ac.jp.

Copyright © 2000 Society for Neuroscience 0270-6474/00/202166-09$15.00/0
changed arginine (R) at the O/R site to glutamine (Q). GluR2Q flop cDNA used in this study was synthesized from GluR2Q flop cDNA by replacing the Bam-HIOL fragment (nucleotide 2278–3300 that contains the flip–flop site) with the corresponding portion of the wild-type GluR2 flop cDNA. The rat wild-type GluR2 flop and GluR2Q flop cDNAs were kind gifts from Drs. Jim Boulter, Stephan F. Heinemann, and Michael Hollmann (Salk Institute, La Jolla, CA). It has been shown that kinetic properties of recombinant AMPARs are affected by RNA editing. In the GluR2 subunit, a codon switch from AGA (arginine) to GGA (glycine) in the primary transcript is determined by intronic elements at a position termed the R/G site that immediately precedes the flip–flop site (Lomeli et al., 1994). In this study, we used the edited form in which the R/G site is occupied by G (glycine) for both flip and flop variants. G form, the edited form, is predominant in both flip and flop variants of the GluR2 subunit in the adult brain (Lomeli et al., 1994).

Expression of cDNAs: Capped cRNAs for GluR2Q and GluR2R were transcribed with T3 RNA polymerase in vitro from linearized plasmids (MEGAscript T3 kit; Ambion, Austin, TX). GluR2Q cRNA (∼50 ng/oocyte) was injected into oocytes of Xenopus laevis. After incubation for 1–2 d in modified Barth’s medium containing (in mM): 88 NaCl, 1 KCl, 2.4 NaHCO3, 0.3 Ca(NO3)2, 0.4 CaCl2, 0.8 MgSO4, and 15 HEPES, pH 7.6 adjusted to 7.2 with KOH. To maintain rectification properties of the AMPAR in the outside-out patch configuration, 100 μM spermine was added to the internal solution (Bowie and Mayer, 1995; Donevan and Rogawski, 1995; Isa et al., 1995; Kamboj et al., 1995; Koh et al., 1995). In some experiments, 2 mM Mg2ATP and 0.2 mM Na3GTP were added to the internal solution. The pipettes had a resistance of 3–5 MΩ when filled with the internal solution. For recording in the outside-out patch mode, the vitelline envelopes were removed with forceps after immersing the oocytes in hypertonic Ringer’s solution for several minutes. All recordings were made using an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, Germany). Current traces were filtered at 3 kHz with an eight-pole low-pass Bessel filter, digitized at 10–250 μs/point, and stored on computer. The pClamp system (Axon instruments, Foster City, CA) was used for data acquisition and analysis.

Fast application of agonists. The experimental arrangement for the fast application of agonists is illustrated in Figure 1A. Double-barreled application pipettes were fabricated from theta glass tubes (2.0 mm outer diameter, TGC200–15; Clark Electromedical Instruments) pulled out to a tip diameter of 200–300 μm. Control solution (normal Ringer’s solution) and test solution (solution containing agonist) was passed continuously through each barrel of the theta glass tube under static pressure. The tip of the patch pipette was positioned close to the interface between the two solutions. To make a clear interface between the stream of the two solutions, 10 mM sucrose was added. The application pipette was operated by a piezoelectric device (PZ-150M; Burleigh Instruments, Fishers, NY) to achieve fast translocation of the interface. The time required for solution exchange was estimated by measuring open tip responses for junction potential between normal Ringer’s solution and 10% normal Ringer’s solution. (Insol. Fig. 1A.) The 20–80% rise time ranged from 50 to 150 μs. Agonist pulses were applied to outside-out patches every 3–5 sec. To induce current responses of AMPARs, l-glutamate or kainate (Wako, Osaka, Japan) was applied at various concentrations. The agonist barrel of the application pipette was connected to three reservoirs containing different drug solutions through miniature solenoid valves so that responses to the three different drugs could be tested in the same outside-out membrane patch. Time required for complete exchange to new drug solution was ∼20 sec. Cyclothiazide (CTZ) (Tocris Cookson, Bristol, UK) was dissolved in DMSO and diluted with normal Ringer’s solution. The final DMSO concentration was 0.1%.

Kinetic model. To predict current responses of outside-out patches to fast application of glutamate, we adopted a kinetic model assuming two binding sites of the agonist on a channel and desensitization of the agonist-bound channel before and after opening of the channel (Dudel et al., 1990, Jonas et al., 1993) (see Fig. 5A). Rate constants in the model were obtained using a combination of trial and error and optimization, as described by Häusser and Roth (1997), but with some modifications. Briefly, starting from the initial values of the rate constants selected arbitrarily, a model-predicted current trace was obtained by digitally solving a set of differential rate equations with 50 μs time intervals using the fourth-order Runge–Kutta method with Excel (Office 97; Microsoft, Seattle, WA) running on an IBM personal computer. The rate constants were changed by trial and error to minimize the differences between the experimental and model-predicted current traces. We then adopted nonlinear least-square method for further automatic minimization. For example, to fit the desensitization time course, we minimized the sum of the square differences between the experimental currents and the predicted ones during the desensitization, putting a partial set of rate constants greatly affecting desensitization as free variables, subject to the
condition of microscopic reversibility. This minimization of the square sum was performed with Solver in Excel with the termination criteria of $<10^{-10}$ relative errors of each rate constant in the successive minimization trials.

**Analysis.** To estimate the open probability at the peak of the current response to glutamate, we performed nonstationary fluctuation analysis (Sigworth, 1980). Transient responses to step applications of 1 or 10 mM glutamate were stored on a computer disk. The mean current and variance for each sample point were calculated across 20–40 current traces. The value of the baseline variance was subtracted from the variance calculated above. The value of the variance ($\sigma^2$) thus obtained was plotted against the mean current ($I$). The resulting plot was fitted with the function: $\sigma^2 = I - I_{\text{ss}}^2/N$, where $i$ is the single-channel current, and $N$ is the number of available channels in the patch. The open probability of channels at the peak of the current was obtained by dividing the amplitude of the peak current by $2N$.

All data are expressed as means ± SEM. Statistical analysis was performed using Student’s two-sided t tests, and differences were considered significant at $p < 0.05$.

**RESULTS**

**Responses to glutamate and kainate in flip and flop splice variants**

Fast application of 1 mM glutamate for 100 msec to outside-out patches excised from *Xenopus* oocytes expressing either flip or flop variant of GluR2Q produced current responses that rose rapidly to a peak and then showed marked desensitization (Fig. 1B, C). The membrane potential was held at −100 mV throughout this study. The 20–80% rise time was much faster in flop (0.28 ± 0.01 msec, $n = 36$) than in flip (0.53 ± 0.03 msec, $n = 32$). The desensitization time constant was also much faster in flop than in flip (see below). The response in flip displayed an appreciable current at steady state, and the ratio of the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.

Kainate at 1 mM produced nondesensitizing currents in both variants. The amplitude of the kainate response was smaller than that of the steady-state current response to glutamate in both flip and flop variants. The peak amplitude of the current activated by each glutamate concentration, with the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.

Dose–response relationship

Figure 2 shows dose–response relationships for the responses to glutamate in both flip and flop variants. The peak amplitude of the response to 1 mM glutamate for 100 msec to outside-out patches excised from *Xenopus* oocytes expressing either flip or flop variant of GluR2Q produced current responses that rose rapidly to a peak and then showed marked desensitization (Fig. 1B, C). The membrane potential was held at −100 mV throughout this study. The 20–80% rise time was much faster in flop (0.28 ± 0.01 msec, $n = 36$) than in flip (0.53 ± 0.03 msec, $n = 32$). The desensitization time constant was also much faster in flop than in flip (see below). The response in flip displayed an appreciable current at steady state, and the ratio of the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.

Kainate at 1 mM produced nondesensitizing currents in both variants. The amplitude of the kainate response was smaller than that of the steady-state current response to glutamate in both flip and flop variants. The peak amplitude of the current activated by each glutamate concentration, with the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.

**Kinetics of desensitization and deactivation**

Figure 3 shows responses to 100 msec glutamate pulses at various concentrations in flip and flop. With increasing concentrations, both activation and desensitization kinetics became faster, although the kinetics were much faster in flip than in flop at all concentrations examined (Fig. 3A, B). The desensitization time constant was obtained by fitting the current decay between 90 and 10% of the peak amplitude of the response with a single exponential function. When the amplitude of the steady-state current was larger than 10% of the peak, fitting was performed between 90% of the peak and the steady state. The value was plotted against the square root of the time constant for desensitization.

90% of the peak and the steady state. The value was plotted against the concentration of glutamate (Fig. 3A, B). It declined gradually up to 1 mM and reached a steady value at 3 mM. We performed the following experiments using 1 mM glutamate, because the peak concentration of glutamate in the synaptic cleft has been estimated to be ~1 mM (Clements et al., 1992; Colquhoun et al., 1992).

Responses to pulses of 1 mM glutamate of brief (1 msec), intermediate (10 msec), and long (100 msec) durations are superimposed in Figure 4A and B. The time constants of the current decay to 100 msec pulses, defined as the desensitization time constants, ranged from 4.00 to 8.59 msec (5.89 ± 0.17 msec; $n = 50$) in flip and from 9.30 to 1.74 msec (1.8 ± 0.05 msec; $n = 37$) in flop (Fig. 4C). When the patch pipette contained 2 mM MgATP and 0.2 mM NaGTP, they were 5.59 ± 0.32 msec ($n = 5$) in flip and 1.08 ± 0.10 msec ($n = 5$) in flop. The results indicated that the presence of ATP and GTP in the internal solution caused no significant change in the time constant for

**Figure 2.** Dose–response relationships for responses of GluR2Q flip (A) and flop (B) AMPA receptor channels to glutamate. The relative peak amplitudes of the current activated by each glutamate concentration, with the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.

Kainate at 1 mM produced nondesensitizing currents in both variants. The amplitude of the kainate response was smaller than that of the steady-state current response to glutamate in flip ($I_{\text{K}} / I_{\text{Glu}} = 0.39 ± 0.04$, $n = 5$), whereas the opposite was true in flop ($I_{\text{K}} / I_{\text{Glu}} = 5.36 ± 1.13$, $n = 5$).

**Dose–response relationship**

Figure 2 shows dose–response relationships for the responses to glutamate in both flip and flop variants. The peak amplitude of the response to 1 mM glutamate was taken as a reference, and the response ratio, i.e., the peak amplitude of the response at various glutamate concentrations divided by that at 1 mM glutamate, was plotted against the log of glutamate concentration. The dose–response curve that fits the data best predicts a half-maximal response at 1.39 mM and a Hill coefficient of 1.08 in flip. The response curve that fits the data best predicts a half-maximal plotted against the log of glutamate concentration. The open probability of channels at the peak of the current was obtained by dividing the amplitude of the peak current by $2N$.

All data are expressed as means ± SEM. Statistical analysis was performed using Student’s two-sided t tests, and differences were considered significant at $p < 0.05$. The 20–80% rise time was much faster in flop (0.28 ± 0.01 msec, $n = 36$) than in flip (0.53 ± 0.03 msec, $n = 32$). The desensitization time constant was also much faster in flop than in flip (see below). The response in flip displayed an appreciable current at steady state, and the ratio of the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current. The kainate amplitude of the current activated by each glutamate concentration, with the amplitude of the steady-state current measured immediately before cessation of the glutamate pulse to that of the peak current was 6.8 ± 0.4% ($n = 53$). In contrast, the steady-state current in flop was almost negligible, being only 1.1 ± 0.1% ($n = 36$) of the peak current.
desensitization. Thus, the desensitization time constant during application of 1 mM glutamate in flip was approximately fivefold slower than that in flop in either experimental condition. In contrast, the decay time constants after 1 msec pulses, tentatively designated as the deactivation time constants in this paper, were similar between the two splice variants. The values ranged from 0.33 to 0.80 msec (0.62 ± 0.06 msec, n = 10) in flip and from 0.41 to 0.70 msec (0.54 ± 0.05 msec, n = 10) in flop (Fig. 4D), and there was no significant difference between these values. However, a possibility cannot be excluded that the difference would have been concealed by the solution exchange time inherent in the fast agonist application system.

Because the flop variant had fast desensitization kinetics, the current response to the intermediate duration (10 msec) exhibited only a single decaying component, indicating that the channels desensitized almost completely within 10 msec. In contrast, there was a marked difference between the desensitization and deactivation kinetics in the flip variant. Therefore, the response induced by 10 msec glutamate pulse exhibited two decaying components. It decayed slowly for the first 10 msec with a time course for desensitization and decayed faster after removal of glutamate. However, the decay after cessation of 10 msec pulse was slower than that observed after a 1 msec pulse. To elaborate on this issue, we applied 1, 5, 10, and 20 msec pulses of 1 mM glutamate and compared the decay kinetics of the deactivation currents among them (Fig. 5B). In six patches tested, deactivation time constants were 0.52 ± 0.07, 0.80 ± 0.10, 1.37 ± 0.20, and 2.37 ± 0.52 msec, respectively, after cessation of 1, 5, 10, and 20 msec pulses. Thus, the deactivation kinetics depended strongly on the extent of desensitization immediately before the removal of glutamate. The prolongation of the deactivation with increasing desensitization may be explained by assuming that the AMPAR in the desensitized state moves back to the undesensitized closed state via the open state. It has been shown that the time required for deactivation after removal of agonist increases in proportion to the extent of desensitization by a similar mechanism in GABA_A receptor channels in cultured rat hippocampal neurons (Jones and Westbrook, 1995).

To provide a better quantitative description of the above finding, a kinetic model was investigated. We adopted a model proposed by Jonas et al. (1993), which could predict current responses of outside-out patches to fast applications of glutamate in CA3 pyramidal neurons. This model assumes fast desensitization upon agonist binding before and after opening of the channel (Fig. 5A). In this model, C is the unliganded closed state, and CA1 and CA2 are single- and double-ligated closed states, respectively. D1, D2, and D3 are desensitized closed states, OA2 is a double-ligated open state, and c indicates the concentration of glutamate. The set of rate constants shown in the legend of Figure 5 determined by a combination of trial and error and optimization accurately predicted the experimentally obtained current traces (Fig. 5B). In this set of rate constants, k15 (1.6 × 10^4 sec^-1) and k16 (1.2 × 10^4 sec^-1), the transition rate constants between D2 and D3, were more than two orders faster than k9 (1.0 × 10^2 sec^-1) and k10 (2.1 sec^-1), those between CA2 and D2, and k11 (3.0 × 10^2 sec^-1) and k12 (1.5 × 10 sec^-1), those between OA2 and D3. Furthermore, k12 was approximately sevenfold faster than k10. Upon cessation of the glutamate pulse, therefore, most of the channels in the desensitized states (D2 and D3) would move back to the liganded and then unliganded closed states via the open state, making the deactivation kinetics of the current response slower.

To assess the validity of the kinetic model shown in Figure 5A, we examined the agreement between measured and predicted values regarding concentration dependence of the peak currents. The values k1–k16 obtained in Figure 5B predicted that the open probabilities at the peak of the current responses to 0.1, 1, and 10
mm glutamate were 0.02, 0.32, and 0.68, respectively. The corresponding values to 0.1 and 10 mM glutamate obtained experimentally in this paper were 0.01 and 0.66 when the value to 1 mM glutamate was set to 0.32. We also estimated the open probabilities at the peaks of current responses to 1 and 10 mM glutamate were 0.02, 0.32, and 0.68, respectively. The correspondence of the peak current reasonably.

Recovery from desensitization

A fraction of AMPARs becomes desensitized during brief exposure to 1 mM glutamate, although desensitization is not a major factor in determining the decay rate after cessation of the brief pulse (Colquhoun et al., 1992; Raman and Trussell, 1995). Significant desensitization of AMPAR is induced by synaptically released glutamate and regulates the strength of synaptic transmission to repetitive stimuli (Trussell et al., 1993; Otis et al., 1996). This regulation may depend at least partially on the functional diversities of postsynaptic AMPARs assembled from different subunit compositions. To examine the extent of desensitization produced by a brief glutamate pulse and the time course of recovery from desensitization, we used a double-pulse protocol in which two successive 1 msec pulses of 1 mM glutamate, separated by intervals of variable durations, were applied to the outside-out patches. Figure 6 shows that the response to the second pulse had a more substantial reduction in flip than in flop. The amplitude of the response to the second pulse given after a 10 msec interval was reduced to 78 ± 12% (n = 10) and 12 ± 2% (n = 10) in the flip and flop variants, respectively. Furthermore, the time course of the recovery was extended for a more prolonged period in the flop variant. The average reduction of the response to the second pulse was plotted against the interval between the two pulses in flip and flop (Fig. 6A,B). The time course of the recovery could be fitted almost satisfactorily by a single exponential for both. The time constant was 11.7 msec in flip and 31.3 msec in flop.

Effects of CTZ on kinetics of desensitization

CTZ strongly potentiates AMPARs by reducing desensitization (Partin et al., 1993; Yamada and Tang, 1993). CTZ enhanced steady-state current responses to glutamate in homomeric GluR1, GluR3, and GluR4 and heteromeric GluR1/GluR2 AMPARs, and the extent of potentiation was more marked for AMPARs assembled from the flip form than from the flop form subunits (Partin et al., 1994, 1996). We examined the effects of CTZ on the GluR2Q AMPARs (Fig. 7).

CTZ at 100 μM completely abolished desensitization caused by a 500 msec pulse of 1 mM glutamate and increased the peak amplitude to 280 ± 37% (n = 5) of control in flip (Fig. 7A). The response in flop was also enhanced by CTZ at 100 μM. However, the effect on flop was less efficient than that on flip. Namely, the peak amplitude was increased to 171 ± 32% (n = 5), and the response to 1 mM glutamate pulse showed substantial desensitization during the application period of 500 msec, although the...
Figure 7. Effects of CTZ on responses to glutamate in GluR2Q flip (A) and flop (B) AMPA receptor channels. Current responses to 500 msec pulses of 1 mM glutamate in the presence and absence of 100 μM CTZ are superimposed. Insets show the initial portion of the response with a faster sweep speed. When CTZ was applied, the control saline also contained 100 μM CTZ. Membrane potential was held at −100 mV.

Figure 8. Recovery from desensitization of GluR2Q flip (A) and flop (B) AMPA receptor channels produced by a brief glutamate pulse in the presence of CTZ. a, Superimposed current traces evoked by two 1 msec pulses of 1 mM glutamate separated by different intervals in the presence of 100 μM CTZ. Each response to the second pulse is the average of three responses. b, Time course of recovery from desensitization. The amplitude of the second response relative to the first is plotted against the time course of recovery. The bars represent the mean ± SEM of the relative amplitude of the second response obtained from four to six experiments. Broken lines indicate the time courses of recovery in the absence of CTZ, which are shown in Figure 6. Note that CTZ almost completely abolished the reduction of the second response in both flip and flop variants. Membrane potential was held at −100 mV.

The rate of desensitization was markedly reduced (Fig. 7B). Furthermore, it was noted that CTZ prolonged the rise time of the response to glutamate in flop (Fig. 7B, inset). We next examined the effects of 100 μM CTZ on the desensitization produced by a brief (1 msec) glutamate pulse. Figure 8 shows the responses to a double 1 msec pulse of 1 mM glutamate separated by intervals of variable durations in the flip and flop variants. No substantial reduction in the response to the second pulse was detected in either variant, indicating that CTZ at 100 μM abolished the desensitization caused by a 1 msec glutamate pulse. The result shown in Figure 7B indicated that the desensitization during the 500 msec glutamate pulse was not completely abolished by 100 μM CTZ in the flip variant. It is likely that in the presence of CTZ the desensitization process of the flop receptor is initiated so slowly that a brief glutamate pulse produces no substantial desensitization.

Kinetic properties of heteromeric receptors assembled from edited and unedited forms of GluR2

The recombinant AMPAR assembled from the GluR2R subunit, the edited form of GluR2, produces no appreciable current in response to glutamate when expressed in Xenopus oocytes (Boultier et al., 1990; Nakanishi et al., 1990; Herlitze et al., 1993). In this study, we examined the kinetic properties of homomeric GluR2 AMPARs by exploiting the fact that the recombinant AMPAR assembled from the GluR2Q subunit, the unedited form of GluR2, is capable of generating a current response sufficiently large for kinetic analysis in outside-out patch configurations. However, because only the edited form of GluR2 is expressed in the adult brain (Sommer et al., 1991; Burnashev et al., 1992), it is desirable to determine whether the kinetic properties of the homomeric GluR2Q AMPAR are similar to those of AMPAR assembled from GluR2R. To address this issue, we examined kinetic properties of the heteromeric receptors assembled from GluR2R and GluR2Q. To increase the probability of incorporation of GluR2R into the receptor, we injected cRNAs of GluR2R and GluR2Q at a ratio of 4:1 into oocytes. Heteromeric AMPARs possessing GluR2R have either a linear or outwardly rectifying I–V relationship, whereas those assembled from GluR1, GluR2Q, GluR3, and/or GluR4 show strong inward rectification (Seeburg, 1993; Hoffmann and Heinemann, 1994). Therefore, we verified the formation of heteromeric AMPARs by determining I–V relationships of responses to glutamate in each outside-out patch. In most patches derived from oocytes into which cRNAs of GluR2R and GluR2Q had been injected at a ratio of 4:1, the I–V relationship of the response to glutamate was either linear or outwardly rectifying with 100 μM spermine in the patch pipette (see Materials and Methods). In a small number of patches, however, the I–V curve displayed a tendency toward inward rectification, even under these conditions. Such patches were discarded from the experiment.

Figure 9A shows examples of the I–V relationships in heteromeric GluR2Qflip/GluR2Rflip (a) and GluR2Qflop/GluR2Rflop (b) receptors, which exhibited outward rectification. Current responses to 1 and 100 msec pulses of 1 mM glutamate were recorded in these patches (Fig. 9B). In the flip receptor, the desensitization and deactivation time constants were 6.40 ± 0.50 and 0.63 ± 0.14 msec (n = 9), respectively. In the flop receptor, they were 1.00 ± 0.07 and 0.58 ± 0.06 msec (n = 11), respectively. These values were similar to those obtained in the homomeric GluR2Q receptor in both flip and flop variants. Furthermore, the response in flip had an appreciable steady-state current, whereas it was almost negligible in flop. The ratio of the amplitude of the steady-state current measured immediately before cessation of the 100 msec glutamate pulse to that of the peak current was 6.7 ± 0.8% (n = 9) in flip and 1.2 ± 0.2% (n = 11) in flop. These values were again similar to those obtained in the homomeric GluR2Q receptor in both flip and flop variants. These results indicated that both desensitization and deactivation properties in the GluR2Q receptor were unaffected by incorporation of GluR2R.

Koike et al. • Desensitization of Splice Variants of GluR2

DISCUSSION

One of the most striking features of the AMPAR is its extremely rapid and strong desensitization (Kiskin et al., 1986; Trussell and Fischbach, 1989; Mosbacher et al., 1994). The desensitization of postsynaptic AMPARs may have a profound effect on synaptic events. Because the kinetics and extent of desensitization differ markedly according to subunit composition of AMPARs, combining different AMPAR subunits may generate postsynaptic receptors finely tuned for encoding special signals in specific neuronal pathways (Trussell et al., 1994). To further clarify the molecular basis of desensitization of the AMPAR, we examined kinetic properties of the homomeric GluR2 AMPAR expressed in Xenopus oocytes using a fast agonist application technique. Differences in desensitization time constants between the flip and flop variants in homomeric GluR1, GluR3, and GluR4 receptors and heteromeric receptors assembled from one of them with GluR2R, the edited form of GluR2, have already been investigated (Mosbacher et al., 1994). However, the kinetic properties of the homomeric GluR2 receptor have not been examined, because no appreciable current response is detected when cRNA of GluR2R alone is injected into oocytes. In this study, we expressed the homomeric GluR2Q receptors in oocytes that produced a sufficiently large current in response to glutamate and examined the kinetic properties of the homomeric GluR2 receptors. We found that the time constant for desensitization during application of 1 mM glutamate in flip (5.89 msec) was approximately fivefold slower than that in flop (1.18 msec), whereas the deactivation time constants were similar. The most prominent difference in the desensitization time constant between flip and flop was reported in GluR4 in which the value during application of 1 mM glutamate in flip (3.6 msec) was approximately fourfold slower than that in flop (0.9 msec) (Mosbacher et al., 1994). Thus, the difference in the desensitization rate between flip and flop was the largest in the GluR2Q homomeric receptor. All experiments in this study were done at a holding potential of −100 mV, whereas those by Mosbacher et al. (1994) were conducted at −40 mV. However, this does not disturb comparison between their data with ours, because there was no voltage dependence in the desensitization kinetics between −40 and −100 mV in both GluR2Q flip and flop receptors (M. Koike, unpublished data).

We also found that there was an appreciable steady-state current during application of 1 mM glutamate in flip, whereas it was negligible in flop. The ratio between the steady-state current relative to the peak current in flip was sixfold to sevenfold greater than that in flop. Furthermore, the amplitude of the steady-state current induced by 1 mM glutamate was greater than that induced by 1 mM kainate in flip, whereas the opposite was true in flop. Using the conventional two-microelectrode voltage-clamp method for oocytes expressing GluR2Q flip, we have confirmed recently that the glutamate-induced steady-state current in whole cell recordings is greater than that induced by the same concentration of kainate at any concentration of the agonist between 100 μM and 10 mM (S. Tsukada, M. Koike, K. Tszuzuki, and S. Ozawa, unpublished observations). This is a unique property of the GluR2Q flip homomeric receptor, because the amplitude of the kainate-induced steady-state current was invariably greater than that induced by glutamate at the same concentrations in any homomeric or heteromeric recombinant AMPARs reported previously (Keinänen et al., 1990; Sommer et al., 1990; Partin et al., 1993).

Only GluR2R mRNA, the edited form of GluR2, is expressed in the adult brain, and extremely low levels of GluR2Q mRNA are present in the fetal brain (Sommer et al., 1991; Burnashev et al., 1992). Therefore, it is important to determine whether the kinetic properties of the GluR2 receptor are altered by the exchange of the single amino acid residue from arginine to glutamine at the Q/R site. In this study, we found no differences in the desensitization or deactivation time constants nor in the
amplitude of the steady-state current between the heteromeric GluR2Q/GluR2R receptor and the homomeric GluR2Q receptors in either flip or flop variants. It is likely that the flip–flop segment located extracellularly between M3 and M4 functions independently of the amino acid species at the Q/R site, which lies on the C terminus of the M2 segment and is considered to lie close to the intracellular surface of the plasma membrane (Hollmann et al., 1994).

When the other GluR subunits are assembled with GluR2, the difference in the kinetic properties between flip and flop of GluR2, i.e., slow and incomplete desensitization in flip and fast and almost complete desensitization in flop, seems to be conferred upon resultant heteromeric AMPARs. Combinations of GluR1, GluR3, and GluR4 subunits, irrespective of flip and flop, with GluR2 flip reduced the desensitization rate of the recombinant heteromeric receptor (Mosbacher et al., 1994). In native neurons, a positive correlation was found between the desensitization time constant and the relative abundance of GluR2 flip mRNAs (Geiger et al., 1995). Furthermore, the steady-state current in response to glutamate was greater when GluR2 flip was coassembled with the other subunits than when GluR2 flop was used (Sommer et al., 1990). Among the neuron species tested, the desensitization time constant was the slowest and the steady-state current was the largest in hilar mossy cells in which the flip form is predominant among the GluR2 mRNAs expressed (Geiger et al., 1995). In contrast, the desensitization time constant was the fastest and the steady-state current was almost negligible in the medial nucleus of the trapezoid body relay neurons in which only flop forms of GluR1, GluR2, and GluR4 mRNAs are expressed (Geiger et al., 1995). In the latter neurons, the dominant expression of GluR4 flop mRNAs (55% of the total mRNA of the GluR, whereas GluR2 flop mRNAs account for 24%) has been considered to be mainly responsible for the fast and complete desensitization (Geiger et al., 1995). However, the fast desensitization kinetics cannot be explained in neocortical nonpyramidal neurons by their GluR4 flop content, because little GluR4 expression was detected in these cells. In these neurons, the flop form is predominant among GluR2 mRNAs, whereas the flip form is predominant in pyramidal neurons that display slower desensitization kinetics (Lambolez et al., 1996). This suggested that the splice variants of GluR2 are important molecular determinants of desensitization kinetics in heteromeric AMPARs in native neurons. Although the desensitization time constant shows a positive correlation with the relative abundance of the GluR2 flip subunit (Geiger et al., 1995), an open question is why the desensitization of native AMPARs containing GluR2 flip abundantly is twofold or threefold slower than that estimated here in the recombinant GluR2 flip receptors. Geiger et al. (1995) have reported that the desensitization time constants of AMPARs in CA3 hippocampal pyramidal cells and hilar mossy cells, in which >30% of the total GluR mRNA are GluR2 flip mRNA, are 15.2 and 16.3 msec, respectively. They have conducted fast application experiments using patch pipettes filled with the internal solution containing 2 mM ATP. In this study, we used the internal solution containing both 2 mM ATP and 0.2 mM GTP in some experiments. However, the presence of both ATP and GTP caused no change in the desensitization time constant in the recombinant GluR2 flip receptors. The reason for extremely slow desensitization kinetics in native AMPARs in CNS neurons with a high relative abundance of GluR2 flip mRNA is presently unknown.

The agonist-binding core of the AMPAR is formed by peptide segments termed S1 and S2. S1 includes ~150 amino acid residues N terminal to M1, and S2 comprises residues between M3 and M4 (Stern-Bach et al., 1994; Wo and Oswald, 1995; Armstrong et al., 1998). The flip–flop region is located downstream of S2, being in close proximity to the M4 domain, which lies on the C terminus of the M2 segment and is considered to lie close to the intracellular surface of the plasma membrane (Hollmann et al., 1994).

REFERENCES


