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Short communication

Fast desensitizing kainate-gated current resolved in whole-cell experiments on isolated rat hippocampal neurons

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Abstract

In whole-cell concentration-jump experiments on dissociated rat hippocampal neurons the existence of a fast desensitizing component of a kainate-gated current was demonstrated. More than 90% of all neurons tested possessed such a receptor. Electrophysiological and pharmacological data suggest an association of this fast kainate-gated current with that mediated through a combination of subunits forming a low-affinity '(*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA)-preferring' receptor. Kinetic data suggest the existence of a compact spatial location of fast desensitizing 'AMPA-preferring' receptors which are naturally expressed on the surface of the neurons. An AMPA-insensitive current component mediated through fast desensitizing high-affinity kainate receptors was not observed.

Keywords: Concentration jump; Hippocampal neuron; Glutamate receptor, ionotropic; AMPA/kainate receptor; Kainate-gated desensitization

1. Introduction

Glutamate (Glu) is the main excitatory neurotransmitter in the mammalian central nervous system (CNS). One of its receptor groups consists of a superfamily of *N*-methyl-D-aspartate (NMDA) and non-NMDA ionotropic Glu receptors (iGlu receptors). Non-NMDA receptors participate in fast signal processing and, since some combinations of non-NMDA subtypes display a Ca^{2+} conductance, possibly also in some forms of plasticity. Glu receptors assembled from Glu_{1-4} receptor subunits are known to be of the (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) type (Sommer and Seeburg, 1992), whereas Glu_5 receptor to Glu_7 receptor, kainate 1 and kainate 2 subunits form the subtypes of a high-affinity kainate receptor (Herb et al., 1992) which normally has non-desensitizing current conductance properties.

A fast desensitizing high-affinity kainate receptor exists, which displays a rapid desensitization, as has been demonstrated in rat dorsal root ganglion neurons (Huettner, 1990). A desensitizing kainate-gated current mediated through fast AMPA/kainate receptors has also been described in experiments on *Xenopus* oocytes expressing rat brain Glu receptors (Arvanov and Usherwood, 1991). In addition, studies of recombinant Glu receptors by Herb et al. (1992) have disclosed subtypes displaying fast desensitizing, kainate-gated currents. Fast desensitizing kainate-gated currents were also observed in 'outside-out' patches excised from the neurons of avian cochlear nucleus (Raman and Trussel, 1992). In cultured rat hippocampal neurons both 'kainate-preferring' (Lerma et al., 1993) and 'AMPA-preferring' (Patneau et al., 1993) fast desensitizing receptors were found. Recently, fast desensitization of kainate-gated currents was demonstrated in patches excised from apical dendrites of hippocampal neurons in slice preparation (Spruston et al., 1995).

In this communication we report on fast desensitizing kainate-gated currents (fast current) in freshly dissociated hippocampal neurons of immature rats. The data suggest that this fast current can be mediated through low-affinity 'AMPA-preferring' receptors.

2. Materials and methods

Methodical procedures were the same as described elsewhere (Pidoplichko and Reymann, 1994). All experiments were performed on neurons dissociated from hippocampal slices of 3-week-old male rats. The ionic composition of

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the extracellular solution was: NaCl 155 mM; $CaCl_2$ 2 mM; HEPES 10 mM; pH 7.4 by Tris. A high Ca^{2+} extracellular solution contained $CaCl_2$ 50 mM (for comparison similar as in Lerma et al., 1993, experiments); *N*-methyl-D-glucamine (NMDG) 110 mM; HEPES 10 mM; pH 7.4 by Tris. The ionic composition of the intracellular solution was: CsF 120 mM; HEPES 10 mM or NMDG-fluoride 120 mM; HEPES 10 mM; pH 7.2 by Tris. The salts, Tris, HEPES, NMDG and concanavalin-A were purchased from Sigma (USA). AMPA and kainic acid (kainate) were from Tocris Neuramin (UK). Cyclothiazide was a gift from Lilly Research Laboratories (USA). Patch pipette resistance was from 1.5 to 2.5 MΩ. The concentration-jump system used in our experiments (Pidoplichko and Reymann, 1994) enabled us to perform a precise and fast solution exchange. The linear flow velocity of 130 μm/ms was similar to that used in high-resolution techniques elsewhere (Colquhoun et al., 1992).

3. Results

When kainate was applied to isolated hippocampal neurons at concentrations greater than 1 mM, a current component displaying fast activation and desensitization was observed in most cells (93% from 109 CA1 and 95% from 37 CA3 neurons). The relative amplitude of the fast current was estimated as the peak current (I_{peak}) minus the steady-state current ($I_{s\text{-}s}$) divided by I_{peak} and was in the range of 20–30% for about 70% of the cells exhibiting a fast current.

The fast current had a steep activation phase, so it was necessary to exclude the possibility of artifacts from the space clamp. Therefore, tetrodotoxin was used to test the sensitivity of the fast current to this drug. In neurons which displayed a fast current, this current was insensitive to tetrodotoxin, thus demonstrating its purely 'ligand-gated' nature. A 'tetrodotoxin test' was always performed prior to the start of an experiment.

The dose-response dependence of I_{peak} is presented in Fig. 1A. The EC_{50} value was 271 ± 12 μM, which is consistent with the low affinity of this receptor to kainate. The Hill coefficient was 1.6 ± 0.1 (mean $\pm$ S.D., data from 3 CA1 neurons).

The non-linearity of the current-voltage relationship (Fig. 1B) was reminiscent of the *I-V* curve recorded from outside-out patches, excised from cultured newborn rat hippocampal neurons (Patneau et al., 1993), although the outward rectification was weaker. High Ca^{2+} affected the fast current preferentially, markedly diminishing the fast current in comparison to other current components (data not shown).

In all neurons displaying a fast current, the fast current component could be completely desensitized by 30 μM AMPA or by pre-application of 100 μM AMPA. Out of 15 (14 CA1 and 1 CA3 cells) neurons displaying a fast current, all were desensitized by 100 μM AMPA. Recovery from desensitization induced by pre-application of 100 μM AMPA was comparatively fast (about 1 s) (Fig. 1C). However, recovery of the fast current following desensitization by pre-application of 2 mM kainate was complete within 50 ms (not illustrated).

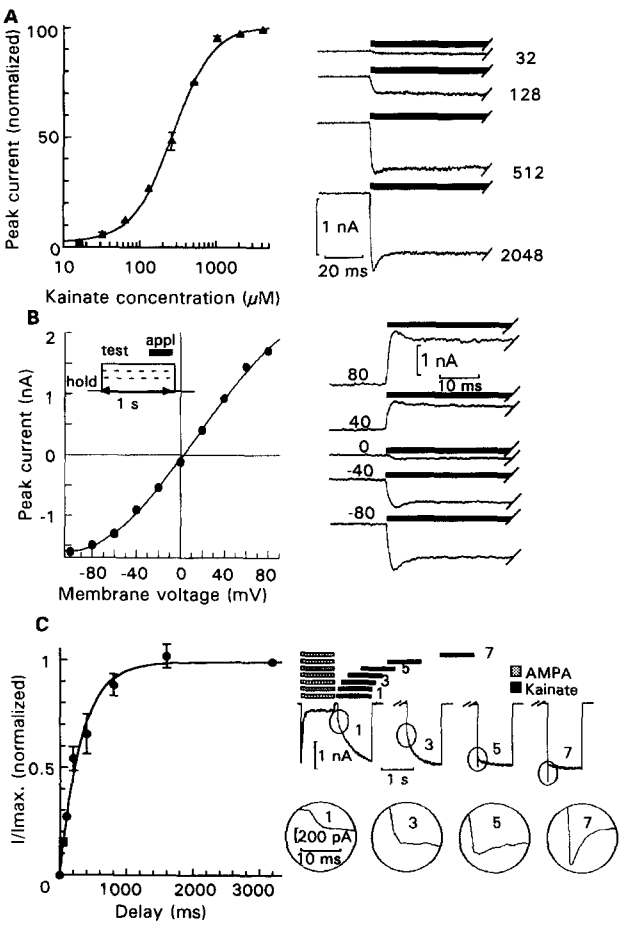

Fig. 1. A: Dose-response dependence of I_{peak}. Mean data for 3 CA1 neurons. EC_{50} value 271 ± 12 μM (mean $\pm$ S.D.), Hill coefficient 1.6 ± 0.1. Sample recordings are presented in the right part of the figure. Kainate concentration (μM) is indicated near application bars. $V_h = -100$ mV. B: Current-voltage dependence of a fast current in a CA1 neuron. The protocol of voltage and agonist applications is presented in the inset. The voltage was stepped in 20-mV increments from $V_h = -100$ mV. Sample traces (currents were gated by 3 mM kainate) are presented in the right part of the figure. Membrane voltages (mV) are shown next to the current traces. C: Kinetics of recovery from desensitization of the fast current. Selected traces demonstrating recovery from desensitization induced by a 1-s application of 100 μM AMPA. 3 mM kainate was applied after a variable (50, 100, 200, 400, 800, 1600 and 3200 ms) delay following washout of AMPA. The protocol for the delivery of concentration pulses to the cell is marked by bars in the upper part of the figure. The kainate-gated current in trace 1 was recorded after a 50-ms washout of AMPA; trace 3: after a 200-ms washout of AMPA; trace 5: after a 800-ms washout of AMPA; trace 7: after a 3200-ms washout of AMPA. Kainate-gated current profiles (during first moments of concentration-pulse delivery) are shown in the expanded time scale in the corresponding insets. A single exponential fit to the curve for fast current recovery from AMPA-induced desensitization is shown on the left. Recovery-time constant (τ_{recov}) are 312 ± 42 ms ($n = 4$, CA1 cells). $V_h = -100$ mV.

Potentiation of kainate-gated currents by cyclothiazide (Fig. 2A,B) was similar to that for patches excised from cultured hippocampal neurons (Patneau et al., 1993) and to that demonstrated for patches excised from nucleus magnocellularis neurons of chicken embryos (Trussel et al., 1993). 100 μM cyclothiazide increased I_{peak} by 2.6 ± 0.4 times (mean $\pm$ S.D., $n = 4$; 3 CA1 and 1 CA3 neurons). The time constant of desensitization (τ_{des}) of the fast kainate-gated current component remained unchanged during washout of cyclothiazide. As shown in Fig. 2B, τ_{des} of the control trace was 3.3 ms. τ_{des} of the fragments 3, 4 and 5 were 3.4, 3.2 and 3.4 ms, respectively (approximating curves not shown in Fig. 2B). The deactivation process was fitted by the sum of two exponentials with τ_{fast} and τ_{slow} for fragments 2/2, 3/3 and 5/5 equal to 3.48 and 22.21, 2.41 and 19.49, 1.12 and 9.69, respectively (approximations not shown in Fig. 2B). However, the fast current was unaffected by a 3-min-long pre-exposure of neurons to concanavalin-A ($n = 3$; 2 CA1 and 1 CA3 neurons; Fig. 2C).

The kinetic parameters for the activation of the fast current were estimated as in Fig. 2D. The parameters activation (95% time to peak value) and desensitization (τ_{des}) of the fast current were correlated with the amplitude (estimated as $I_{peak} - I_{s\text{-}s}$) of the fast current. The amplitude of the fast current was assumed to be proportional to the area occupied by active channels. Data (95% time to peak and τ_{des}) obtained for neurons displaying similar fast current amplitudes were plotted vs. fast current amplitude and fitted by an empirical equation (Fig. 2E). It is worth noting that some cells displayed low-amplitude fast currents, the kinetic parameters of which were close to those observed

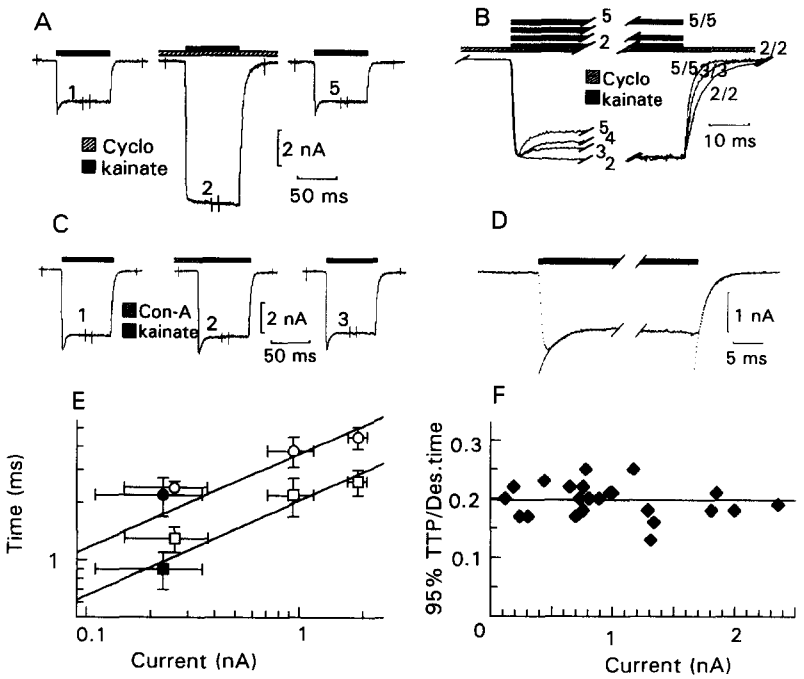

Fig. 2. A–C: Effects of modulatory substances on whole-cell kainate-gated currents. A: Potentiation of 3 mM kainate-gated current by 100 μM cyclothiazide (Cyclo). Trace 1: control. Trace 2: current gated by the 70-ms application of kainate during application of cyclothiazide. Trace 5: recovery after a 4-min washout of cyclothiazide. The protocols for application of substances to the cell are marked by bars over the corresponding traces. B: Kainate-gated current fragments (same cell as in A) during activation and deactivation (normalized to the peak cyclothiazide potentiated current). Fragment 2: profile of the current at the maximal potentiation by 100 μM cyclothiazide (corresponding deactivation fragment 2/2). Fragment 3: current profile after 20-s washout of cyclothiazide (corresponding deactivation fragment 3/3). Fragment 4: further recovery from cyclothiazide action. Kainate-gated current after a 40-s washout. Fragment 5: full recovery after a 4-min washout (corresponding deactivation fragment 5/5). Protocols of exposure to substances are shown above the fragments of current traces. C: The absence of concanavalin-A (Con-A) action on whole-cell kainate-gated current. Trace 1: control. Trace 2: current recorded after a 3-min-long exposure to 0.3 mg/ml of Con-A. Trace 3: recovery; 3 mM kainate was applied after 3-min washout of Con-A. Protocols of exposure to substances are marked by bars over the corresponding current traces. A–C: Holding potential -100 mV, CA1 neurons. D–F: Kinetics of fast current. Example of 3 mM kainate-gated current recorded from a CA3 neuron. 110-ms concentration pulse. Activation/desensitization and deactivation phases are shown in the expanded time scale. 95% time-to-peak (time to peak) 1.4 ms, $\tau_{des} = 2.4$ ms (mono-exponential fit). The deactivation process was fitted by the equation $A + B * \exp(\text{time}/\tau_{slow}) + C * \exp(\text{time}/\tau_{fast})$ where $A = -0.131$ nA, $B = -0.113$ nA, $\tau_{slow} = -18.69$ ms, $C = -1.429$ nA and $\tau_{fast} = -1.53$ ms. $V_h = -90$ mV. E: Comparison of the kinetic data (95% time to peak and τ_{des} values) obtained for patches (adapted from Patneau et al., 1993; filled symbols) and cells with different weights of expression of active fast kainate receptors, based on an empirical equation. Kinetic data from cells, demonstrating similar current amplitudes (estimated as $I_{peak} - I_{s\text{-}s}$) were pooled together for low amplitude ($n = 5$, 4 CA1 and 1 CA3 neurons), medium amplitude ($n = 14$, 11 CA1 and 3 CA3 neurons) and high amplitude ($n = 4$, 3 CA1 and 1 CA3 cells) currents which were gated by 3 mM kainate. A solid line marks the fitting of data points by the equation 95% time to peak $= 2.06\sqrt{(I_{peak} - I_{s\text{-}s})}$ (lower curve, squares) and by the equation $\tau_{des} = 3.65\sqrt{(I_{peak} - I_{s\text{-}s})}$ (upper curve, circles). F: Ratio 95% time to peak/$3\tau_{des}$ plotted vs. $I_{peak} - I_{s\text{-}s}$ ($n = 23$, 18 CA1 and 5 CA3 neurons).

for patches (Patneau et al., 1993). The data from the Patneau et al. (1993) paper are included in Fig. 2E (filled symbols). 95% time to peak was taken equal to 10–90% of fast current rise time multiplied by the correction factor 2.1 estimated separately. In Fig. 2F the ratio of 95% time to peak over the time to complete desensitization ($3\tau_{des}$) is plotted against fast current amplitude.

4. Discussion

In this study we have demonstrated the existence of a fast desensitizing kainate-gated current mediated by Glu receptors in rat hippocampal neurons. This evidence does not allow precise conclusions about the Glu receptor subunits which are responsible for these currents. However, the presumably low Ca^{2+} permeability of channels that mediate the fast current and the shape of the *I-V* curve suggest that they may incorporate the edited Glu_2 receptor subunit (Burnashev et al., 1992). Although the low-amplitude fast current in high Ca^{2+} solution is similar to that observed by Lerma et al. (1993) for a high-affinity 'kainate-preferring' receptor, other characteristics of the fast current suggest that 'AMPA-preferring', low-affinity receptor subunits may be involved (Patneau et al., 1993). Cross-desensitization with AMPA and fast recovery (τ_{recov} = 312 ms) from AMPA-induced desensitization support this conclusion. The fast current recovered after a 50-ms washout of kainate. Therefore, the time constant of recovery from kainate-induced desensitization could be less than 17 ms. Such a short recovery time contrasts with a long-lasting recovery from desensitization (about 1 min) for high-affinity kainate receptors (Lerma et al., 1993). Further support comes from the similar voltage dependence of the fast current and the 'AMPA-preferring' kainate receptor in patches (Patneau et al., 1993). Finally the fast current was potentiated by cyclothiazide but not by concanavalin-A. This observation argues again in favor of 'AMPA preference' of the fast current.

Another important observation was the close similarity of the kinetic parameters to those described by Patneau et al. (1993). This is surprising because they used a patch preparation, arguing that in whole-cell preparations the fast kainate current is too rapid to be detected. However, in our study we were able to resolve these events in whole-cell concentration jump experiments. A possible explanation for this discrepancy is that receptors mediating the fast kainate response are not distributed evenly over the cell surface. If fast kainate receptors were evenly distributed, such a desensitizing kainate-gated current would not be resolved. More evidence of an uneven receptor distribution came from measurements in the whole-cell mode, where we were able to record responses similar to the nucleated patch responses seen in the experiments of Patneau et al. (1993) on cultured newborn rat neurons.

The resolution of the fast current indicates that fast 'AMPA-preferring' receptors, which are naturally expressed in 3-week-old rat hippocampal neurons, occupy a compact area on a cell surface. Thus the current from such a region(s) with high-receptor density could be a dominating one in our fast current recordings.

In earlier somatic-macropatch experiments on adult rat hippocampal slice preparations (Jonas and Sakmann, 1992) the fast desensitizing kainate receptor was not resolved. The presence of a low-amplitude fast desensitizing kainate-gated current was demonstrated recently in outside-out patches excised from apical dendrites of hippocampal neurons (Spruston et al., 1995). It is possible that if these receptors occupied a compact area on the surface of immature and adult rat hippocampal neurons, the probability of patching a region with a high density of fast receptors (i.e. having the area of a macropatch) would be extremely low. This possibility offers an alternative to the explanation of Patneau et al. (1993) for the absence of the fast component in kainate-gated current recordings from somatic patches excised from hippocampal slice preparations. It is therefore possible to speculate that in adult animals fast desensitizing 'AMPA-preferring' receptors are expressed in a dominant compact region of the cell surface.

In addition we found an interesting correlation between current amplitude (Fig. 2E) and the kinetic parameters. Our experimental findings indicate that a larger size of the preparation (i.e. current amplitude) correlates with slower kinetics. If we assume that currents of higher amplitude originate in a larger number of active receptor channels, we then have to assume that this means that a greater surface area was occupied by these receptors. Fig. 2F agrees with these considerations. If we assume that the mosaics of receptors on CA1 neurons are a reproducible entity, then we must accept that there is a parameter that can standardize the kinetic characteristics of a certain receptor channel population. Indeed, if we calculate the 95% time to peak/desensitization time we get a relative constant factor, independent of the current amplitude (i.e. the cell size, Fig. 2F, solid line).

Beyond doubt, further efforts are needed to elucidate the functional significance of the low-affinity fast 'AMPA-preferring receptors' for kainate and fast high-affinity kainate receptors. Given the present state of our knowledge, the only definite conclusion one can make is in support of the domination of naturally expressed 'AMPA-preferring receptor' subunits mediating fast desensitizing kainate-gated currents in isolated mature hippocampal neurons.

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References

Arvanov, V.L. and P.N.R. Usherwood, 1991, Concentration jump studies of intracellularly dialysed *Xenopus* oocytes show desensitization of kainate receptors, Neurosci. Lett. 129, 201.

Burnashev, N., H. Monyer, P.H. Seeburg and B. Sakmann, 1992, Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit, Neuron 8, 189.

Colquhoun, D., P. Jonas and B. Sakmann, 1992, Action of brief pulses of glutamate on AMPA/kainate receptors in patches from different neurones of rat hippocampal slices, J. Physiol. (London) 458, 261.

Jonas, P. and B. Sakmann, 1992, Glutamate receptor channels in isolated patches from CA1 and CA3 pyramidal cells of rat hippocampal slices, J. Physiol. (London) 455, 143.

Herb, A., N. Burnashev, P. Werner, B. Sakmann, W. Wisden and P.H. Seeburg, 1992, The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits, Neuron 8, 775.

Huettner, J.E., 1990, Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by con A, Neuron 5, 255.

Lerma, J., A.V. Paternain, R.J. Naranjo and B. Mellström, 1993, Functional kainate-selective glutamate receptors in cultured hippocampal neurons, Proc. Natl. Acad. Sci. USA 90, 11688.

Patneau, D.K., L. Vyklicky, Jr. and M. Mayer, 1993, Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate, J. Neurosci. 13(8), 3496.

Pidoplichko, V.I. and K.G. Reymann, 1994, Abscisic acid potentiates NMDA-gated currents in hippocampal neurons, NeuroReport 5, 2311.

Raman, I.M. and L.O. Trussel, 1992, The kinetics of the response to glutamate and kainate in neurons of the avian cochlear nucleus, Neuron 9, 173.

Sommer, B. and P.H. Seeburg, 1992, Glutamate receptor channels: novel properties and new clones, Trends Pharmacol. Sci. 13, 291.

Spruston, N., P. Jonas and B. Sakmann, 1995, Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons, J. Physiol. (London) 482(2), 325.