Kinetics and Activation of Postsynaptic Kainate Receptors at Thalamocortical Synapses: Role of Glutamate Clearance

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Kidd, Fleur L. and John T. R. Isaac. Kinetics and activation of postsynaptic kainate receptors at thalamocortical synapses: role of glutamate clearance. J Neurophysiol 86: 1139–1148, 2001. Kainate (KA) receptor-mediated excitatory postsynaptic currents (EPSCs) exhibit slow kinetics at the great majority of synapses. However, native or heterologously expressed KA receptors exhibit rapid kinetics in response to agonist application. One possibility to explain this discrepancy is that KA receptors are extrasynaptic and sense glutamate diffusing from the synaptic cleft. We investigated this by studying the effect of three manipulations that change glutamate clearance on evoked KA EPSCs at thalamocortical synapses. First, we used high-frequency stimulation to increase extrasynaptic glutamate levels. This caused an apparent increase in the relative contribution of the KA EPSC to transmission and slowed the decay kinetics. However, scaling and summing the EPSC evoked at low frequency reproduced this, demonstrating that the effect was due to postsynaptic summation of KA EPSCs. Second, we applied inhibitors of high-affinity glutamate transport. This caused a depression in both AMPA and KA EPSC amplitude due to the activation of a presynaptic glutamatergic auto-receptor. However, transport inhibitors had no selective effect on the amplitude or kinetics of the KA EPSC. Third, to increase glutamate clearance, we raised temperature during recordings. This shortened the decay of both the AMPA and KA components and increased their amplitudes, but this effect was the same for both. Therefore these data provide evidence against glutamate diffusion out of the synaptic cleft as the mechanism for the slow kinetics of KA EPSCs. Other possibilities such as interactions of KA receptors with other proteins or novel properties of native synaptic heteromeric receptors are required to explain the slow kinetics.

INTRODUCTION

Fast excitatory synaptic transmission in the CNS is mediated predominantly by the ionotropic glutamate receptors that can be divided into three classes: α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate (KA) receptors (Hollmann and Heinemann 1994; Watkins and Evans 1981). Although AMPA and NMDA receptors have been extensively studied, KA receptors have received less attention and their physiological functions remain relatively unexplored. The recent development of pharmacological agents that distinguish between AMPA and KA receptors (BLEakman and Lodge 1998; Chittajallu et al. 1999; Frerking and Nicoll 2000) have now enabled the roles of KA receptors in the CNS to be studied.

KA receptors are made up of different combinations of the GluR5–7 and KA1 and KA2 subunits, which are widely expressed throughout the CNS (Hollmann and Heinemann 1994). The synaptic activation of KA receptors has been described in a number of brain regions (Chittajallu et al. 1999; Frerking and Nicoll 2000). Presynaptic KA receptors are involved in regulating transmitter release at both excitatory and inhibitory synapses in the hippocampus (Chittajallu et al. 1996; Clarke et al. 1997; Rodriguez-Moreno et al. 1997) and at inhibitory synapses in the hypothalamus (Lui et al. 1999). Postsynaptic KA receptor-mediated excitatory postsynaptic currents (EPSCs) were first described at mossy fiber synapses in the CA3 region of the hippocampus (Castillo et al. 1997; Vignes and Collingridge 1997). Since these first two reports, KA receptor-mediated EPSCs (KA EPSCs) have been described in a variety of other preparations such as hippocampal interneurons (Cossart et al. 1998; Frerking et al. 1998), amygdala (Li and Rogawski 1998), retina (DeVries and Schwartz 1999), spinal cord (Li et al. 1999), barrel cortex (Kidd and Isaac 1999), and cerebellum (Bureau et al. 2000). The precise roles of KA receptors in many of these brain regions still remain to be determined. Most is known about KA receptor function in the hippocampus where they have been shown to be important for regulating excitability (e.g., Chittajallu et al. 1996; Clarke et al. 1997; Contractor et al. 2001; Cossart et al. 1998; Frerking et al. 1998, 1999; Kamiya and Ozawa 2000; Min et al. 1999; Mulle et al. 2000; Rodriguez-Moreno et al. 1997; Schmitz et al. 2000). They have also been shown to be important for the induction of mossy fiber LTP (Bortolotto et al. 1999).

At the great majority of synapses studied, KA EPSCs exhibit slow kinetics (Bureau et al. 2000; Castillo et al. 1997; Cossart et al. 1998; Frerking et al. 1998; Kidd and Isaac 1999; Li and Rogawski 1998; Li et al. 1999; Vignes and Collingridge 1997; but see DeVries and Schwartz 1999). This might suggest that KA receptors are of high affinity, similar to N-methyl-D-aspartate receptors (NMDARs). However, this idea is not supported by studies in which agonists were exogenously applied to either native or heterologously expressed KA receptors. Under these conditions, KA receptors always exhibit rapid kinetics similar to AMPA receptors even when high-affinity subunits (KA1 and KA2) are present (e.g., Cui and Mayer 1999; Herb et al. 1992; Huettner 1990; Lerma et al. 1993; Paternain et al.

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1995; Patneau et al. 1994; Schiffer et al. 1997; Swanson and Heinemann 1998; Swanson et al. 1996). In some of these studies, differences in kinetics were observed with certain combinations of subunits (Cui and Mayer 1999; Herb et al. 1992), and another study has shown that the association of GluR6 and KA2 with the postsynaptic density proteins SAP90 and SAP102 also causes alterations in kinetics (Garcia et al. 1998). However, these relatively small differences fall far short of being able to explain the large discrepancy between the kinetics of synthaptically activated KA receptors and KA receptors activated by exogenously applied agonists.

Therefore another possibility that must be considered is that it is the time course of the application of the agonist that differs between these types of studies. That is, while exogenously applied agonist arrives rapidly at the receptors, synthaptically released glutamate arrives at KA receptors more slowly, and this influences the time course and extent of their activation. Although this is not a new idea (see: Castillo et al. 1997; Lerma 1997; Mayer 1997; Vignes and Collingridge 1997), the original proposal was used to explain the slow kinetics of the KA EPSCs at the mossy fiber–CA3 synapse, which are only observed after high-frequency stimulation (Castillo et al. 1997; Vignes and Collingridge 1997), a condition likely to favor a build up of glutamate in the synaptic cleft and its slow diffusion to receptors. However, more recent studies have demonstrated equally slow kinetics for KA EPSCs evoked by a single stimulus (Bureau et al. 2000; Cossart et al. 1998; Frerking et al. 1998; Kidd and Isaac 1999; Li and Rogawski 1998; Li et al. 1999), making the kinetics much more perplexing. Figure 1 shows some possibilities for the location of KA receptors under which conditions diffusion of glutamate from the synaptic cleft could influence the extent of their activation and kinetics. They could be present on the spine but on the edge of the synapse (Fig. 1A) existing as perisynaptic receptors sensing “spill-out” of glutamate from one synapse. They could be present in the center of the postsynaptic density of a synapse that has a nonfunctional presynaptic bouton and only sense glutamate released from other synapses (Fig. 1B), thus sensing spillover of glutamate. Another possibility is that they could be entirely extrasynaptic and sense spillover (Fig. 1C).

These models could explain the slow kinetics of the KA EPSC; however, there is one problem. Data from recent studies modeling the spatiotemporal properties of glutamate diffusion indicate that glutamate is unlikely to remain in the extrasynaptic space for long enough to activate KA receptors for \( \geq 100 \) ms (Barbour and Haussner 1997; Rusakov and Kulman 1998; Wahl et al. 1996), particularly at a concentration to activate a low-affinity receptor. However, these studies also show that the profile of extrasynaptic glutamate is critically dependent on a number of unknowns, such as the glutamate diffusion coefficient, for which there are currently no accurate estimates and which may vary from region to region of the brain. Furthermore when synaptic transporter currents are studied in brain slices, it is noticeable that there is a slow component to the current that persists for \( > 150 \) ms (e.g., Bergles and Jahr 1997). This presumably reflects the long-lasting presence of glutamate in the extracellular space and indicates that modeling studies may not provide a full description of glutamate diffusion in the brain. For example, uncertainties remain about the location of high-affinity glutamate transporters and the possibility that transporters act as a buffers for released glutamate and slowly re-release glutamate over a prolonged period (Diamond and Jahr 1997). Furthermore these glutamate diffusion studies have been performed using hippocampal CA1 synapses; therefore without further studies and more detailed analysis of glutamate diffusion in other areas of the CNS, it is unclear whether glutamate diffusion alone can account for the very slow kinetics of the KA EPSC.

The aim of this study was to investigate whether the kainate receptors underlying the KA EPSC at thalamocortical synapses are located outside the synaptic cleft and whether this contributes to the slow kinetics of the KA EPSC. In this situation, receptors would be activated by glutamate diffusing from the synaptic cleft, and hence the extent and kinetics of their activation should be altered by manipulations that alter extrasynaptic glutamate clearance. In only two studies to date has there been a systematic evaluation of an extrasynaptic diffusion model for the activation of kainate receptors. One, on the mossy fiber–CA3 KA EPSC, found that slowing diffusion with a high-molecular-weight dextran caused a potentiation in the amplitude of the KA EPSC with little effect on the kinetics (Min et al. 1998b). This suggests that the glutamate build up during the high-frequency stimulation necessary to evoke the current determined the amplitude but not the kinetics of the response. In the other study, the effects of glutamate transport inhibitors and a glutamate scavenger were investigated on the KA EPSC at cerebellar parallel fiber–Golgi cell synapses (Bureau et al. 2000). No effect of these manipulations was observed on transmission under physiological conditions, although it wasn’t possible to simultaneously investigate the effects on AMPA receptor-mediated EPSCs in this preparation. In the present study, we took advantage of the large size of the KA component to transmission at developing thalamocortical synapses (Kidd and Isaac 1999) to compare the effects of manipulating glutamate diffusion on both AMPA and KA receptors simultaneously at the same population of synapses. Because the affinity and synaptic localization of AMPA receptors is well known, this enabled us to make stronger conclusions on the affinity and localization of synthaptically activated...
KA receptors than has previously been possible (Bureau et al. 2000; Frerking et al. 1998). Using this approach, we found that there was no selective effect on the size or kinetics of the KA receptor-mediated component to transmission when synapses were activated at high frequency, glutamate transport was inhibited, or temperature was changed. This suggests that the synaptic activation of KA receptors is not strongly influenced by extrasynaptic glutamate diffusion.

**METHODS**

**Electrophysiology**

Thalamocortical slices (Agmon and Connors 1991) were prepared as previously described (Feldman et al. 1998; Kidd and Isaac 1999) from Wistar rat pups aged between postnatal day 3 and 9 (day 0 is the day of birth). Slices were bathed in an extracellular solution of the following composition (mM): 119 NaCl, 2.5 KCl, 1.0 NaH2PO4, 26.2 NaHCO3, 2.5 CaCl2, 1.3 MgSO4, and 11 glucose, saturated with 95% O2-5% CO2 at room temperature (23–25°C, unless otherwise indicated). The extracellular solution also contained t-2-amino-5-phenolphonopentanoic acid (t-AP5, 100 μM) and picrotoxin (50 μM) to block NMDA receptor- and GABA receptor-mediated currents, respectively. Whole cell patch-clamp recordings were made from neurons in layer IV of the somatosensory (barrel) cortex (Feldman et al. 1998; Kidd and Isaac 1999) using electrodes (3–5 MΩ) containing the following solution (mM): 135 CsMeSO4, 8 NaCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, and 0.3 Na-GTP, pH 7.25, 285 mOsm. In some experiments, 5 mM lidocaine N-ethyl bromide (QX-314) and 1 mM QX-314, in addition, in some experiments, blocked postsynaptic GABA receptor-mediated responses. EPSCs were evoked by electrical stimulation (0.1–0.2 Hz) of thalamocortical afferents in the thalamus or the white matter (in most experiments, 5 stimuli at 100 Hz; Fig. 2A) (Kidd and Isaac 1999). The relatively large size of the KA EPSC and the good kinetic separation between this and the AMPA EPSC allowed us to measure the charge transfer and the decay kinetics of both components simultaneously.

**RESULTS**

In the presence of d-AP5 and picrotoxin, electrical stimulation of thalamocortical axons evokes EPSCs in layer IV neurons in the barrel cortex of neonatal rats mediated by both AMPA and KA receptors (Kidd and Isaac 1999). As previously described, these two components are kinetically and pharmacologically distinct; the fast component is mediated by AMPA receptors (AMPA EPSC), and the slow component by KA receptors (KA EPSC). Therefore the decay of the KA EPSC is best fit by the sum of two exponentials (Fig. 2A) (Kidd and Isaac 1999). The relatively large size of the KA EPSC and the good kinetic separation between this and the AMPA EPSC enabled us to measure the charge transfer and the decay kinetics of both components simultaneously.

**High-frequency stimulation potentiates KA EPSCs due to postsynaptic summation and a buildup of glutamate**

Our first approach was to compare EPSCs evoked at low frequency (EPSC1F) with EPSCs evoked by a train of five stimuli at high frequency (100 Hz; Fig. 2A). The high-frequency train caused a depression in peak EPSC amplitude (amplitude of last EPSC in train = 66 ± 6% of first EPSC amplitude, P < 0.005, n = 16; Fig. 2B) as has been reported for thalamocortical synapses onto layer II/III pyramidal neurons in barrel cortex (Gil et al. 1997). When the last EPSC of the train was compared with the preceding averaged EPSC1F, there was a slowing in the decay of the KA component (EPSC1F 7 decay = 138.6 ± 19.1, last EPSC of train τ decay = 211.6 ± 34.4 ms, P < 0.05, n = 16; Fig. 2C). An increase in the proportion of charge transfer through the KA component of the dual EPSC in response to the train was also observed (KA EPSC charge contribution as percentage of total EPSC charge, EPSC1F 60 ± 7%, last EPSC of train = 85 ± 4%, P < 0.005, n = 16; Fig. 2D). Using pharmacologically isolated KA EPSCs, similar results were obtained; the decay time constant for the isolated KA EPSC under these conditions was very similar to that estimated from the dual component EPSC, and a similar increase in the decay time constant was also observed in response to the 100-Hz trains (isolated KA EPSC1F 7 decay = 149.6 ± 18.4, isolated KA EPSC train τ decay = 248.2 ± 43.1
ms, \( P < 0.05, n = 9; \) Fig. 2C). This demonstrates that the double-exponential fitting procedure reliably reports changes in the kinetics of the KA component of the dual EPSC.

To investigate whether the increase in charge transfer and the slowing of the KA EPSC were due to a buildup of extrasynaptic glutamate during the stimulus train or simply an effect of postsynaptic summation, we reconstructed the response to the burst using the preceding averaged EPSC_LF (Fig. 3A). The response reconstructed from the EPSC_LF was very similar to the response to the high-frequency train in all cells (mean \( \tau_{\text{decay}} \) values for KA receptor-mediated component: train = 211.6 ± 34.4 ms, reconstructed train = 202.5 ± 34.0 ms, \( P = 0.8, n = 16 \); KA EPSC charge contribution: train = 85 ± 4%, reconstructed train = 83 ± 5%, \( P = 0.7 \); Fig. 3, B and C). Therefore the slowing of the decay kinetics and the increased contribution of the KA EPSC at the end of the train were due to the summation of the EPSCs evoked during the train rather than increased activation of KA receptors by a buildup of glutamate.

We also compared the summation of the AMPA receptor and KA receptor mediated components of transmission during trains of stimuli by evoking dual component EPSCs or pharmacologically isolated KA EPSCs with trains of five stimuli at 33 Hz. Due to its slow kinetics, the pharmacologically isolated KA EPSC exhibited much greater postsynaptic summation during the trains than the AMPA receptor-dominated dual component EPSC (amplitude of last isolated KA EPSC in train = 293 ± 26% of 1st EPSC, \( n = 3 \); amplitude of dual EPSC = 83 ± 11% of 1st, \( n = 9 \); Fig. 3D). A similar small amount of paired-pulse facilitation (PPF) was observed for both types of EPSCs (isolated KA EPSC = 113 ± 19%, \( n = 3 \); dual EPSC = 109 ± 14%, \( n = 9 \); \( P = 0.9 \)). This indicates that both had a similar probability of release, and the lack of significant PPF also indicates that probability of release at these synapses is high.

**FIG. 2.** High-frequency stimulation potentiates the kainate (KA) component to transmission at thalamocortical synapses in the neonatal barrel cortex. A: excitatory postsynaptic currents (EPSCs) from a representative experiment in which the response to low-frequency stimulation (left) was compared with the response to 5 stimuli at 100 Hz (middle and right, the 2 responses superimposed and scaled). Superimposed black lines (left and middle) represent a double exponential fit of the decay (\( \tau_{\text{decay}} \) values indicated). For these and traces in all following figures, the stimulus artifact has been digitally subtracted. B: change in peak EPSC amplitude (normalized to the amplitude of the 1st response; postsynaptic summation not subtracted) during the 100-Hz train from 16 pathways/12 cells (asterisks represent \( P < 0.005 \) vs. the 1st EPSC in the train, \( n = 16 \)). C: mean \( \tau_{\text{decay}} \) values of the KA EPSC for the low-frequency EPSC (black) and last EPSC in the train (gray) estimated from the slow exponential fit to the decay (\( \tau_{\text{KA}} \)) of the dual component (left; asterisk indicates \( P < 0.05, n = 16 \)) or the pharmacologically isolated KA EPSC (right; asterisk indicates \( P < 0.05, n = 9 \)). D: charge contribution of the AMPA and KA receptor-mediated components to the dual component EPSC (normalized to total EPSC charge) for low-frequency EPSC (black) and the last EPSC in the train (gray; asterisks indicate \( P < 0.005, n = 16 \)).
However, subtraction of postsynaptic summation revealed that the KA EPSCs did not depress during the train (last isolated KA EPSC = 128 ± 32% of 1st, n = 3). This suggests that sufficient receptors at the same population of synapses were available during repetitive stimulation to produce multiple KA EPSCs of similar sizes even though a significant proportion of receptors was still activated by the preceding release of glutamate. This indicates that only a fraction of the available KA receptors are occupied in response to a single synaptic release of glutamate.

**Glutamate transport inhibitors do not have a selective effect on KA EPSCs**

Another approach we used was to slow extrasynaptic glutamate clearance by inhibiting high-affinity glutamate transport. We used a combination of the transport inhibitors, D,L-threo-β-hydroxyaspartate (THA; 300 µM) (Balcar et al. 1977) and dihydrokainate (DHK; 300 µM) (Johnston et al. 1978) to assess the effect of blocking glutamate uptake on the KA EPSC. However, in contrast to studies using these transport inhibitors, the apparent potentiation of the KA EPSC by 100-Hz stimulation is reproduced by scaling and summing individual EPSCs evoked at low frequency. A: EPSCs from a representative experiment in which the averaged EPSC evoked at low frequency (A1) was compared with the response to a train of stimuli at 100 Hz (A2) by reconstructing the train by scaling and summing the low-frequency EPSC (A3). A4: A, 2 and 3, superimposed. B: mean τ_decay values for AMPA and KA receptor-mediated components from the last EPSC in the 100-Hz train (black) and from the train reconstructed from the low-frequency EPSC (gray) for 16 pathways/12 cells. C: charge contribution of the AMPA and KA receptor-mediated components (normalized to total EPSC charge) for the 100-Hz train (black, n = 16) and reconstructed train (gray, n = 16). D: EPSC amplitude (% 1st EPSC in train) for pharmacologically isolated KA EPSC (filled circles, n = 3) or dual component EPSC (open circles, n = 9) during 33-Hz train stimulation. Inset: examples of trains of dual EPSCs (top) and pharmacologically isolated KA EPSCs (bottom).
inhibitors on hippocampal slices (Bergles and Jahr 1997; Lüscher et al. 1998), we subsequently found that THA (300 μM) alone or another transport inhibitor, L-trans-pyrrolidine-2,4-dicarboxylate (tPDC; 300 μM) (Bridges et al. 1991), alone had very similar effects to the combination of inhibitors. Therefore all these data were pooled. Application of the transport inhibitors caused a rapid depression in the amplitude of the dual component EPSC (EPSC amplitude = 14 ± 7% of baseline, P < 0.001, n = 21) that was reversible on wash-out (Fig. 4A). This depression was not due to postsynaptic desensitization because cyclothiazide (100 μM), which blocks AMPA receptor desensitization (Yamada and Tang 1993), did not prevent the depression (Kidd and Isaac 2000). However, the depression was significantly antagonized by the selective KA receptor antagonist LY382884 (10 μM) (Bortolotto et al. 1999), indicating that it was due to the activation of an inhibitory presynaptic kainate autoreceptor (EPSC amplitude in presence of transport inhibitors and LY382884 = 47 ± 12% of baseline, n = 8; P < 0.05 vs. EPSC amplitude with transport inhibitors alone). This activation of the presynaptic autoreceptor suggests that the transport inhibitors alter the profile of glutamate in the synaptic cleft and hence also very likely have an effect on the extrasynaptic profile of released glutamate. This is in agreement with other studies demonstrating that transport inhibitors can cause the activation of presynaptic receptors due to increased ambient levels of glutamate (Maki et al. 1994; Zorumski et al. 1996). In addition it has been shown that altering glutamate clearance with transport blockers, glutamate scavengers, or a high-molecular-weight dextran has direct effects on the level of activation of postsynaptic receptors (Bureau et al. 2000; Min et al. 1998b).

Due to the presynaptic inhibition, glutamate transport inhibitors are likely to actually reduce the amount of spillover of glutamate from neighboring thalamocortical synapses (Fig. 1, B and C). However, this manipulation is useful for evaluating whether KA receptors are perisynaptic because it would be predicted to enhance the spill-out of glutamate from the synaptic cleft when transmitter is released (see Fig. 1A). The transport inhibitors had no effect on the decay of the KA EPSC (Fig. 4C) or the relative contribution of KA receptors to the dual component EPSC (KA EPSC charge transfer values: baseline = 875.1 ± 214.4 fC, inhibitors = 199.3 ± 54.2 fC, P < 0.005, n = 20; KA EPSC charge percentage total EPSC: baseline = 7% ± 5%, inhibitors = 7.5%, P = 0.5, n = 20; τdecay values: baseline = 28.1 ± 2.2 ms, n = 17, inhibitors = 26.5 ± 1.9 ms, n = 16, P = 1.0; Fig. 4D). The combination of inhibitors caused a rapid depression in the amplitude of the dual component EPSC (KA EPSC charge transfer values: baseline = 58.5 ± 7.1%, inhibitors = 53.2 ± 7.5%, P = 0.5, n = 20; τdecay values: baseline = 28.1 ± 2.2 ms, n = 17, inhibitors = 26.5 ± 1.9 ms, n = 16, P = 1.0; Fig. 4E). This depression was not due to postsynaptic desensitization because cyclothiazide (100 μM), which blocks AMPA receptor desensitization (Yamada and Tang 1993), did not prevent the depression (Kidd and Isaac 2000). However, the depression was significantly antagonized by the selective KA receptor antagonist LY382884 (10 μM) (Bortolotto et al. 1999), indicating that it was due to the activation of an inhibitory presynaptic kainate autoreceptor (EPSC amplitude in presence of transport inhibitors and LY382884 = 47 ± 12% of baseline, n = 8; P < 0.05 vs. EPSC amplitude with transport inhibitors alone). This activation of the presynaptic autoreceptor suggests that the transport inhibitors alter the profile of glutamate in the synaptic cleft and hence also very likely have an effect on the extrasynaptic profile of released glutamate. This is in agreement with other studies demonstrating that transport inhibitors can cause the activation of presynaptic receptors due to increased ambient levels of glutamate (Maki et al. 1994; Zorumski et al. 1996). In addition it has been shown that altering glutamate clearance with transport blockers, glutamate scavengers, or a high-molecular-weight dextran has direct effects on the level of activation of postsynaptic receptors (Bureau et al. 2000; Min et al. 1998b).

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Therefore inhibition of high-affinity glutamate transport, although having an effect on the peak amplitude of the dual component EPSC due to raised ambient levels of glutamate, had no selective effect on the KA EPSC. This provides evidence against a perisynaptic location for KA receptors.

Increasing temperature does not selectively affect KA EPSCs

Having used two approaches to increase the amount of extrasynaptic glutamate and observed no change in the KA EPSC, we were concerned that the lack of a detectable effect could reflect the fact that KA receptors are fully saturated during basal conditions. Under these conditions, reducing extrasynaptic glutamate clearance would be expected to have no additional effect. Therefore we studied the effect of raising temperature during recordings. This manipulation increases the rate of glutamate uptake because transport is temperature sensitive (van der Kloot and Molgo 1994). For example, it produces effects on glutamatergic synaptic transmission in hippocampal slices that have been attributed to a reduced extrasynaptic glutamate concentration (e.g., Asztely et al. 1997). Increasing temperature from room temperature (22.8 ± 0.3°C, range = 22–24°C, n = 8 cells) to close to physiological temperature (35.4 ± 1.1°C, range = 29–38°C, n = 8 cells) caused an increase in peak EPSC amplitude (191.0 ± 25.6% of baseline, P < 0.005; n = 14) that was reversible on returning to room temperature (Fig. 5A). The increase in temperature also caused a decrease in τdecay of both the AMPA and KA EPSCs (AMPA EPSC τdecay values: 23°C = 7.1 ± 1.1 ms, 35°C = 3.9 ± 0.4 ms, P < 0.005, n = 14; KA EPSC τdecay values: 23°C = 130.0 ± 15.2 ms, n = 13, 35°C = 60.4 ± 7.9 ms, n = 12, P < 0.005; Fig. 5, B and C). However, temperature had no effect on the charge transfer through the two components (AMPA EPSC charge values: 23°C = 330.2 ± 53.3 fC, 35°C = 402.7 ± 137.7 fC, P = 0.5, n = 14; KA EPSC charge values: 23°C = 1,077.7 ± 267.7 fC, 35°C = 1,122.1 ± 298.1 fC, P = 0.7, n = 14; Fig. 5D) and therefore did not affect the relative contribution of the KA EPSC to transmission (KA EPSC charge percentage of total EPSC charge: 23°C = 63.2 ±
7.3%, 35°C = 56.8 ± 8.6, P = 0.1, n = 14; Fig. 5E). The lack of change in charge transfer in either component therefore indicated that the same relative numbers of KA and AMPA channels contributed to the EPSC at both temperatures. The changes in peak EPSC amplitude and decay were very likely due to the strong temperature dependence of channel kinetics (Hestrin et al. 1990; Magleby and Stevens 1972). However, the increase in temperature did not decrease the charge transfer of the KA EPSC as would be expected for the glutamate diffusion increase in temperature. However, the due to the strong temperature dependence of channel kinetics changes in peak EPSC amplitude and decay were very likely channels contributed to the EPSC at both temperatures. The indicated that the same relative numbers of KA and AMPA of change in charge transfer in either component therefore therefore did not cause a change in the KA EPSC. We think this is unlikely for three reasons: 1) glutamate transport inhibitors had a marked effect on EPSC amplitude due to increased ambient glutamate levels activating an inhibitory presynaptic glutamate receptor as has been reported in other studies (Asztely et al. 1997; Maki et al. 1994; Min et al. 1998; Scanziani et al. 1997; Zorumski et al. 1996). There are other possible explanations for the effect of transport inhibitors on EPSC amplitude, but we think these are less likely. Desensitization of postsynaptic receptors is unlikely because cyclothiazide did not block the effect of the inhibitors (Kidd and Isaac 2000). THA is also an NMDA receptor agonist (Tong and Jahr 1994); however, direct activation of NMDA receptors can be ruled out because all recordings were performed in 100 μM d-AP5. Furthermore tPDC, which had a similar effect, is not an NMDA receptor agonist. It is possible that both tPDC and THA are agonists at a presynaptic metabotropic glutamate (mGlut) receptor. However, while there is some evidence for tPDC as an agonist at one mGlut receptor subtype in astrocytes (Miller et al. 1994), other studies provide evidence against such an action for both tPDC and THA (Fitzsimonds and Dichter 1996; Thomsen et al. 1994). An alternative possibility is the direct activation of the presynaptic kainate receptor by the transport inhibitors; however, binding studies show that both THA and tPDC do not displace the kainate receptor ligands, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or KA (Balcar et al. 1995). 2) The use of increasing temperature as a method to speed up the clearance of glutamate has been shown to have effects on glutamate diffusion in hippocampal culture (Tong and Jahr 1994) and has been reported to reduce spillover of glutamate at synapses in hippocampal slices (Asztely et al. 1997; Min et al. 1998a). 3) A recent study of synaptically activated transporter currents (STCs) in hippocampal astrocytes (Diamond and Jahr 2000) showed a progressive change in the rise and decay kinetics of STCs during a 100-Hz train at room temperature with effects observed by the fourth stimulus. This indicates that brief trains of 100-Hz stimulation alter the clearance of glutamate.

All this suggests that the manipulations used in the present study alter extrasynaptic glutamate clearance. Indeed similar manipulations have been used at mossy fiber-CA3 synapses, CA1 synapses and cerebellar synapses (Asztely et al. 1997; Bureau et al. 2000; Min et al. 1998; Scanziani et al. 1997). These manipulations have been shown to change the activation of both presynaptic and postsynaptic receptors, demonstrating that glutamate transport can play an important role in determining the level of activation of glutamate receptors at synapses.

KA receptors appear to be far from fully occupied following a single synaptic release of glutamate, an observation also reported for cerebellar parallel fiber–Golgi cell synapses (Bureau et al. 2000). This suggests that the receptors are of low affinity or are located far from the site of release. The lack of effects on the KA EPSC of alterations in glutamate clearance reported in the present study provides evidence against this latter possibility. The lack of a selective effect of manipulating glutamate clearance also suggests that AMPA receptors have a similar low level of occupancy. This would be consistent with both AMPA and KA receptors having similar affinities for glutamate and being at similar locations in the synapse.

**Mechanisms for the slow kinetics of the KA EPSC**

The lack of effect of manipulating glutamate clearance on the KA EPSC provides evidence against glutamate diffusion as the mechanism for the slow kinetics of the KA EPSC. Therefore what other mechanisms might explain the slow kinetics? One possibility is that native KA receptors interact with proteins at the synapse and this causes a change in their kinetic properties. A recent study has provided evidence for this by showing that interactions of GluR6 homomers or GluR6/KA2 heteromers with the postsynaptic density protein SAP 90 produced receptors that did not fully desensitize in response to glutamate (Garcia et al. 1998). Another possibility is that synaptically KA receptors are heteromers that have novel properties and that these combinations of subunits are not found for the native extrasynaptic or heterologically expressed KA receptors so far studied. For thalamocortical synapses, although the subunit composition of the native receptors is not known, a prediction can be made from in situ hybridization studies. During the first postnatal week GluR5 is expressed at high levels in layers II/III and IV of the rat barrel cortex, GluR7 is expressed in layers IV–VI, and KA2 is expressed throughout the neocortex (Bahn et al. 1994). KA receptors at thalamocortical synapses during this developmental period also exhibit a strongly rectifying I-V relationship (Kidd and Isaac 1999), suggesting they contain a substantial proportion of unedited subunits. Therefore the native KA receptors at thalamocortical synapses in neonatal tissue are likely to be heteromeric com-
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The exact stoichiometry will depend on the relative levels of expression of these subunits and their targeting to synapses. The properties of GluR5 homomers are well known; they exhibit rapid kinetics similar to AMPA receptors (Herb et al. 1992; Swanson and Heinemann 1998; Swanson et al. 1996). While there is no information on GluR5(Q)/GluR7/K2A homomers, it is known that GluR5(Q)/KA2 also exhibits rapid kinetics (Herb et al. 1992). However, co-assembly of different subunits can cause alterations in the properties of the receptor complex (Cui and Mayer 1999; Herb et al. 1992), and GluR5-containing receptors exhibit variations in kinetics, for example, in the rate of recovery from desensitization (Swanson and Heinemann 1998). These properties could provide potential mechanisms for the slow kinetics of synaptic KA receptors.

In summary, we have found that manipulations that affect the clearance of glutamate have no selective effect on the KA EPSC at thalamocortical synapses. This provides evidence against glutamate diffusing out of the synaptic cleft and activating peri- or extrasynaptic kainate receptors. Further work is necessary to determine if single-channel properties or interactions with synaptic proteins can explain the slow kinetics of the KA EPSC.

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