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Presynaptic Kainate Receptors that Enhance the Release of GABA on CA1 Hippocampal Interneurons

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Summary

We report that kainate receptors are present on presynaptic GABAergic terminals contacting interneurons and that their activation increases GABA release. Application of kainate increased the frequency of miniature inhibitory postsynaptic currents recorded in CA1 interneurons. Local applications of glutamate but not of AMPA or NMDA also increased GABA quantal release. Application of kainate as well as synaptically released glutamate reduced the number of failures of GABAergic neurotransmission between interneurons. Thus, activation of presynaptic kainate receptors increases the probability of GABA release at interneuron–interneuron synapses. Glutamate may selectively control the communication between interneurons by increasing their mutual inhibition.

Introduction

Inhibitory GABAergic interneurons play a crucial role in shaping cortical activity (Cobb et al., 1995; Buzsaki, 1997; Tamas et al., 2000). They are densely interconnected via chemical (GABAergic) and electrical synapses, and this interconnectivity is believed to enhance the computational power of cortical networks (Galarreta and Hestrin, 1999; Gibson et al., 1999; Fukuda and Kosaka, 2000). In that respect, the regulation of presynaptic GABAergic terminals is crucial to consider, since an up- or downregulation of GABA release can modify neuronal network function/behavior, as occurs in synaptic plasticity (Wigstrom and Gustafsson, 1983), oscillations (Hajos et al., 2000), or epilepsy (Prince and Jacobs, 1998; Hirsch et al., 1999).

Transmitter release at presynaptic GABAergic terminals can be regulated by a variety of neuromodulators and neurotransmitters including GABA itself and the ubiquitous excitatory neurotransmitter glutamate (MacDermott et al., 1999). The control of GABA release by glutamate receptors is best documented for metabotropic receptors. The activation of presynaptic glutamatergic metabotropic receptors at GABAergic terminals generally leads to a decrease of transmitter release (Poncer et al., 1995; Rodriguez-Moreno and Lerma, 1998; Semyanov and Kullmann, 2000). A possible control of GABA release by ionotropic glutamatergic receptors has only emerged recently. Thus, AMPA and NMDA receptors are present on presynaptic GABAergic terminals in the cerebellum, and their activation leads to an increase (Bureau and Mulle, 1998; Glitsch and Marty, 1999) or a decrease (Satake et al., 2000) of GABA release.

The purpose of this study was to investigate whether GABA quantal release is modulated by presynaptic glutamate receptors at various classes of interneuron–interneuron synapses in the hippocampus. Attention was focused on kainate because these receptors are widely expressed in most classes of interneurons (Bahn et al., 1994; Petralia et al., 1994; Siegel et al., 1995), raising the possibility of their expression in presynaptic GABAergic terminals, and because kainate receptors are hypothesized to play an important role as modulators of GABAergic activity (Fisher and Alger, 1984; Cossart et al., 1998; Rodriguez-Moreno et al., 1997; Bureau et al., 1999; Frerking et al., 1999).

We report that kainate receptors are present on GABAergic terminals contacting various classes of morphologically identified CA1 interneurons. Bath application of kainate or glutamate but not of any other agonist for ionotropic or metabotropic glutamate receptors increased the frequency of miniature inhibitory postsynaptic currents (mIPSCs) on interneurons without affecting their amplitude. We also show that bath-applied kainate or synaptically released glutamate selectively decreased the failure rate of evoked GABAergic responses on CA1 interneurons. Therefore, the activation of presynaptic kainate receptors increases the efficacy of GABAergic transmission between interneurons.

Results

Since presynaptic receptors can differently regulate action potential–dependent and -independent release of neurotransmitter (Glitsch and Marty, 1999), two types of experiments are available to assess the presence of presynaptic receptors: (1) the recording of miniature events, a change in their frequency but not of their amplitude, indicating a presynaptic effect, and (2) assessing the probability of failure of synaptic transmission evoked by the electrical activation of a presynaptic axon. Both approaches were used in this study.

Low Concentrations of Kainate Increase the Frequency of Miniature GABAergic Currents in CA1 Interneurons

To determine whether kainate modulates miniature GABAergic currents at GABAergic synapses on interneurons, we have recorded mIPSCs (Vhold, + 10mV) from 37 visually identified interneurons of stratum radiatum and stratum oriens in the CA1 region of the hippocampus. In all experiments, we used a low concentration of kainate (250 nM) that selectively activates kainate

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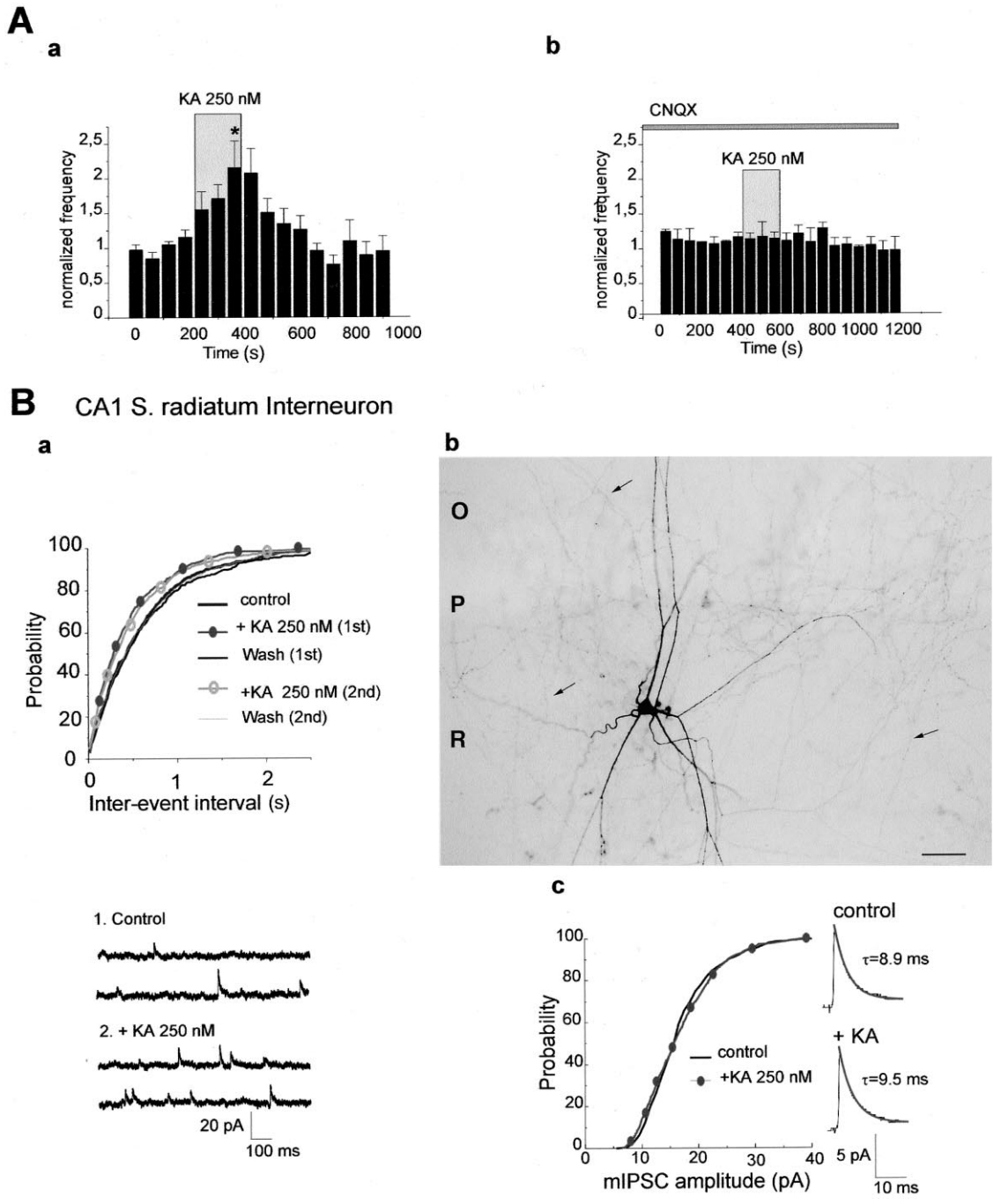

Figure 1. Kainate Increases the Frequency of Miniature GABAergic Events on CA1 Interneurons via the Activation of Presynaptic Kainate Receptors

(Aa and Ab) The bar graphs show mean ± SEM values. *$p < 0.01$.

(Aa) Mean frequency of mIPSCs (Vh +10 mV) as a function of time for the entire population of CA1 interneurons ($n = 18$) for a 3 min bath application of kainate (250 nM). For each cell, mIPSC frequency was normalized to the value of mean IPSC frequency before application of kainate (bin = 60 s). Experiments were performed in the presence of TTX (1 μM) and of AMPA and NMDA receptor antagonists, D-APV (50 μM) and GYKI 53655 (30 μM).

(Ab) Pooled results for the mean mIPSCs frequency as a function of time, showing that the effect of a second application of kainate (250 nM) on mIPSCs is blocked by the mixed AMPA/kainate antagonist CNQX (50 μM) in the presence of D-APV (50 μM) and GYKI 53655 (50 μM).

(Ba) Two successive bath applications of kainate (250 nM) in the presence of GYKI 53655 (30 μM) and D-APV (50 μM) reversibly increased the frequency of miniature IPSCs (Vh + 10mV). (Upper trace) Cumulative probability plots of miniature IPSC intervals show a significant reversible shift of the distribution towards smaller time intervals between events for two successive applications of kainate. (Bottom traces) Miniature GABAergic events recorded before (1) and during (2) the first application of kainate.

(Bb) The photomicrograph illustrates the interneuron recorded in (Ba) and (Bc). The soma is in stratum radiatum, and the axon (arrows) extensively innervates stratum oriens and stratum radiatum (Schaffer-associated interneuron in stratum radiatum). Scale bar = 50 μm.

receptors without affecting AMPA receptors (Cossart et al., 1998; Bureau et al., 1999). Furthermore, kainate application was performed in the presence of GYKI 53655 (30 μM) and D-APV (50 μM) to block AMPA- and NMDA receptor–mediated responses, respectively. In the presence of TTX (1 μM) to suppress action potential–dependent IPSCs, the mean frequency of miniature GABAergic currents was 5.8 ± 0.9 Hz (mean ± SEM; n = 37, range: 0.9–14.7 Hz). Bath application of kainate caused a significant increase of the mean mIPSC frequency (178% ± 19% of control values; n = 37, $p < 0.01$) but no change in the mean amplitude, decay time constant, or amplitude distribution of mIPSCs (Figure 1B). Figure 1A shows the frequency of mIPSCs as a function of time, pooled for the entire population of cells for a 3 min kainate application. The frequency of mIPSCs was increased by more than 20% in the majority of cells (80%). In the other 20% of cells, the increase in mIPSCs frequency was not statistically significant. Morphologically, the kainate-responsive cells included the following groups of previously described GABAergic neurons (Freund and Buzsaki, 1996; Vida et al., 1998): oriens–lacunosum moleculare (O-LM) cells (n = 4), Schaffer-associated interneurons in stratum radiatum (n = 4; Figure 1B), bistratified interneurons in stratum oriens (n = 1), perisomatic interneurons in stratum oriens (n = 1), and perforant path–associated interneurons at stratum radiatum/lacunosum moleculare border (n = 3). The other neurons were unequivocally identified as interneurons but could not be classified, as their axons were incompletely labeled. The kainate-nonresponsive cells did not belong to any specific morphological class.

The increase of mIPSC frequency persisted throughout the application of kainate (3–10 min) and was fully reversed after the drug was washed out (Figure 1). There was no effect of kainate on the holding current of the interneurons, as cells were voltage clamped at the reversal potential for kainate receptor–mediated currents. A second application of kainate following wash out of the first produced the same reversible increase in mIPSC frequency (Figure 1B; n = 3). Since, when AMPA and NMDA receptors are blocked, CNQX, the AMPA/kainate antagonist, can be used as a kainate receptor antagonist, we tested the effects of kainate in the presence of CNQX. The effect of kainate on mIPSC frequency (148% ± 12%; n = 3, $p < 0.01$) was antagonized by CNQX (50 μM; 107% ± 8%; n = 3, $p > 0.1$; Figure 1A). Furthermore, the frequency of mIPSCs was similar before and during CNQX application (0.81 ± 0.32 Hz and 0.95 ± 0.7 Hz, respectively; n = 5, $p > 0.05$), suggesting an absence of endogenous activation by glutamate of presynaptic kainate receptors at GABAergic terminals in vitro.

The enhanced rate of mIPSCs was not mediated by calcium influx through voltage-sensitive calcium channels. Cadmium (100 μM) was applied to block voltage-sensitive calcium channels. Cadmium application did not affect mIPSC frequency (n = 6, $p > 0.1$). The kainate-induced increase of the frequency of mIPSCs in the presence of cadmium (250% ± 80% of control values; n = 6, $p < 0.01$; Figure 2A) was not significantly different ($p < 0.05$, chi-square test) from that in the absence of cadmium.

Since a metabotropic link between kainate receptors and the GABA release machinery involving the phospholipase C (PKC) pathway has been suggested to explain the kainate-induced depression of evoked IPSCs (eIPSCs) in CA1 pyramidal cells (Rodriguez-Moreno and Lerma, 1998; Cunha et al., 2000; Rodriguez-Moreno et al., 2000), we have also investigated the effects of kainate on mIPSCs in the presence of the PKC/PKA inhibitor staurosporine (500 nM). Bath application of staurosporine did not prevent the effect of kainate on mIPSCs, although it reduced the induction of LTP, which is dependent upon the activation of the PKC pathway (Malenka and Nicoll, 1993) (30 min after the tetanus, the increase of the field potential was 64% ± 9% in control, n = 6, $p < 0.01$, versus 31% ± 5% in the presence of staurosporine, n = 10, $p < 0.1$). Indeed, in the presence of staurosporine, kainate increased mIPSCs frequency to 152% ± 23% of control values (n = 7, $p < 0.01$; Figure 2B), an increase not statistically different ($p < 0.05$, chi-square test) from that obtained in the absence of staurosporine.

These two former observations suggest that the mechanisms responsible for the enhancement of GABA release by kainate most likely imply the direct activation of ionotropic kainate receptors, as they do not involve a metabotropic pathway or the indirect activation of voltage-sensitive calcium channels.

The Effects of Kainate Are Mediated by Receptors that Do Not Contain the GluR5 Subunit

Since the postsynaptic activation of kainate receptors in CA1 interneurons is mostly mediated by receptors containing the GluR5 subunit (Cossart et al., 1998), we tested whether the presynaptic effect of kainate was also mediated by GluR5-containing receptors. Bath applications of ATPA (1 μM), the selective agonist for kainate receptors containing the GluR5 subunit (Clarke et al., 1997), did not induce any significant change in the rate of mIPSCs (105% ± 12% of control; n = 7, $p > 0.05$; Figure 4A), showing that the kainate-induced increase of GABA quantal release at interneuron–interneuron synapses is not mediated by kainate receptors containing the GluR5 subunit.

Glutamate Increases the Frequency of mIPSCs through Activation of Kainate but Not of Other Ionotropic Receptors

If the activation of presynaptic kainate receptors leads to an increase of quantal release of GABA, similar effects should be produced by the endogenous ligand glutamate. We therefore investigated the effects of focal applications of glutamate on the frequency of mIPSCs recorded in interneurons. In the presence of TTX (1 μM),

(Bc) Cumulative probability plots of miniature IPSC amplitudes are not modified when kainate is applied. Right traces represent the averaged miniature IPSCs in control and during kainate application. Neither the amplitude nor the kinetics of the averaged traces are significantly different.

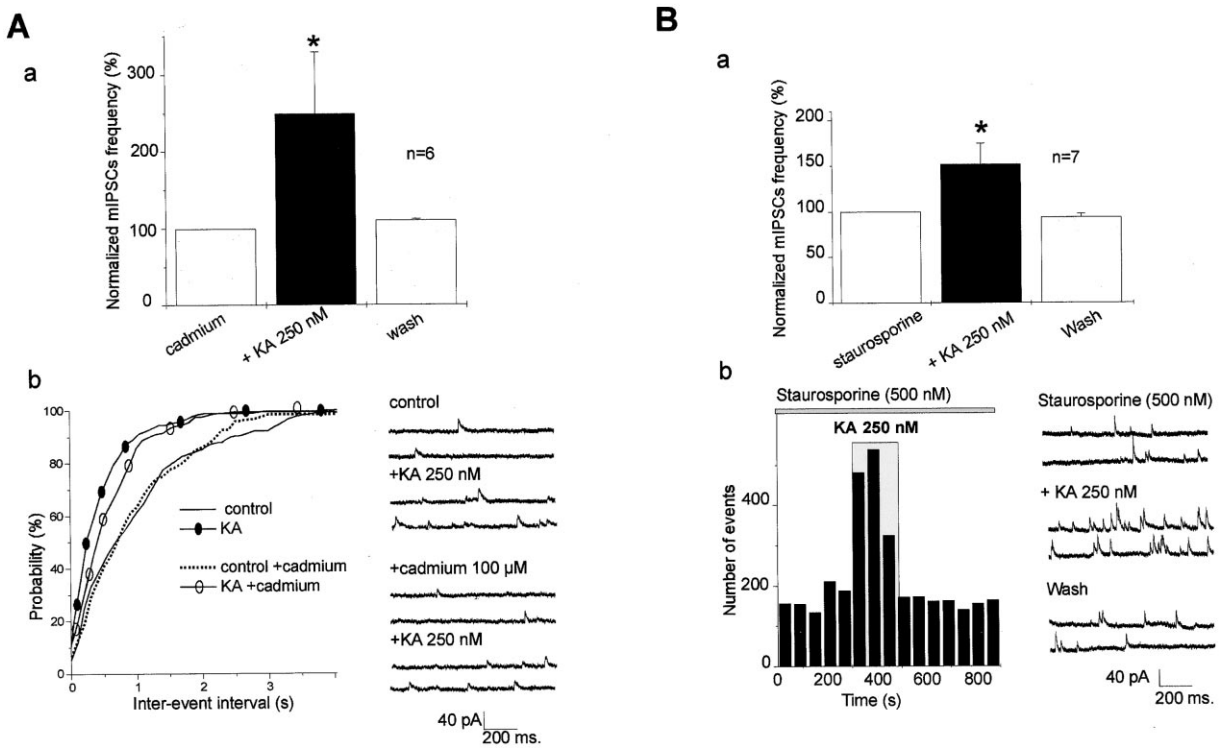

Figure 2. Possible Mechanisms to Explain the Effect of Kainate on mIPSCs in Interneurons

(Aa) The effect of kainate on mIPSCs in interneurons does not involve voltage-dependent calcium channels. Pooled results histogram for the effect of kainate (250 nM) on mIPSCs in the presence of cadmium (100 μM) (n = 6 cells). The bar graph shows mean ± SEM values. *$p < 0.01$.

(Ab) (Left) Cumulative probability plots for interevent intervals show the same shift toward smaller interval values during kainate (250 nM) application in the presence of cadmium (100 μM) than in control conditions (GYKI 53655 [30 μM] and D-APV [50 μM]). (Right) Traces show mIPSCs recordings in the same cell (Vhold + 10mV).

(Ba) The effect of kainate on mIPSCs in interneurons does not involve a metabotropic pathway. Pooled results histogram for the effect of kainate (250 nM) on mIPSCs in the presence of staurosporine (500 nM) (n = 7 cells). The bar graph shows mean ± SEM values. *$p < 0.01$.

(Bb) (Left) Mean frequency of mIPSCs as a function of time (bin = 60 s) in a representative stratum radiatum interneuron showing the effect of kainate (250 nM) application in the presence of staurosporine (500 nM), GYKI 53655 (30 μM), and D-APV (50 μM). (Right) Traces show mIPSCs recordings in the same cell.

glutamate (3 mM) was pressure applied through a pipette located close to the soma of the recorded interneuron (within 50 μm). Brief application of glutamate (20 ms) in the presence of GYKI 53655 (30 μM) and D-APV (50 μM) induced a robust increase of the frequency of mIPSCs (530% ± 155%, n = 8, $p < 0.01$) without any significant change in the holding current. This increase in frequency outlasted the puff for around 30 s (Figure 3). Repeated applications of glutamate (n = 2–5) produced the same effect on mIPSC frequency but did not produce any change of their amplitude (Figure 3). These effects persisted in the presence of a cocktail of antagonists for metabotropic glutamate receptors (MCPG [500 μM] and MSOP [100 μM]; 637% ± 200%, n = 4, $p < 0.01$, an increase not statistically different from that obtained in the absence of mGluRs antagonists, $p < 0.05$, chi-square test) but were completely blocked when the mixed AMPA-KA receptor antagonist CNQX (50 μM; 114% ± 26%; n = 5, $p > 0.05$; Figure 3) was added to the saline. Therefore, the endogenous agonist glutamate increases the frequency of mIPSCs on interneurons through the activation of presynaptic kainate receptors.

To determine whether the effect of glutamate was specific for the kainate type of ionotropic glutamate receptor, we tested the effects of AMPA and NMDA on the frequency of mIPSCs in interneurons. Neither bath application (3–10 min) of AMPA (10 μM) nor NMDA (5 μM) produced any significant change in mIPSC frequency (96% ± 9% for NMDA application, n = 5, $p > 0.1$, and 93% ± 2% for AMPA application, n = 5, $p > 0.1$; see Figure 4A) or amplitude (data not shown). Finally, we also tested the effect of the activation of metabotropic glutamate receptors on mIPSCs in interneurons. Bath application of tACPD (20 μM), the broad spectrum agonist for mGluRI–II, did not significantly affect the frequency of mIPSCs (113% ± 27%, n = 5, $p > 0.1$; Figure 4B). Furthermore, application of the selective group III metabotropic glutamate receptor agonist L-AP4 (100 μM) reversibly decreased the frequency of mIPSCs in interneurons (52% ± 9% of control; n = 3, $p < 0.01$; Figure 4B), which is consistent with the previously described depression of spontaneous and evoked IPSCs on CA1 interneurons (Semyanov and Kullmann, 2000).

Therefore, the positive modulation of GABA release is selective for kainate receptors and does not involve the other main ionotropic or metabotropic glutamate receptors.

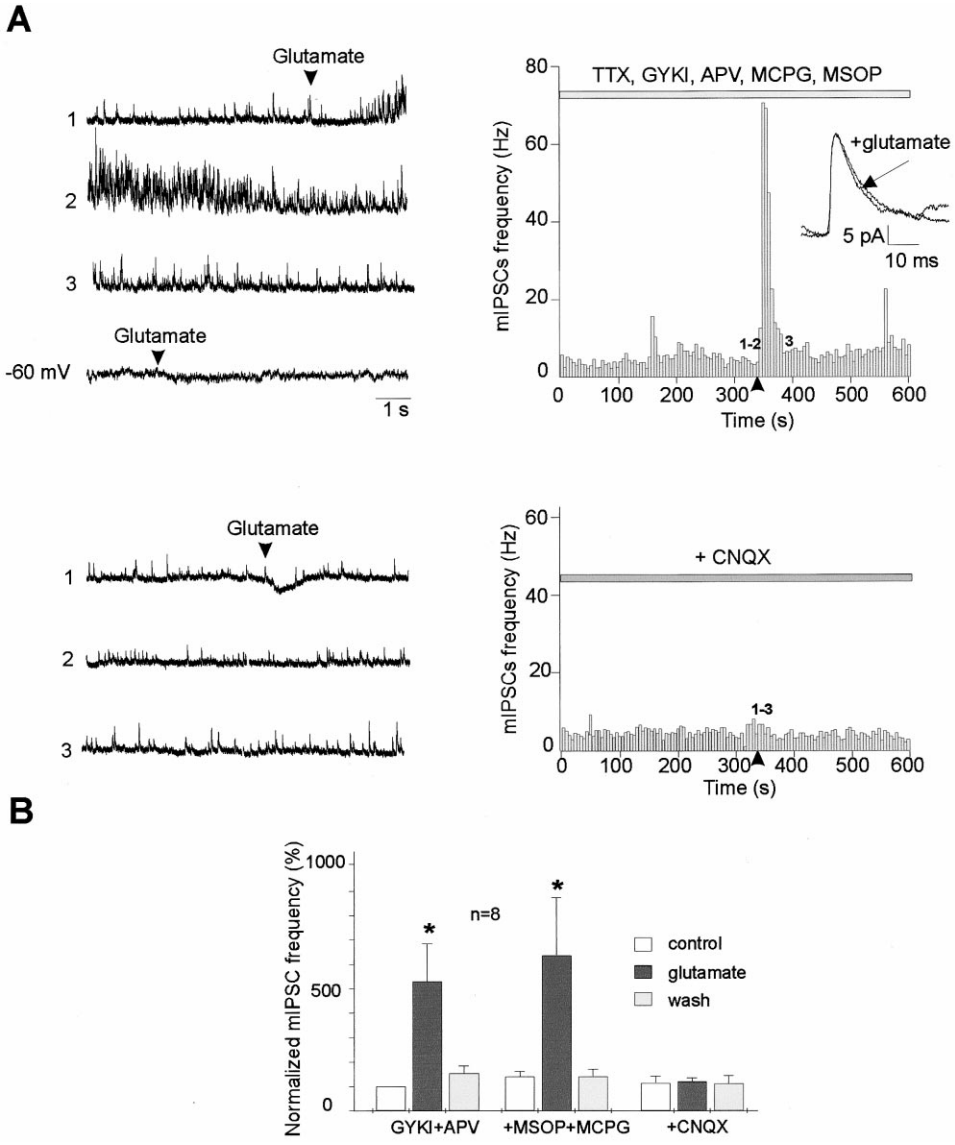

Figure 3. Bath Application of Glutamate Increases the Frequency of mIPSCs on Interneurons via the Activation of Kainate Receptors

(A) Focal application of glutamate increases mIPSCs frequency in interneurons via the activation of kainate receptors. Puff application of glutamate (3 mM; 100 kPa for 50 ms) 50 μm away from the soma of the recorded interneuron (GYKI 53655 [30 μM], D-APV [50 μM], MCPG [500 μM], and MSOP [100 μM]) induced a large increase in the frequency of mIPSCs (Vhold + 10mV), as shown by the three consecutive trace recordings (1–3, top left) and frequency versus time plot histogram (top right). The amplitude and kinetics of the events are not changed by the application of glutamate, as shown by the superimposition of average traces (n = 100 events averaged each). Miniature EPSCs (Vhold − 60mV) are totally suppressed in the presence of the blockers, and the same application of glutamate did not produce any current at the reversal potential for GABAergic events (−60mV). The effect of glutamate on mIPSCs is blocked when CNQX (50 μM) is added to the saline (see bottom traces and histogram). The small deflection of the baseline is most likely produced by the pressure applied close to the recording site. The recorded interneuron is an O-LM cell.

(B) Pooled results for the effect of pressure-applied glutamate on mIPSCs in interneurons (n = 8 cells). Pressure-applied glutamate in the presence of GYKI 53655 (30 μM) and D-APV (50 μM) significantly increased the frequency of mIPSCs ($p < 0.01$). This effect is not prevented by addition of a cocktail of metabotropic glutamate receptor antagonists (MCPG [500 μM] and MSOP [100 μM]; $p < 0.01$), but it is completely prevented by addition of CNQX (50 μM). The bar graphs show mean ± SEM values. *$p < 0.01$.

Kainate Selectively Decreases the Number of Failures of Synaptically Evoked GABAergic Transmission on Interneurons

Since action potential–dependent and -independent release can be differentially modulated (Glitsch and Marty, 1999), we investigated the effects of kainate on the transmission probability at GABAergic synapses on interneurons by analyzing the failure rate of eIPSCs. Evoked IPSCs were obtained by minimal stimulation with a glass electrode placed close to the recorded cell body in the presence of GYKI (30 μM) and D-APV (50 μM). Kainate (250 nM) reversibly decreased the percentage of failures from 60.2% ± 7.8% to 21.2% ± 7.9% ($n = 10$, $p < 0.01$; Figure 5A) in interneurons. As expected

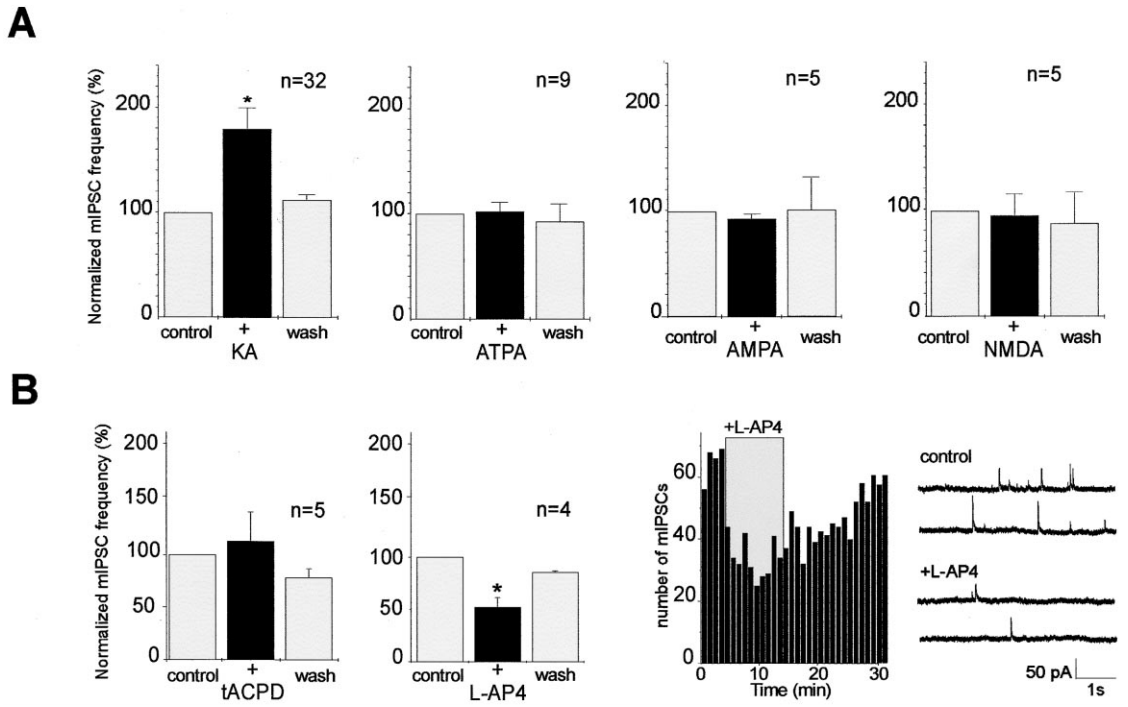

Figure 4. The Kainate Receptor Family Is the Only Glutamate Receptor–Type Activation of which Leads to an Increase of GABA Quantal Release on CA1 Interneurons

(A) Pooled results for the effect of bath-applied kainate (250 nM; n = 32 cells), ATPA (1 μM; n = 9 cells), AMPA (10 μM; n = 5 cells), and NMDA (5 μM; n = 5 cells) on mIPSC frequency recorded in CA1 interneurons (Vhold + 10mV). The bar graphs show mean ± SEM values. *$p < 0.01$.
(B) Pooled results for the effect of bath-applied agonists for metabotropic glutamate receptors on mIPSC frequency recorded in CA1 interneurons (Vhold + 10mV). Trans-ACPD (20 μM; n = 5 cells; $p > 0.1$) did not significantly alter mIPSCs frequency, whereas application of L-AP4 (100 μM; n = 3 cells; *$p < 0.05$) reversibly decreased the frequency of mIPSCs, as shown by the histogram plotting the number of mIPSCs as a function of time (bin = 60 s) when L-AP4 is applied and by the trace recordings of mIPSCs from the same stratum radiatum interneuron in control and in L-AP4. The bar graphs show mean ± SEM values.

from previous observations, we also observed an increase in the frequency of sIPSCs due to the membrane depolarization and the increased firing rate of interneurons induced by kainate (Cossart et al., 1998; Frerking et al., 1998). Interestingly, kainate always decreased the number of failures of eIPSCs in the different populations of interneurons recorded.

Since kainate (250 nM) could decrease the number of failures through the direct depolarization of interneurons and not via an action at GABAergic terminals, we have done the following experiments. We have examined the effects of kainate on the rate of failures of evoked GABAergic currents recorded in CA1 pyramidal cells. Kainate did not significantly alter the number of failures of eIPSCs on pyramidal cells (from 41.1% ± 10.8% in control conditions to 34.1% ± 6.8% when kainate was applied; n = 8, $p > 0.1$; Figure 5B), while it increased the frequency of sIPSCs as previously reported (Cossart et al., 1998; Frerking et al., 1998). While on average kainate did not affect GABAergic transmission on pyramidal cells, it is worth noting that these cells responded heterogeneously to kainate, since, (1) in 25% of the cells, kainate increased by more than 20% the number of failures; (2) in contrast, in 25% of the cells, kainate decreased the number of failures by more than 20%; and, (3) in 50% of the cells, the percentage of failures was not modified. The basis for this heterogeneity is presently not known. Still, the global absence of effect of kainate on failures on pyramidal neurons indicates that depolarization of presynaptic interneurons cannot alone account for the kainate-induced decrease in the number of failures we report on interneurons.

Furthermore, the application of AMPA (1 μM), NMDA (1 μM), or ATPA (1 μM), which depolarized interneurons to the same extent as kainate but did not modify the frequency of mIPSCs, did not alter the rate of failures of evoked GABAergic currents on interneurons (Figure 5C; $p > 0.1$). This indicated that the somatic depolarization of interneurons is not the mechanism responsible for the decrease of failures induced by kainate. Besides, the latter observation further confirms the results obtained with the analysis of miniature activity that AMPA-, NMDA-, or GluR5-containing receptor activation does not modify the release of GABA on interneurons.

We conclude that the activation of kainate receptor leads to a general enhancement of GABAergic transmission (quantal and evoked) at interneuron–interneuron synapses.

The Activation of Kainate Receptors by Synaptically Released Glutamate Decreases the Failure Rate of GABAergic Transmission on CA1 Interneurons

Finally, to determine the physiological relevance of the kainate receptor–dependent enhancement of GABA release, we examined whether synaptically released gluta-

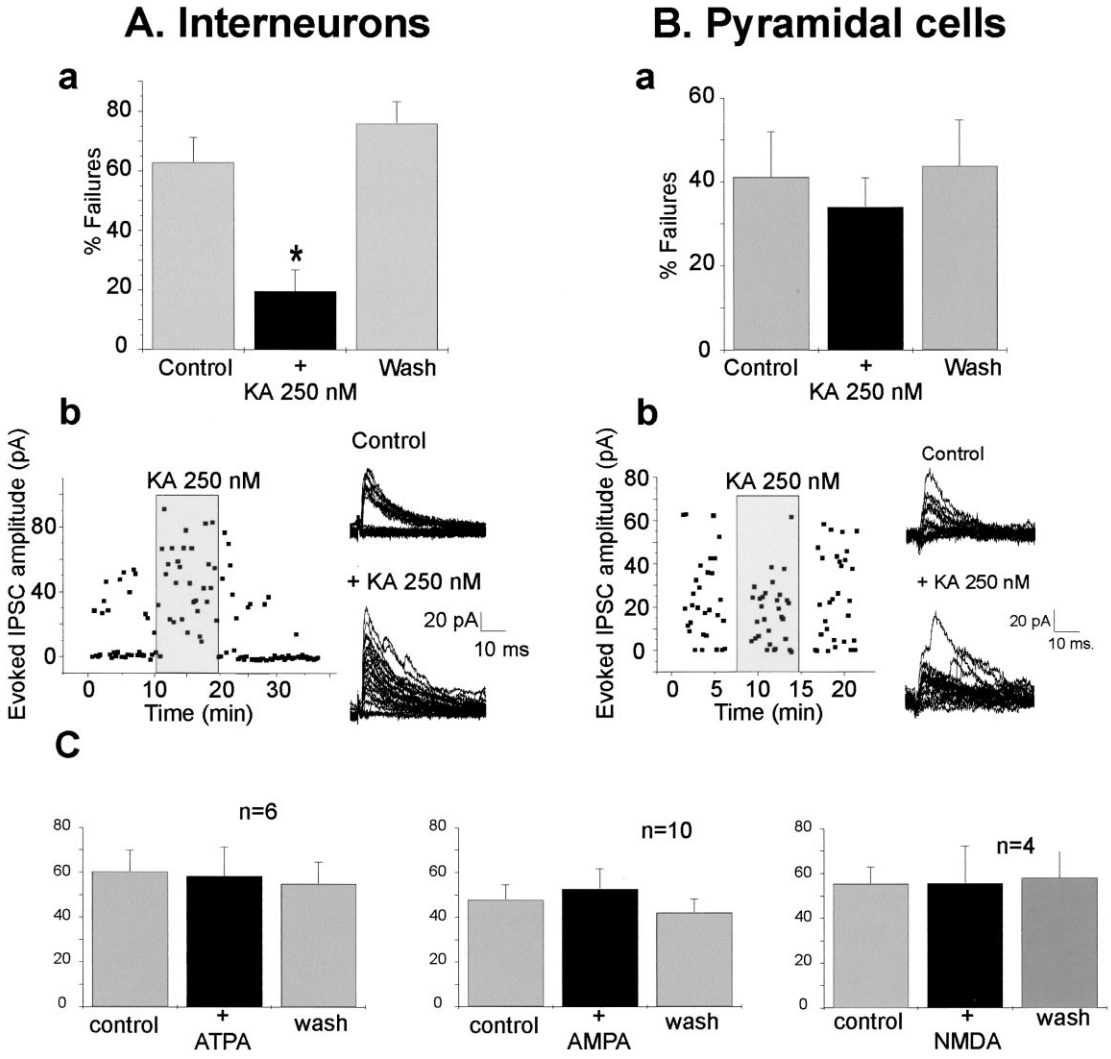

Figure 5. Kainate Decreases the Number of Failures of Evoked GABAergic Responses in Interneurons but Not in Pyramidal Cells

(Aa and Ab) Interneurons.

(Aa) Pooled results for the entire population of recorded interneurons showing that kainate application (250 nM; n = 10 cells) reversibly and significantly decreases the percentage of failures of evoked IPSCs in interneurons in the presence of GYKI 53655 (30 μM) and D-APV (50 μM). The percentage of failures was calculated for 30 minimal electrical stimulations (0.03 Hz) delivered by a glass electrode placed close to the recorded cell body before, during, and after kainate application.

(Ab) Evoked IPSC amplitude as a function of time, showing the effect of kainate application on failures. Traces show the 30 superimposed evoked responses (Vhold + 10mV) for the same cell in control conditions and during kainate application. Note the kainate-induced increase in the frequency of sIPSCs. The interneuron recorded is located at the stratum radiatum/lacunosum moleculare border, and its axon innervates essentially LM (perforant path–associated interneuron).

(Ba and Bb) Pyramidal cells.

(Ba) Pooled results for the entire population of recorded CA1 pyramidal cells, showing that on the whole kainate has no significant effect on failure probability (same stimulation as interneurons, see [A]).

(Bb) Evoked IPSC amplitude as a function of time for a representative pyramidal cell, showing that kainate does not modify the failure rate. Traces show 30 superimposed evoked responses from the same cell. Note that kainate application increased the frequency of sIPSCs.

(C) Pooled results showing that bath application of ATPA (1 μM; n = 6 cells), AMPA (1 μM; n = 10 cells), and NMDA (1 μM; n = 4 cells) has no significant effect ($p > 0.1$) on the failure probability of evoked IPSCs recorded in CA1 interneurons. The bar graphs show mean ± SEM values. *$p < 0.01$.

mate could modify GABAergic neurotransmission. To assess the effect of synaptically released glutamate on the probability of failures of eIPSCs in interneurons, we have adapted the experimental design proposed by Min et al. (1999) (Figure 6A). Experiments were carried out in the presence of GYKI 53655 (30 μM) and D-APV (50 μM) to block AMPA and NMDA receptors. A "distal" electrode was placed in stratum radiatum (Figure 6A) far enough to initiate glutamate release without evoking any $GABA_A$ or kainate receptor mediated current. Fifty milliseconds after the release of glutamate induced by high-frequency stimulation of the distal electrode (50 Hz; 400 ms), we recorded eIPSCs in interneurons elicited by minimal stimulation of a "proximal" glass electrode.

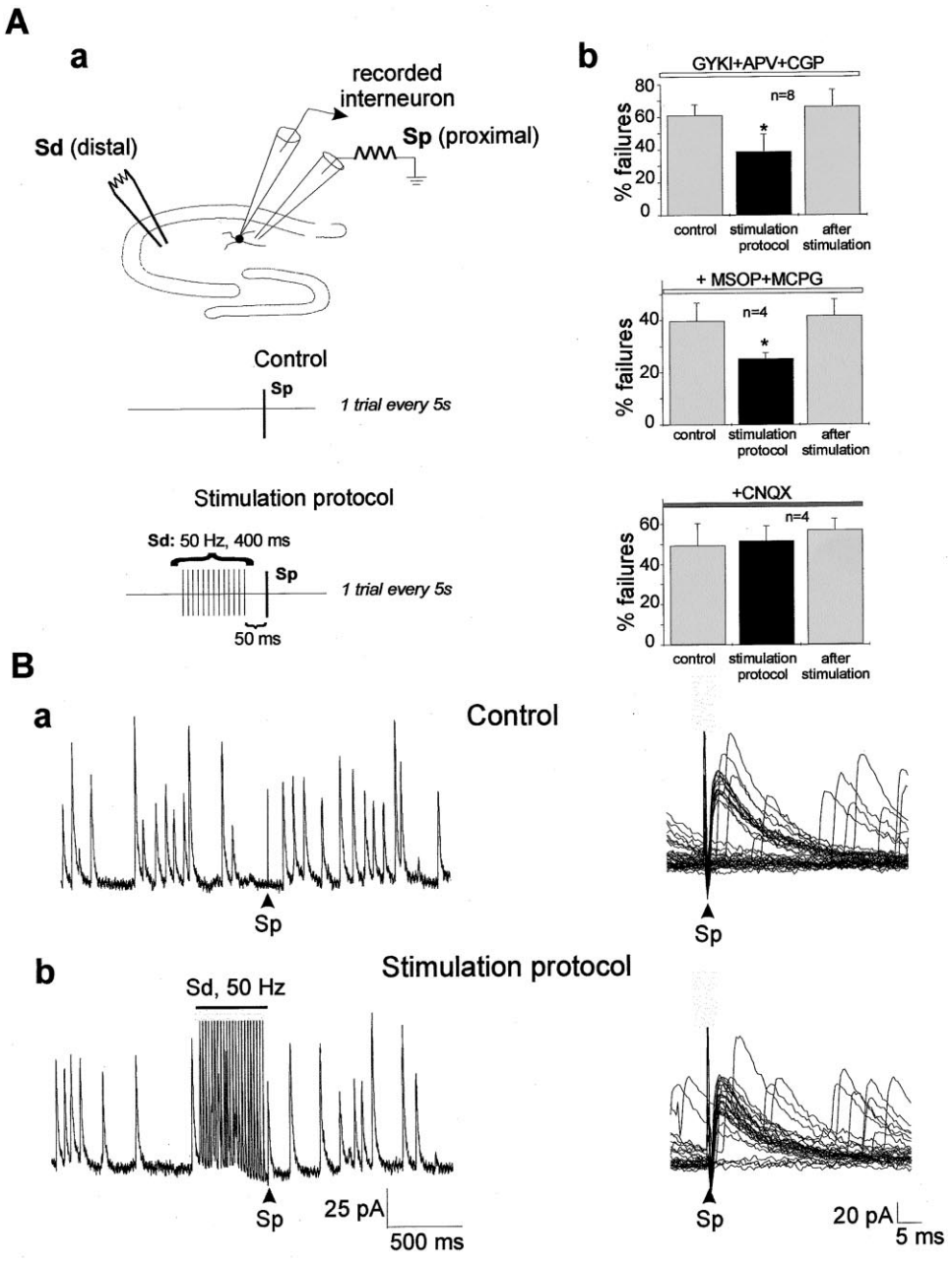

Figure 6. Synaptically Released Glutamate Decreases the Percentage of Failures of Evoked GABAergic Responses in Interneurons via the Activation of Kainate Receptors

(Aa) Schematic illustration of the experimental design showing the placement on the hippocampal slice of the recording pipette and of the two stimulating electrodes (Sd, distal bipolar electrode; Sp, proximal glass electrode). The stimulating protocol is schematized below.

(Ab) Pooled results histograms showing that the percentage of eIPSCs (Vhold + 10mV) failures (calculated over 30 trials) decreases reversibly during glutamate release by Sd stimulation (GYKI 53655 [30 μM], D-APV [50 μM]; $n = 8$, $^*p < 0.01$). The decrease in the probability of failures is not blocked by the metabotropic glutamate receptors antagonists (MCPG [500 μM] and MSOP [100 μM]; $n = 4$, $^*p < 0.01$) but is fully prevented ($p > 0.1$) when CNQX (50 μM) is added to the saline. The bar graphs show mean ± SEM values.

(Ba) Minimal stimulations in control conditions (GYKI [30 μM], D-APV [50 μM], MCPG [500 μM], MSOP [100 μM], and CGP 55845A [1 μM]). Arrow indicates time of proximal stimulation (Sp). (Left) Continuous recording of sIPSCs (Vhold + 10mV) shows a failure of the evoked IPSC following proximal stimulation. (Right) Superimposition of evoked IPSCs ($n = 30$) in the same cell. The thick baseline is due to the high proportion of failures. The cell recorded is an interneuron with the soma in CA1 stratum oriens.

(Bb) Minimal stimulations preceded by the distal stimulation protocol in the same cell as in (Ba) and in the same perfusion saline. (Left) Continuous recording of sIPSCs (Vhold + 10mV) shows a success of GABAergic transmission evoked by (Sp) stimulation following the high-frequency distal stimulation protocol (Sd; 50 Hz; 400 ms). (Right) Superimposition of evoked IPSCs ($n = 30$) following the distal stimulation protocol in the same cell. Note that baseline trace is considerably thinner, showing that the number of failures has considerably decreased.

We observed a significant and reversible decrease in the rate of failures following the 50 Hz tetanus (39% ± 10%, as compared to 61% ± 6% in control conditions; $n = 8$, $p < 0.01$; Figure 6B). This decrease in the number of failures persisted in the presence of metabotropic glutamate receptors antagonists (α-MCPG, 500 μM; MSOP, 100 μM; $n = 4$, $p < 0.01$; Figure 6A), but it was fully blocked by the addition of the mixed AMPA/KA antagonist (CNQX, 50 μM; $n = 3$, $p > 0.1$; Figure 6A), indicating that synaptically released glutamate can also stimulate GABAergic transmission on interneurons, most likely via kainate receptors.

Therefore, the activation of kainate receptors by synaptically released glutamate stimulates communication between interneurons.

Discussion

Kainate Receptor Activation Stimulates GABA Release on CA1 Interneurons

The main finding of this study is that glutamate (bath applied or synaptically released) can increase GABA quantal and evoked release at interneuron–interneuron synapses in a majority of morphologically identified interneurons in the CA1 area of the hippocampus. This effect is mediated by only one class of glutamate receptors, the kainate type, since (1) kainate increased mIPSC frequency and decreased the failure rate of eIPSCs at a concentration (250 nM) that activates kainate but not AMPA receptors (Bureau and Mulle, 1998; Cossart et al., 1998); (2) the increase of GABA quantal release was blocked by CNQX, a mixed AMPA/KA receptor antagonist, but not by GYKI 53655, a selective antagonist for AMPA receptors; (3) it was not mimicked by the other ionotropic glutamate receptor agonists, AMPA or NMDA; and (4) it was not mediated by the activation of metabotropic glutamate receptors because the effect of glutamate on mIPSC frequency was not blocked by a cocktail of antagonists for metabotropic glutamate receptors (MCPG + MSOP). Furthermore, although group III but not group I or II metabotropic glutamate receptors are present at interneuron–interneuron synapses, their activation leads to a decrease of GABAergic neurotransmission (Semyanov and Kullmann, 2000; and present results).

Our conclusion that the effects of kainate are presynaptic is based on two converging observations. (1) Kainate increased the probability of occurrence of TTX-resistant events with no change in their amplitude distribution; this type of effect is considered to be mediated by the activation of receptors at a presynaptic site (MacDermott et al., 1999). (2) Kainate reduced the probability of failures of IPSCs evoked in interneurons by minimal electrical stimulation, an indication of a change in the probability of transmitter release (MacDermott et al., 1999). Another protocol to assess a presynaptic effect is to measure the amplitude of evoked synaptic responses. Application of kainate (250 nM) in the presence of GYKI and D-APV did not significantly alter the amplitude of evoked IPSCs in interneurons (to 92% ± 7% of control $n = 4$, $p > 0.1$; data not shown), but interpreting these experiments in relationship to a presynaptic mechanism is difficult because bath application of kainate also produces a high-frequency firing of interneurons and a dramatic increase of the frequency of IPSCs in CA1 pyramidal neurons and interneurons (Cossart et al., 1998; Frerking et al., 1998). The subsequent activation of presynaptic $GABA_B$ receptors, exhaustion of GABAergic terminals, and shunt of postsynaptic membrane results in a direct decrease of the amplitude of evoked GABAergic responses in CA1 pyramidal cells, thus masking possible presynaptic effects (Frerking et al., 1999; but see Rodriguez-Moreno et al., 2000).

Similar potentiation of GABA release involving presynaptic ionotropic glutamate receptors has been reported in other brain regions (Bureau and Mulle, 1998; Glitsch and Marty, 1999; Liu et al., 1999). In the cerebellum, this effect is mediated by presynaptic NMDA (Glitsch and Marty, 1999) and AMPA receptors (Bureau and Mulle, 1998) located on GABAergic terminals. In hypothalamic cultured neurons, micromolar doses of kainate produce an increase of GABA quantal release in 70% of the cells (Liu et al., 1999). It will be interesting to determine whether the responsive hypothalamic cells are interneurons or principal cells. In the CA1 area of the hippocampus from adult rats, our results indicate that kainate receptors are the only subtype of glutamate receptors capable of increasing the release of GABA on interneurons. Indeed, presynaptic group III metabotropic glutamate receptors have also been reported to modulate the release of GABA on interneurons (Semyanov and Kullmann, 2000) but in the opposite direction, since their activation rather decreased GABA release. Here we provide additional data showing that activation of group III metabotropic glutamate receptors also decreases GABA quantal release on interneurons. It is worth noting that synaptically released glutamate, even when metabotropic receptors are not blocked, could still increase GABA release.

Our observation that the increase in mIPSC frequency is not mediated by the activation of the GluR5 subunit is rather unexpected, as the GluR5 gene is highly expressed among hippocampal interneurons (Wisden and Seeburg, 1993; Petralia et al., 1994; Bureau et al., 1999). Since, in an earlier study (Cossart et al., 1998), we showed that GluR5-containing kainate receptors mediate the postsynaptic excitation of interneurons, the present observations suggest, as previously reported for $GABA_B$ (Deisz et al., 1997; Yamada et al., 1999) and metabotropic glutamate receptors (Poncer et al., 1995), that different subunits or kainate receptors with a different pharmacology are involved at pre- and postsynaptic sites. Other kainate receptor subunits (KA2, GluR6, and GluR7) could mediate the presynaptic effect of kainate. Indeed, KA2 is widely expressed throughout the brain (Herb et al., 1992; Wisden and Seeburg, 1993) and stratum radiatum, and oriens interneurons express the GluR6 and GluR7 subunit mRNA (Bureau et al., 1999; Paternain et al., 2000) and protein (Petralia et al., 1994).

Presynaptic Kainate Receptors that Enhance GABA Release on Interneurons Are Not Coupled to a PKA/PKC Signaling Pathway

Although the precise determination of the mechanism responsible for the enhancement of GABA release by kainate receptor activation was beyond the scope of

this study, the fact that the effect is not blocked by the PKC/PKA inhibitor staurosporine argues against the involvement of a PKC/PKA signaling pathway as previously suggested for interneuron–pyramidal cell synapses (Rodriguez-Moreno and Lerma, 1998; Cunha et al., 2000). Hence, the potentiation of GABA release is due to the activation of ionotropic kainate receptors most probably located on GABAergic axons. Two alternate possibilities would be a passive propagation to the axon terminals of the depolarization resulting from the activation of postsynaptic kainate receptors and/or an enhancement of fiber excitability resulting from the activation of presynaptic kainate receptors. The first mechanism requires a small electrotonic distance between the somatodendritic compartment and the axon terminals (Glitsch and Marty, 1999), an unlikely feature in the hippocampus, where the axonal arborization of interneurons extends over considerable distances (Freund and Buzsaki, 1996). Moreover, the fact that NMDA (1 μM) and AMPA (1 μM) or ATPA (1 μM), which produce the same sustained depolarization on the membrane of interneurons as kainate, did not affect GABA release excludes this possibility. The second mechanism, an increased excitability of the axon, has been recently described in the CA3 region (Kamiya and Ozawa, 2000; Schmitz et al., 2000). The activation of presynaptic kainate receptors leads to an increase of mossy fibers excitability (Schmitz et al., 2000), and a similar mechanism cannot be ruled out to explain the decreased probability of failure of evoked GABA release we report. This would imply that the failure to evoke a postsynaptic IPSC following minimal stimulation is always due to a failure to activate the en passant axons and not to a failure of neurotransmission per se. The existence of high-failure GABAergic synapses in the CA1 region (Jiang et al., 2000; Maccaferri et al., 2000) rather argues against this interpretation. Therefore, we conclude that the kainate receptor–induced potentiation of GABA quantal release is due to the activation of presynaptic kainate receptors located on GABAergic terminals, and we propose that the same receptors may control the action potential–driven release of GABA. Since calcium-permeable kainate receptors can be formed (Burnashev et al., 1996) and voltage-dependent calcium channels are not involved in the kainate-induced potentiation of GABA release, the effects of kainate we describe may be due to Ca^{2+} influx through calcium-permeable kainate receptors.

The Increase of GABA Release by Activation of Presynaptic Kainate Receptors Is Specific for Interneurons

Our results lead to an important observation: in CA1, low doses of kainate (250 nM) consistently increase the probability of quantal and evoked GABA release on most interneurons. The situation seems to be more complex at GABAergic synapses on pyramidal cells. We report that the number of eIPSCs failures measured in pyramidal cells during kainate application is either increased, decreased, or unchanged. Therefore, the modulation of GABA release by kainate may be heterogeneous and depends on the properties of the GABAergic synapse. This heterogeneity makes it difficult to interpret experiments and may be the main reason for the controversy on the presence and function of presynaptic kainate receptors at interneuron–pyramidal cell connections (Rodriguez-Moreno et al., 1997; Cossart et al., 1998; Bureau et al., 1999; Frerking et al., 1999; Min et al., 1999). Clearly, this issue needs to be further investigated in order to conclude whether the expression of kainate receptors on GABAergic synapses is indeed target specific, as is the case for metabotropic receptors, for example (Poncer et al., 1995; Scanziani et al., 1998; Semyanov and Kullmann, 2000). At present, it is also not known whether the kainate receptor–induced increase of GABAergic transmission on interneurons depends on the inhibitory pathway. Indeed, interneurons are known to be densely interconnected in the hippocampus, and, since they also receive a GABAergic projection from interneuron-specific interneurons (Freund and Buzsaki, 1996), the possibility that presynaptic kainate receptors are preferentially expressed at these synapses cannot be ruled out.

The precise function of these presynaptic kainate receptors remains to be explored. A tonic activation of kainate receptors by glutamate in vitro conditions is not likely, since the mixed AMPA/kainate antagonist CNQX did not affect GABA quantal release. The situation could be different in vivo, where the endogenous glutamatergic activity is considerably greater than in vitro (Pare et al., 1998). Whatever the underlying mechanisms, our results suggest that glutamate released during high-frequency inputs will act via the activation of kainate receptors to increase the release of GABA on interneurons. A modulation of GABAergic transmission by glutamate spillover via metabotropic receptors has also been suggested recently (Semyanov and Kullmann, 2000). That such spillover may also occur in physiological conditions is supported by the fact that, in the hippocampus, most GABA neurons display a pattern of axonal distribution that closely parallels that of glutamatergic afferent pathways (Gulyas et al., 1993; Buhl et al., 1994; Vida et al., 1998; Gulyas et al., 1999; Vida and Frotscher, 2000). Thus, it is likely that during high levels of glutamatergic activity communication between interneurons should be facilitated by the activation of presynaptic kainate receptors, which will inhibit some interneurons and tend to synchronize their activities. Indeed, the presynaptic regulation of GABAergic terminals is particularly important to control network oscillations (Hajos et al., 2000).

In conclusion, kainate receptors assembled from different subunits exert a dual control on inhibition in the CA1 region: a widespread increase of the tonic inhibitory drive via the activation of postsynaptic GluR5 subunit–containing receptors in interneurons and a local, targeted stimulation of GABAergic transmission between interneurons via the presynaptic activation of a different type of kainate receptor. This heterogeneity of kainate receptors opens a wide range of possible modulations of hippocampal network function according to the type of receptor activated.

Experimental Procedures

Electrophysiology

Adult Wistar rat slices were obtained as previously described (Esclapez et al., 1997). Slices (350 μm thick) were prepared with a Leica

VT 1000E tissue slicer and immediately transferred to an incubation chamber. ACSF contained (in mM) NaCl, 126; KCl, 3.5; NaH_2PO_4, 1.2; $NaHCO_3$, 26; $MgCl_2$, 1.3; $CaCl_2$, 2; D-glucose,10; and was continuously aerated with 95% O_2 and 5% CO_2. After rest (>1 hr), slices were transferred to a chamber mounted on the headstage of an upright microscope (DM-FLS, Leica). CA1 interneurons and pyramidal cells were identified using IR-DIC microscopy through a 40× water immersion objective. Whole-cell recordings were obtained at 32°C and filtered at 3 kHz using an EPC-9 amplifier (HEKA-Electronics, Liambrecht, Germany). Microelectrodes had a resistance of 4–12 MΩ and internal solution (osmolarity 265–275 mOsm) of the following composition was used (in mM): Cs-gluconate, 135; $MgCl_2$, 10; $CaCl_2$, 0.1; EGTA, 1; HEPES, 10; and 0.5% biocytin (pH 7.25). The series resistance ranged between 15 and 40 MΩ and was not allowed to vary by more than 20% during the course of the experiment. Miniature IPSCs in interneurons were measured at the reversal potentials for glutamatergic events (+10mV) as described previously (Esclapez et al., 1997). A picospritzer (General Valve Corporation, Fairfield, NJ) was used to puff-apply glutamate. The pressure varied from 100 to 200 kPa, and the duration of the puff varied from 10 to 40 ms. Stimulation of interneurons and pyramidal cells was performed via a glass electrode filled with saline placed near the cell body (around 100 μm). Distal stimulation was positioned to recruit Schaffer collateral axons and was performed via a bipolar nickel–chrome electrode. Staurosporine was bath applied 15 min before the experiment. When cadmium was applied, the complete blockade of evoked currents was used as a positive control for the efficacy of this drug. MSOP and α-MCPG were applied 20 min before each experiment involving these drugs. CGP55845A (1 μM) was added to the saline in all experiments examining failures of evoked GABA release to prevent any possible effect of presynaptic $GABA_B$ receptor activation. LTP of field EPSP was induced by three trains of stimulation at 100 Hz (duration: 1s, every 20 s) and measured 30 min after the induction protocol.

During recordings, all neurons were filled passively with biocytin for post hoc morphological identification. Morphological identification is necessary, since even when IR-DIC microscopy was used to select interneurons, 5% of the recordings were performed from pyramidal cell dendrites. Recordings were digitized (10 kHz) online with a Labmaster interface card (Axon Instrument, USA) to a personal computer and analyzed offline with Acquis1 program (G. Sadoc, 1996, BioLogic, Claix, France). Statistical deviations from mean values given in the text and error bars in figures indicate ± SEM across experiments; n being the number of experiments. Unless otherwise stated, statistical differences between two sets of data were assessed with a student's t test or a paired student's t test for paired values.

Data Analysis

Miniature events were detected offline using Acquis1 program. Detection thresholds were adjusted between 6 and 12 pA.

To examine the effect of a drug, miniature currents were recorded and counted as a function of time during the whole experiment. The average value of mIPSCs frequency during the control period (typically 3–5 min) was calculated, and the frequencies of all the events during and after drug application (3–10 min) were normalized to this value. The effect of a drug was quantified by the percentage of increase in mIPSCs frequency obtained for the last minute of drug application compared to control value.

Morphology

Slices were fixed overnight at 4°C in a solution containing 4% paraformaldehyde in 0.12 M phosphate buffer (PB [pH 7.4]). After fixation, slices were rinsed in PB, cryoprotected in sucrose for 16 hr, and quickly frozen on dry ice. Detection of biocytin-filled neurons was performed on unsectioned slices. To neutralize endogenous peroxidase, slices were pretreated for 30 min in 1% H_2O_2. After several rinses in saline phosphate buffer (0.12 M PBS [pH 7.4]), slices were incubated for 24 hr at 4°C in 1/100 Avidin-biotinylated peroxydase complex diluted in PBS containing 0.3% Triton X-100. After 30 min rinses in PBS, slices were processed with 0.06% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.006% H_2O_2 diluted in PBS, rinsed, mounted on gelatin-coated slides, and coverslipped in an aqueous medium (Crystal/Mount, Biomeda, Foster City, CA).

Chemicals

GYKI 53655 (LY303070) and ATPA were kindly provided by Dr. Leander (Lilly Research Center, Ltd). CGP55845A was a gift from Novartis. Kainate, AMPA, NMDA, CNQX, D-APV, L-AP4, staurosporine, and biocytin were obtained from Sigma; tACPD, α-MCPG, and MSOP from Tocris Cookson; and TTX from Latoxan.

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