Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus

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Kainate (KA) receptor activation depresses stimulus-evoked \(\gamma\)-aminobutyric acid (GABA-mediated) synaptic transmission onto CA1 pyramidal cells of the hippocampus and simultaneously increases the frequency of spontaneous GABA release through an increase in interneuronal spiking. To determine whether these two effects are independent, we examined the mechanism by which KA receptor activation depresses the stimulus-evoked, inhibitory postsynaptic current (IPSC). Bath application of the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/KA receptor agonist GYKI 53655 caused a large increase in spontaneous GABA release and a coincident depression of the evoked IPSC. The depressant action on the evoked IPSC was reduced, but not abolished, by the GABA\(_a\) receptor antagonist SCH 50911, suggesting that the KA-induced increase in spontaneous GABA release depresses the evoked IPSC through activation of presynaptic GABA\(_a\) receptors. KA had no resolvable effect on the potassium-induced increase in miniature IPSC frequency, suggesting that KA does not act through a direct effect on the release machinery or presynaptic calcium influx. KA caused a decrease in pyramidal cell input resistance, which was reduced by GABA\(_a\) receptor antagonists. KA also caused a reduction in the size of responses to iontophoretically applied GABA, which was indistinguishable from the SCH 50911-resistant, residual depression of the evoked IPSC. These results suggest that KA receptor activation depresses the evoked IPSC indirectly by increasing interneuronal spiking and GABA release, leading to activation of presynaptic GABA\(_a\) receptors, which depress GABA release, and postsynaptic GABA\(_a\) receptors, which increase passive shunting.

Excitatory synaptic transmission in the central nervous system is mediated primarily by the release of the neurotransmitter glutamate. There are two families of glutamate receptors, each with distinct functions: metabotropic glutamate receptors, which activate G proteins and intracellular signaling cascades, and ionotropic receptors, which are ligand-gated cation channels (1). Ionotropic glutamate receptors can be divided into two classes, \(N\)-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors, which are readily distinguished by numerous pharmacological agents (2). Non-NMDA receptors can be further subdivided into \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate (KA) receptors (2, 3), which can be distinguished by the AMPA receptor antagonist, GYKI 53655 (4). AMPA and NMDA receptors contribute to excitatory postsynaptic currents at nearly all glutamatergic synapses described so far, and KA receptors also have been shown to generate excitatory postsynaptic currents in a limited distribution of cells (5–11). Recently, however, KA receptors also have been proposed to play a second role as modulators of synaptic transmission at both excitatory (12, 13) and inhibitory (14–16) synapses in the hippocampus. This second role of KA receptors has been studied most extensively at inhibitory GABAergic synapses onto pyramidal cells in the hippocampus, where the evoked IPSC (eIPSC) is reduced by KA receptor activation, either by the non-NMDA receptor agonist KA (7, 8, 14–17) or by synaptically released glutamate (18), both in the presence of GYKI 53655. Surprisingly, this depressant action of KA appears to be at least in part presynaptic (14, 15, 17). Even more surprisingly, the KA-induced depression is reduced by manipulations affecting metabotropic signaling pathways, including blockade of G protein function (16). From these results, it has been suggested that there are KA receptors on the presynaptic terminal at GABAergic synapses, which depress the eIPSC through a novel, direct, metabotropic action that reduces the probability of release.

Interpretation of these results has been recently complicated by the observation that KA strongly potentiates the frequency of spontaneous miniature IPSCs (mIPSCs), coincident with the reduction in eIPSC amplitude (7, 8, 17). This increase in mIPSC frequency is caused by somatic/dendritic KA receptors, which depolarize GABAergic interneurons and increase the frequency of interneuronal spiking. This result suggests an alternative explanation for the depression of eIPSC amplitude: that the KA-induced increase in interneuronal activity leads ultimately to a use-dependent depression of the eIPSC (7). In principle, this alternative model could be ruled out if the KA-induced depression persisted even in the absence of interneuronal spiking, but results examining action potential-independent miniature IPSCs (mIPSCs) have been controversial, with initial reports of a strong depressant effect of KA on mIPSC frequency (15, 16) and subsequent reports suggesting little or no effect of KA on mIPSC frequency (7, 8, 17).

In this study, we examine the mechanisms underlying the KA-induced depression of the eIPSC. We find that the depression is caused by two major effects: first, activation of presynaptic metabotropic GABA\(_a\) receptors, and second, a decrease in postsynaptic responsiveness to GABA. We propose that both of these effects are, at least in part, a downstream consequence of the KA-induced increase in spontaneous GABA release.

Methods

Slice Preparation and Recording Techniques. Hippocampal slices 300 to 500 \(\mu\)m thick were prepared from 2- to 3-wk-old rats as described (7). In brief, slices were transferred to a recording chamber at room temperature perfused at 2 ml/min with a solution consisting of 119 mM NaCl, 26.2 mM NaHCO\(_3\), 11 mM glucose, 2.5 mM KCl, 2.5 mM CaCl\(_2\), 1.3 mM MgSO\(_4\), and 1.0 mM NaH\(_2\)PO\(_4\) and then bubbled with 95% O\(_2\)-5% CO\(_2\) at least 1 hr after slicing. Patch electrodes (2–5 M\()\) were filled with a solution adjusted to pH 7.2, 270–290 mOsm containing 117.5 mM cesium gluconate, (10 mM), tetraethylammonium chloride, 10 mM HEPES, 8 mM NaCl, 5 mM QX-314Cl, 4 mM MgATP, 2.5 mM CsCl, 0.3 mM Na\(_3\)GTP, and 0.2 mM EGTA for pyramidal neurons.
cells and 140 mM K gluconate, 5 mM Hepes, 4 mM MgATP, 2 mM MgCl2, 1.1 mM EGTA, and 0.3 mM Na3GTP for interneurons. In all experiments, NMDA receptors were blocked with 100 μM d-2-amino-5-phosphonovaleric acid, AMPA receptors were blocked with 100 μM GYKI 53655, and postsynaptic GABA_B receptor-activated conductances were blocked by internal QX-314 (19). Cells were held between −10 and +10 mV.

Whole-cell patch clamp recordings from pyramidal cells and interneurons were made by visual identification of these cells using differential interference contrast microscopy. Stimulation and recording techniques were similar to those described previously (7). Stimulation-evoked whole-cell currents and voltages were filtered at 2 kHz and digitized at 2.5–5 kHz. Data were analyzed online with Igor Pro (Wavemetrics, Lake Oswego, OR). Series resistances were generally between 10 and 25 MΩ, and input resistances were 200–400 MΩ (pyramidal cells) or 300–800 MΩ (interneurons).

Current responses to brief (1–5 msec) iontophoretic GABA applications were measured at the peak of the postsynaptic response. Iontophoretic electrodes (80–100 MΩ) were filled with 1 M GABA and placed in stratum radiatum, and backing current response. Iontophoretic electrodes (80–100 MΩ) were filled with 1 M GABA and placed in stratum radiatum, and backing current was applied between iontophoretic current injections to prevent basal GABA leakage.

Data Analysis. All data are presented as the mean ± SEM. Data were compared by using the Student’s t test. Significance was assessed at P < 0.05. Miniature IPSCs (mIPSCs) in tetrodotoxin under normal conditions were identified by using an automated detection routine (J. H. Steinbach, Washington University School of Medicine, St. Louis) described previously (7). Because this program underestimated the number of events at higher frequencies, mIPSC frequency for experiments in high extracellular K+ was calculated by counting the number of visually identified events occurring in 0.5- to 2-sec blocks measured at regular intervals throughout the experiment.

Results

In the presence of d-2-amino-5-phosphonovaleric acid and GYKI 53655, extracellular stimulation in stratum radiatum evoked an eIPSC in hippocampal CA1 pyramidal cells, which was subsequently depressed by bath application of KA (10 μM) (Fig. 1 A1 and B). Concomitant with this depression, as reported previously (7, 8, 17), KA caused a large increase in the frequency of sIPSCs (see Figs. 2B and 4A), which is caused by an increase in interneuronal spiking. We reasoned that the KA-induced increase in spontaneous GABA release might lead to an increase in the activation of presynaptic GABA_B receptors, which are known to depress stimulus-evoked release through a metabotropic reduction in release probability (20). To test this hypothesis, we compared the KA-induced depression of the eIPSC in the presence and absence of the GABA_B receptor antagonist SCH 50911 (21). We found that, although SCH 50911 (did not abolish the depression of KA on the eIPSC, (as reported, ref. 7), it did reduce this effect (Fig. 1 A2 and 2B). On average, KA induced a 73 ± 9% depression of the eIPSC under control conditions (n = 7), but in the presence of SCH 50911, the depression was reduced to 39 ± 6% (n = 8; P < 0.01; Fig. 1C).

The KA-induced increase in sIPSC frequency was not qualitatively affected by SCH 50911 (not shown), nor was the KA-induced decrease in input resistance significantly affected by SCH 50911 (see below; control: 43 ± 7%, n = 7; SCH 50911: 49 ± 7%, n = 8), suggesting that SCH 50911 does not act directly on KA receptors or reduce KA-induced interneuronal spiking. To assess the effectiveness of SCH 50911 at blocking GABA_B receptor function, we examined the effects of SCH 50911 on the depressant action of the GABA_B receptor agonist baclofen (10 μM). Although the baclofen-induced depression of the eIPSC was similar in magnitude to that induced by KA (81 ± 8%, n = 3) in control conditions, baclofen had no significant effect on eIPSC amplitude in the presence of SCH 50911 (8 ± 8%, n = 3, Fig. 1C). These results indicate that part, but not all, of the KA-induced depression is mediated by the activation of presynaptic GABA_B receptors.

This result seems, at first glance, to be inconsistent with our previous observation (7) that KA does not affect paired-pulse depression (PPD), because GABA_B receptor activation has been shown to affect this parameter (20, 22, 23). However, the effect of GABA_B receptor activation during KA application accounts for only approximately one-half of the total action of KA on the eIPSC, corresponding to a 34 ± 11% reduction in release probability. This effect is smaller than previously considered and may be too small to generate a resolvable change in PPD. We found that in cells in which baclofen (1–10 μM) caused a large depression of the eIPSC (69 ± 2%, n = 5), a robust reduction in PPD was observed (from 33 ± 3% to 4 ± 10%, P < 0.05); however, in cells in which baclofen (1 μM) depressed the eIPSC to an extent comparable with that calculated for the GABA_B receptor-mediated component of the KA-induced depression (baclofen: 32 ± 3%, n = 6), baclofen had no significant effect on PPD (from 39 ± 5% to 30 ± 7%, P = 0.1). We conclude that PPD is not sufficiently sensitive to detect the KA-induced change in release probability caused by GABA_B receptor activation, although it can detect larger changes in release probability.

Next, we considered the possibility that the SCH 50911-resistant component of the KA-induced depression might be mediated by a direct, frequency-dependent depression of the eIPSC caused by spiking in the presynaptic interneuron. To address this possibility, we recorded from synaptically connected

![Fig. 1. The KA-induced depression of eIPSC amplitude is reduced by the GABA_B receptor antagonist SCH 50911. (A) Representative examples of the depression of the eIPSC induced by 10 μM KA in the absence (A1) or presence (A2) of the GABA_B receptor antagonist SCH 50911 (20 μM). eIPSCs (n = 20) were averaged together in each of the displayed traces. (B) On average, KA reduced eIPSC amplitude to a greater extent in the absence of SCH 50911 (●) than in its presence (○). The depressant action of KA is significantly reduced by SCH 50911, but a residual action remains. In contrast, the depressant action of the GABA_B receptor agonist baclofen (10 μM) is abolished by SCH 50911.](image-url)
interneuron-pyramidal cell pairs, in which we held the interneuron in voltage-clamp to prevent the KA-induced increase in interneuronal excitability. We recorded from ~60 pairs of interneurons and pyramidal cells. Of these 60, three cell pairs were connected with sufficiently large and stable unitary IPSCs (uIPSCs) to allow for comparison before and after KA application. A representative experiment is shown in Fig. 2 A–C. Brief (3–5 msec) depolarizing steps in the interneuron induced action potentials (Fig. 2A1), which caused time-locked uIPSCs in the pyramidal cell (Fig. 2B1). The average uIPSC for this connection is shown in Fig. 2C1. Upon KA application in the presence of SCH 50911, presynaptic action potentials were still reliably induced (Fig. 2A2), but postsynaptic uIPSCs were smaller than seen in control conditions (Fig. 2B2 and C2). KA still caused a large increase in sIPSC frequency under these conditions (Fig. 2B2) because, as expected, other interneurons synapsing onto the pyramidal cell were activated by KA. Similar results are shown for uIPSC amplitudes measured in another cell pair with a weaker connection (Fig. 2D). On average, the residual depressant action of KA was no different in synaptically connected cell pairs than when IPSCs are evoked by extracellular stimulation.

Fig. 2. The KA-induced depression of the eIPSC is not dependent on depolarization of the presynaptic cell. (A) Consecutive current traces for a representative interneuron held in voltage clamp at −70 mV, with a brief depolarizing step to induce a single action potential, shown before (A1) and during (A2) the application of 10 μM KA. (B) Consecutive current traces for the pyramidal cell connected to the interneuron shown in A, showing IPSCs evoked by the presynaptic depolarizing step before (B1) and during (B2) KA application. Note the large increase in spontaneous IPSCs after KA application. (C) Averages of the IPSCs shown in B, before (C1) and during (C2) the KA application. (D) Amplitude measurements from a different cell pair with a weaker connection, showing successes and failures of synaptic transmission before KA addition and a decrease in uIPSC amplitude upon KA application. The noise level increases dramatically upon KA application, presumably due to the increase in sIPSCs, preventing resolution of successes and failures during the KA application. (E) The SCH 50911-resistant depressant action of KA on eIPSCs is not significantly different from that on uIPSCs.

Fig. 3. KA has no detectable effect on K⁺-evoked miniature IPSCs. (A) Representative current traces from an experiment in which the effect of KA on the frequency of K⁺-evoked mIPSCs was examined. (B) On average, K⁺-evoked mIPSC frequency was unaffected by KA application when compared with control experiments in which KA was not added. (C) Summary of experiments on mIPSCs. mIPSC frequency was unaffected by KA when mIPSCs were evoked by high K⁺, by normal K⁺ in low Ca²⁺, or under normal conditions in Wistar rats.

(Fig. 2E). These results suggest that a direct frequency-dependent depression of the IPSC does not contribute significantly to the effect of KA on the eIPSC.

We also examined the possibility that KA depresses the eIPSC through a direct action on the presynaptic terminal. Such an action, monitored with mIPSCs, should be resistant to blockade of interneuronal spiking with the sodium channel blocker, tetrodotoxin (1 μM), but previous results have been controversial using this approach, and we have been unable to observe an effect of KA on mIPSCs (7). One possible explanation for the controversy is that KA selectively depresses Ca²⁺ influx, and in our conditions (7), mIPSC frequency is independent of Ca²⁺ influx (see ref. 24), whereas in the studies of Lerma and coworkers (15, 16), Ca²⁺ influx contributed to the mIPSC frequency. We therefore recorded depolarization-evoked mIPSCs in the presence of d-2-amino-5-phosphonovaleric acid, GYKI 53655, and tetrodotoxin. Upon addition of an external solution containing 20 mM K⁺ to depolarize the GABAergic terminals, a large increase in mIPSC frequency was observed (Fig. 3 A and B). Subsequent addition of KA had no effect on mIPSC frequency; however, the Ca²⁺ channel blocker Cd²⁺ (100 μM) reversed the increase in mIPSC frequency, confirming that the majority of mIPSCs recorded in these conditions were Ca²⁺ influx-dependent (Fig. 3 A and B, n = 6). To ensure that a KA-induced change in mIPSC frequency was not masked by drift in depolarization-evoked mIPSC frequency, we repeated these experiments with sham KA applications (Fig. 3B, n = 6). Comparison of these control experiments with those in which KA was applied revealed no difference in mIPSC frequency. We then examined mIPSC frequency in normal external K⁺ concentrations but reduced external Ca²⁺ concentrations (0.5 mM Ca²⁺/3.3 mM Mg²⁺, n = 4), as the effects of KA have been reported to be inversely related to Ca²⁺ concentration (16).
also examined the effects of KA on mIPSC frequency in Wistar rats (n = 3), the strain of rats used by Lerma and coworkers (15, 16). None of these parameters revealed a significant effect of KA on mIPSC frequency (Fig. 3C). These results suggest that KA, in our hands, has no direct effect on the GABA release machinery or the presynaptic Ca\(^{2+}\) current.

Because KA has been reported to increase the holding current of pyramidal cells in voltage-clamp recordings (15, 17), another possible explanation for the residual action of KA is that KA might affect passive membrane properties in the postsynaptic cell. Fig. 4A (Top) shows the current responses to 5-mV hyperpolarizing steps in a pyramidal cell. Upon addition of KA, the holding current of the cell increased substantially (Fig. 4A and B) and the average steady-state current during the step was increased (Fig. 4A Bottom), suggesting that the increase in holding current is due to an opening of postsynaptic conductances. We reasoned that the KA-induced change in holding current might be caused by tonic GABA\(_A\) conductances, resulting from the large KA-induced increase in sIPSC frequency. Consistent with this idea, the GABA\(_A\) receptor antagonist bicuculline (2 \(\mu\)M) reduced both the increase in holding current (Fig. 4B\(_1\)) and the decrease in input resistance caused by KA (Fig. 4B\(_2\)).

The averaged current response in KA shows a larger steady-state value (Bottom), indicating an increase in resting conductance. (B) The KA-induced increase in sIPSC frequency causes a decrease in input resistance that correlates with the decrease in eIPSC amplitude. (B2) The large increase in \(I_h\) upon KA application (●) is reduced when the KA is applied in the presence of 2 \(\mu\)M bicuculline (○). (B3) The average KA-induced depression of input resistance as a function of time (●) is not significantly different from the average KA-induced depression of eIPSC amplitude as a function of time (○). (C) KA alters eIPSC kinetics. (C1) KA depresses the eIPSC. (C2) eIPSCs in C1 before and after KA application are shown after normalization to their peak amplitudes. (D) On average, KA reduces the half-width of IPSCs. Individual experiments (●) are shown.

Fig. 4. The KA-induced increase in sIPSC frequency alters postsynaptic passive membrane properties. (A) Eight consecutive current traces in response to a 5-mV hyperpolarizing step are shown before (Top) and during (Middle) KA application, after subtraction of average holding current from each trace. Note the increased sIPSC activity in the presence of KA and the associated large change in holding current (\(I_h\)). The averaged current response in KA shows a larger steady-state value (Bottom), indicating an increase in resting conductance. (B) The KA-induced increase in sIPSC frequency causes a decrease in input resistance that correlates with the decrease in eIPSC amplitude. (B2) The large increase in \(I_h\) upon KA application (●) is reduced when the KA is applied in the presence of 2 \(\mu\)M bicuculline (○). (B3) The average KA-induced depression of input resistance as a function of time (●) is not significantly different from the average KA-induced depression of eIPSC amplitude as a function of time (○). (C) KA alters eIPSC kinetics. (C1) KA depresses the eIPSC. (C2) eIPSCs in C1 before and after KA application are shown after normalization to their peak amplitudes. (D) On average, KA reduces the half-width of IPSCs. Individual experiments (●) are shown.

Fig. 5. The KA-induced decrease in eIPSC amplitude in the presence of SCH 50911 is postsynaptic. (A) Average responses to synaptic stimulation (A\(_1\)) or iontophoretic GABA application (A\(_2\)) are shown before and during KA application. There are 15 sweeps included in each average. (B) The effects of KA on the eIPSC (●) are not significantly different from the effects of KA on the GABA response (○) in six cells in which the two responses were compared. (C) There is no significant action of KA on eIPSC amplitude in the presence of SCH 50911 once the postsynaptic effects are accounted for.
accounting of the entire residual effect of KA by passive membrane properties would require a perfect correlation between these parameters, and it is not clear that such a correlation is applicable. For example, it is possible that the eIPSC amplitude is less sensitive to changes in input resistance than the responses to GABA, owing to the faster kinetics of the eIPSC (25); this would lead us to overestimate the role of input resistance in the effect of KA on the eIPSC. However, the measured change in input resistance is likely to be less than the actual change in the dendrites because the KA-activated conductances are at least in part at poorly clamped synapses distant from the soma; this would lead us to underestimate the role of input resistance in the effect of KA on the eIPSC. These quantitative reservations leave open the possibility of an additional small depressant action of KA on the eIPSC.

Finally, we considered possible effects of KA on fiber excitability because an effect of KA on eIPSC latency (15) in cultured neurons has been reported. We examined the eIPSC latency in the presence and absence of KA, to assess any possible KA-induced decrease in fiber reliability. We found no effect of KA on latency in most cells (16 of 19); however a minority of cells (3 of 19) did show a lengthening of eIPSC latency, which ranged between 0.5 and 2.5 msec (data not shown). Additionally, we found in one cell that recovery from the KA-induced depression was characterized by fluctuations between two distinct levels, separated by 300 pA (data not shown). These amplitude fluctuations are too large to be explained by the recovery of probabilistic release from single release sites (see refs. 7 and 24) but could be explained by intermittent firing in a single presynaptic axon connected to the pyramidal cell through multiple release sites. Because the majority of cells show neither of these effects, we are reluctant to propose a substantial role of loss of fiber excitability in the action of KA; however, our data are consistent with such an action in a minority of interneurons.

Discussion

Our purpose in this work has been to determine the mechanism by which KA acts to depress the eIPSC. We found that approximately one-half of this depression is removed by antagonism of GABA_B receptors, and the other one-half is accompanied by a similar change in the input resistance and is observed not only in the eIPSC, but also in response to iontophoretic GABA applications. We have been unable to find any effect of KA on mIPSC frequency under a number of different conditions, nor were we able to block the depressant action of KA on eIPSCs by holding individual presynaptic interneurons in voltage clamp to prevent the KA-induced increase in presynaptic spiking. We have seen, in a small subpopulation of cells, evidence for a KA-induced reduction in interneuronal fiber reliability.

These results are synthesized into a working model for KA action in Fig. 6. KA receptor activation causes a somatic depolarization of a population of interneurons, which leads to an increase in interneuronal spiking. This increase in spiking increases spontaneous GABA release, which has two major depressant effects. First, the increase in GABA leads to activation of presynaptic metabotropic GABA_B receptors, which reduce the probability of release. Second, the increase in GABA causes a tonic activation of postsynaptic GABA_A receptors, causing a decrease in input resistance and an increase in shunting. A minor portion of the measured decrease in postsynaptic input resistance is bicuculline-resistant, presumably caused by KA receptors on the CA1 pyramidal cells (17), but the absence of a detectable effect of KA on the amplitude of mIPSCs in our hands (7) suggests that this secondary effect is not on its own a major contributor to the depression of the eIPSC. An additional action of KA on interneuronal fiber excitability remains a possibility, but we have been unable to obtain consistent evidence for such an action, and therefore we propose that its contribution to the overall depressant effect of KA on the eIPSC is negligible; in any case, we cannot exclude the possibility that this minor action is a result of the electrotonic spread of interneuronal somatic depolarization down the axon, which would inactivate axonal sodium channels and cause failure of spike propagation. There is no obvious direct effect of KA on GABA release in our hands because we have been unable to detect any effect on the frequency (this study and ref. 7) of mIPSCs under a variety of conditions.

We briefly consider the ability of this model to explain the available data from previous work. The model predicts that KA should be largely ineffective on the eIPSC if the increase in spontaneous firing is prevented for the entire interneuronal population, and this result has been observed (7). Because we propose that the action of KA is in part presynaptic, through indirect activation of GABA_B receptors, the model is also consistent with observations that KA application increases the failure rate and variability of eIPSCs (15, 17) and depresses GABA_A and GABA_B eIPSCs in parallel (14). The observation that KA does not resolvably alter PPD (7) might seem incompatible with a presynaptic effect of KA, but we have shown that paired-pulse assays are not sufficiently sensitive to detect the small change in release probability consistent with the GABA_B receptor-dependent depression induced by KA.

The model also raises the possibility that evidence for a metabotropic action of KA on the eIPSC (blockade by pertussis toxin and protein kinase C modulators; ref. 16) can be explained by activation of metabotropic GABA_B receptors. The mechanisms by which GABA_B receptors depress GABA release are largely unknown, but at least some evidence suggests that...
pertussis-toxin sensitive G proteins may be involved (27, 28), and an interaction between the effectors of presynaptic GABA \(_B\) receptors and protein kinase C has been also described (27, 28). Further elucidation of the signaling pathways by which GABA \(_B\) receptors depress release will be of interest.

Not all available data on the effect of KA on eIPSCs can be readily reconciled with our model. First, our model predicts no effect of KA on mIPSCs; while we (this study and ref. 7) and others (8, 17) have reported modest or no effects of KA on mIPSCs, initial reports of the depressant action of KA on the eIPSC also reported a strong (>50%) depressant action on mIPSC frequency (15, 16). The reasons for this discrepancy are unclear, but our data suggests that simple experimental variables (e.g., composition of bathing medium and animal type) are unlikely to account for the difference. Second, our model predicts that KA should have no effect on GABA release from isolated presynaptic terminals; however, a depression of GABA release from synaptosomes has been reported (29). It is noteworthy that this synaptosomal effect is sensitive to the GluR6-selective antagonist NS-102, although we have seen no effect of this drug on the depression of the eIPSC (7), and the KA-selective antagonist NS-102, although we have seen no effect of KA on the depression of the eIPSC (7), and the KA-selective antagonist NS-102, although we have seen no effect of KA on GABA release from GluR6-deficient mice (17). This mechanistic difference makes the synaptosomal data difficult to incorporate into a model compatible with the data from slices. Finally, our model predicts that KA should have little or no effect on isolated interneuronal-pyramidal cell pairs in culture; however, a KA-induced depression has been reported in this system. We note that in the culture system, KA causes a remarkable increase in eIPSC latency (~10 msec; see, e.g., ref. 15, figure 1). We suggest that in this system, KA receptors might have a much stronger effect on fiber excitability than in the slice preparation, possibly due to altered morphology or changes in KA receptor localization in the culture preparation.

In conclusion, we have presented evidence suggesting that KA acts to depress the eIPSC mainly by increasing spontaneous GABA release and subsequently activating presynaptic GABA \(_B\) receptors, which depress release, and postsynaptic GABA \(_A\) receptors, which increase shunting. We propose that these actions represent a negative feedback loop that limits the increase in inhibitory drive induced by KA receptor activation. Our results indicate that the effects of KA receptor activation described at these synapses can be explained in terms of the conventional, postsynaptic, and ionotropic actions of these receptors. It remains to be seen whether the depressant action of KA on excitatory synapses (12, 13) can be similarly explained through indirect mechanisms.

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