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Metabotropic-Mediated Kainate Receptor Regulation of I_{sAHP} and Excitability in Pyramidal Cells

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Summary

Kainate receptors (KARs) on CA1 pyramidal cells make no detectable contribution to EPSCs. We report that these receptors have a metabotropic function, as shown previously for CA1 interneurons. Brief kainate exposure caused long-lasting inhibition of a postspike potassium current (I_{sAHP}) in CA1 pyramidal cells. The pharmacological profile was independent of AMPA receptors or the GluR5 subunit, indicating a possible role for the GluR6 subunit. KAR inhibition of I_{sAHP} did not require ionotropic action or network activity, but was blocked by the inhibitor of pertussis toxin-sensitive G proteins, N-ethylmaleimide (NEM), or the PKC inhibitor calphostin C. These data suggest how KARs, putatively containing GluR6, directly increase excitability of CA1 pyramidal cells and help explain the propensity for seizure activity following KAR activation.

Introduction

Kainate is an excitotoxic glutamate analog that has well-known actions to increase excitability and produce seizure-like activity in the hippocampus (Westbrook and Lothman, 1983, Fisher and Alger, 1984; Ben-Ari and Cossart, 2000). Although these actions of kainate have been studied for many years, our understanding of the kainate receptor (KAR) itself has lagged behind that of other ionotropic glutamate receptors. It is now apparent that, in many early studies, kainate was used in ways that did not discriminate between actions on AMPA receptors and KARs. However, recent evidence has revealed how KAR modulation of synaptic mechanisms may contribute to hippocampal hyperexcitability (reviewed in Frerking and Nicoll, 2000).

Kainate receptors have been shown to mediate excitatory postsynaptic potentials or currents in CA1 interneurons (Cossart et al., 1998; Frerking et al., 1998) and CA3 pyramidal neurons (Vignes and Collingridge, 1997; Castillo et al., 1997). In CA1 pyramidal neurons, knockout studies have demonstrated that GluR6-containing KARs produce inward currents (Bureau et al., 1999), yet KARs make no detectable ionotropic contribution to synaptic responses in these cells. In the meantime, a metabotropic function has been reported for the presynaptic KARs on CA1 interneurons (Rodríguez-Moreno and Lerma, 1998). To resolve the enigma of a functional receptor without any apparent function, we tested the hypothesis that KARs have a metabotropic action in CA1 pyramidal cells.

A candidate target for any metabotropic action is the slow afterhyperpolarization (sAHP), which follows short bursts of action potentials and is generated by a voltage-independent, Ca^{2+}-dependent K^+ current (Lancaster and Adams, 1986). The sAHP (or current, I_{sAHP}) lasts up to several seconds and is activated proportionally to the number and frequency of action potentials within a burst. It thus acts as a negative feedback mechanism that limits repetitive spiking (Madison and Nicoll, 1984) and burst firing (Traub et al., 1993) during hyperexcitability. A spectrum of neurotransmitters like noradrenaline, acetylcholine, or glutamate, via G protein-coupled receptors, will inhibit sAHP (Nicoll, 1988). The transduction mechanism for noradrenaline action involves the cAMP/protein kinase A pathway (Madison and Nicoll, 1986; Pedarzani and Storm, 1993), whereas acetylcholine has been suggested to act through Ca^{2+}-calmodulin-dependent protein kinase II (Müller et al., 1992). Protein kinase C (PKC) activation also has been shown to inhibit the sAHP (Malenka et al., 1986), but the transmitter linked to this action remains to be identified.

Although kainate is known to promote network oscillations (Buhl et al., 1998) as well as seizure-like activity, an understanding of kainate action against intrinsic pyramidal cell conductances (Robinson and Deadwyler, 1981; Ashwood et al., 1986; Gho et al., 1986) has not kept pace with advances in the field of glutamate receptor pharmacology. We show here that GluR6-containing kainate receptors in CA1 pyramidal neurons are the likely trigger for PKC-mediated inhibition of sAHP.

Results

The Slow AHP in Pyramidal Cells Is Inhibited by High-Affinity Kainate Receptors

The first series of experiments tested whether kainate inhibition of I_{sAHP} was mediated by a direct postsynaptic action of a kainate-preferring glutamate receptor. Bath application of 200 nM kainate for 5–15 min caused a long-lasting inhibition of the peak amplitude of I_{sAHP} (34% ± 6%, mean ± SEM, n = 7; Figures 1A and 2A). This action reached a plateau regardless of prolonged kainate application (15 min, Figure 1A), and lasted for more than 1 hr after kainate application (data not shown). I_{sAHP} inhibition was not accompanied by significant changes in holding current.

To test if I_{sAHP} inhibition was an indirect effect, caused by action potential-dependent transmitter release, further experiments were conducted in the presence of 1 μM tetrodotoxin (TTX) and 5 mM tetraethylammonium

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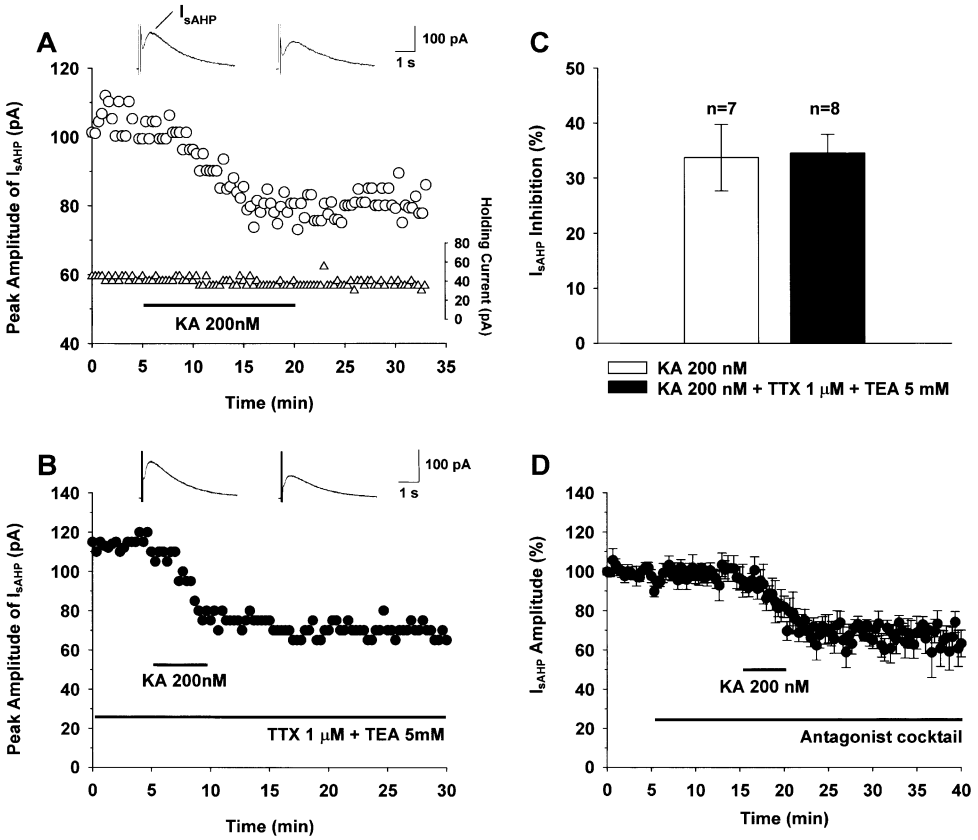

Figure 1. Kainate Inhibits I_{sAHP} by a Direct Action on CA1 Pyramidal Cells

(A) 200 nM kainate application reduced the amplitude of I_{sAHP}. The peak amplitudes of I_{sAHP} (circles) and the holding current values (triangles) are plotted against time. Sample traces were obtained before and after kainate application in the same neuron (averages of 15 trials).

(B) The inhibition of I_{sAHP} by kainate was reproduced in the presence of 1 μM TTX and 5 mM TEA.

(C) Comparison of the 200 nM kainate evoked inhibition with and without 1 μM TTX plus 5 mM TEA. The data presented are the average percentage inhibition of I_{sAHP} (mean ± SEM).

(D) Summary time course of I_{sAHP} inhibition from five experiments (mean ± SEM) recorded in the presence of the cocktail of antagonists: DL-AP5 (100 μM), GYKI52466 (100 μM), MCPG (1 mM), MSOP (250 μM), picrotoxin (100 μM), 2-OH-saclofen (200 μM), atropine sulfate (1 μM), DPCPX (0.1 μM), AM 251 (2 μM), and naloxone (10 μM).

(TEA) (Figure 1B). TEA was added in these experiments in order to facilitate calcium spikes so that I_{sAHP} could be evoked reliably. The kainate inhibition of I_{sAHP} observed in the presence of TTX was indistinguishable from controls (35% ± 3% in eight cells; Figure 1C).

It has been shown that kainate can increase spontaneous GABA release from CA1 interneurons (Cossart et al., 1998). In order to test whether the effect of kainate on I_{sAHP} depended on any secondary activation of receptors other than the kainate subtype, we used a cocktail of antagonists. Blockers were used for the NMDAR (100 μM DL-AP5), AMPAR (100 μM GYKI52466), mGluR (1 mM MCPG and 250 μM MSOP), $GABA_AR$ (100 μM picrotoxin), $GABA_BR$ (200 μM 2-OH-saclofen), muscarinic AchR (1 μM atropine sulfate), opioid (10 μM naloxone), cannabinoid CB_1 (2 μM AM 251), and adenosine receptors (0.1 μM DPCPX). Under these conditions, a 5 min kainate application inhibited the peak amplitude of I_{sAHP} (32% ± 5%, n = 5; Figure 1D) with a time course similar to that recorded in control experiments (n = 7; Figure 2A).

The effect of kainate showed a requirement for AMPA/kainate receptors, since it was completely abolished by prior application of CNQX (20 μM, n = 6; Figures 2B and 3). In contrast, application of 20 μM CNQX after kainate did not relieve I_{sAHP} inhibition (n = 3; Figure 2C). Therefore, the prolonged action is unlikely to be due to persistent receptor activation or slow washout of the kainate but may, instead, reflect the action of an intracellular messenger. The effect of kainate on I_{sAHP} was concentration dependent, reaching a plateau of 34% at 100 nM with an IC_{50} = 15 nM (Figure 2D). A more complete block was observed with high concentrations of kainate (84% at 10 μM), which were associated with large inward currents. We have restricted our analysis to concentrations that were not associated with clear ionotropic effects.

In order to identify the receptor subtype responsible for kainate-induced inhibition of I_{sAHP}, we tested the effects of different AMPA and kainate receptor agonists and antagonists. Figure 3 shows a summary histogram for these effects. Domoate (200 nM), which is a more potent agonist of kainate receptors than kainate itself (Debonnel et al., 1989), also caused substantial inhibition of I_{sAHP} (51% ± 6% versus 34% ± 6%, respectively).

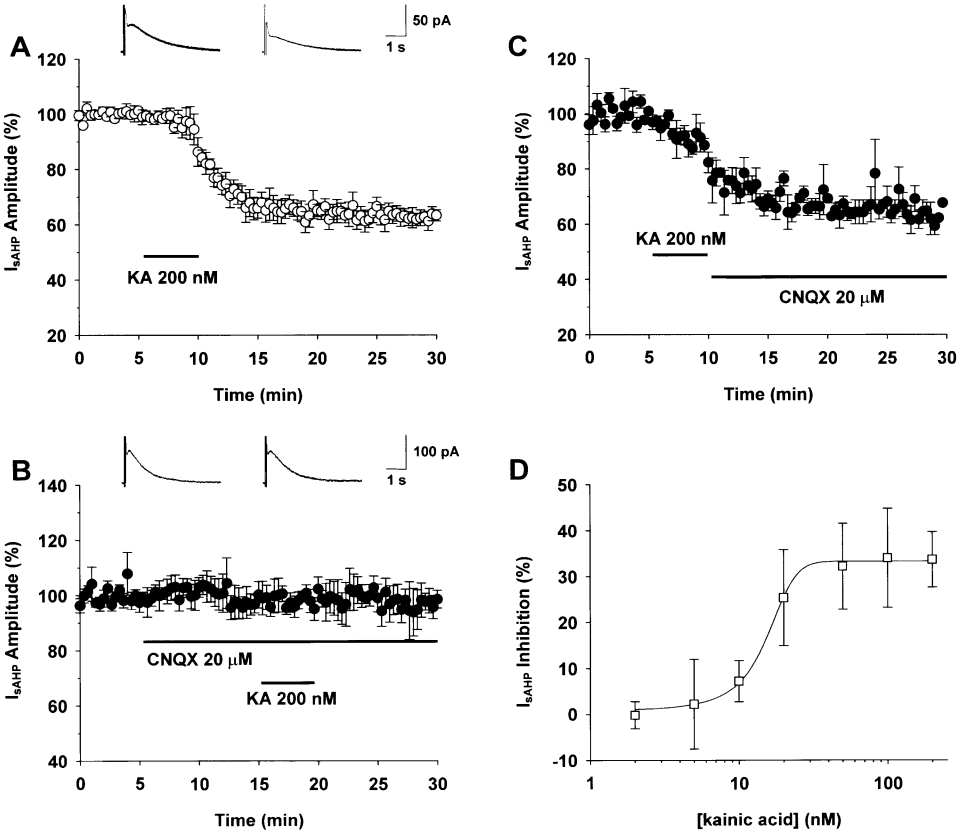

Figure 2. I_{sAHP} Inhibition Is Mediated by AMPA/KA Receptor Activation

(A) Summary time course of I_{sAHP} inhibition from seven experiments (mean ± SEM). The traces illustrate data from one neuron before and after 200 nM kainate application.

(B) Prior application of 20 μM CNQX blocked the effect of kainate. Summary time course of I_{sAHP} inhibition (n = 6). The traces (averages of 15 trials) were recorded in the presence of 20 μM CNQX before and after 200 nM kainate was added to the solution.

(C) 20 μM CNQX applied after 200 nM kainate did not relieve I_{sAHP} inhibition. Summary time course of I_{sAHP} inhibition (n = 3).

(D) Dose-response curve for kainate-induced inhibition of I_{sAHP}. Each symbol represents average of at least four cells. The data were fitted with a logistic curve which gives an IC_{50} = 15 nM.

I_{sAHP} was not affected by the potent AMPA receptor agonist (S)-5-fluorowillardiine (300 nM, n = 7) (Wong et al., 1994; Jane et al., 1997). The nonselective AMPA/kainate receptor antagonist CNQX (20 μM) blocked the effect of kainate. Kainate activation of AMPA receptors was then tested with the blocker GYKI52466 (100 μM) (Paternain et al., 1995), which was ineffective at five to ten times the reported IC_{50} (Bleakman et al., 1996). Kainate receptors found in CA1 interneurons have been reported to contain GluR5 subunits (Cossart et al., 1998). Therefore, we used the GluR5 subtype-preferring agonist ATPA (Clarke et al., 1997) to test for the involvement of this subunit. At concentrations known to be effective against GluR5 subunits (Clarke et al., 1997), ATPA (2 μM) did not produce any changes in I_{sAHP} amplitude (n = 5, Figure 3). These data are consistent with a direct action of kainate on CA1 pyramidal cells, acting via GluR6-containing kainate receptors to modulate I_{sAHP}.

Kainate Action in Pyramidal Cells Involves Metabotropic Function

The inhibition of I_{sAHP} was not associated with clear ionotropic effects of the kainate receptor. A metabotropic function of kainate receptors acting through pertussis toxin-sensitive G proteins and protein kinase C was described for presynaptic kainate receptors on CA1 interneurons (Rodríguez-Moreno and Lerma, 1998). We tested for metabotropic actions using an inhibitor of pertussis toxin-sensitive G proteins, N-ethylmaleimide (NEM) (Heuss et al., 1999; Frerking et al., 2001), and the protein kinase C inhibitor, calphostin C. Prior application of NEM (50 μM) prevented the inhibitory action of kainate (200 nM) on I_{sAHP} in seven experiments (Figure 4). NEM caused some reduction of I_{sAHP} amplitude, possibly due to calcium current rundown, which has been reported with this reagent (Shapiro et al., 1994; Viana and Hille, 1996; Delmas and Gola, 1997). Preincubation of the slices with 1 μM calphostin C for 2–4 hr prevented I_{sAHP} inhibition by kainate (200 nM, n = 10, Figure 4). In four of these cells, subsequent application of 10 μM noradrenaline blocked I_{sAHP}. This demonstrates that the calphostin C treatment was not causing a broad spectrum kinase inhibition because it did not block sensitivity to noradrenaline, which is known to reduce I_{sAHP} via PKA (Pedarzani and Storm, 1993). Therefore, these data suggest that kainate inhibition of I_{sAHP} has a specific requirement for PKC.

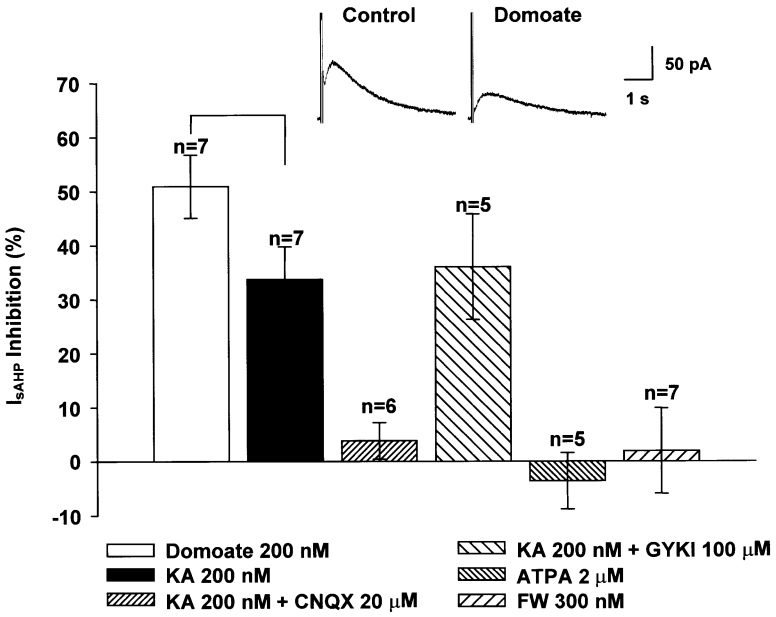

Figure 3. Summary Histogram of the Effects of AMPA and Kainate Receptor Agonists and Antagonists on I_{sAHP}

The traces show the effect of 200 nM domoate on I_{sAHP}. Each trace represents the average of 15 trials recorded before and after domoate application. The bars are the average percentage inhibition of I_{sAHP} (mean ± SEM). Kainate (200 nM) and domoate (200 nM) both produce inhibition of I_{sAHP}. The action of kainate can be blocked by CNQX (20 μM), but not by GYKI52466 (100 μM). (S)-5-fluorowillardiine (300 nM) and ATPA (2 μM) were ineffective.

Kainate Acts Downstream of Ca^{2+} Influx to Enhance Excitability

Kainate has been reported to inhibit voltage-dependent Ca^{2+} currents (Nistri and Cherubini, 1991) and the inhibition of I_{sAHP} might, in principle, be a simple consequence of this action. This was tested in two ways, first by examining the effect of kainate on Ca^{2+} action potentials. In current-clamp experiments in the presence of 1 μM TTX and 5 mM TEA, depolarizing current injection (50–80 ms 0.1–1.0 nA) generated a calcium action potential. The addition of kainate at concentrations that inhibit I_{sAHP} (200 nM, Figure 5A) did not affect either the width or the amplitude of calcium spikes; this was quantified by measuring the area under the calcium spikes. Figure 5B shows the time course of the calcium spike area for the data in Figure 5A; the average data (inset, Figure 5B) from eight experiments shows that there was no change in calcium spike area (−5% ± 3%) 10 min after

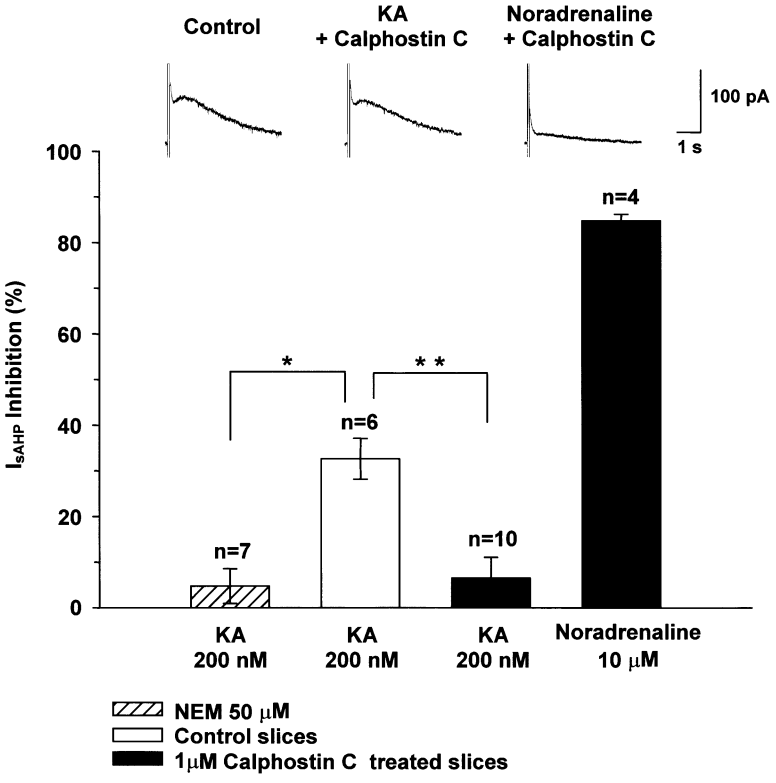

Figure 4. Kainate-Induced Inhibition of I_{sAHP} Involves Metabotropic Function

Sample traces (averages of 15 trials) were recorded in calphostin C-treated slices before and after kainate application, and after subsequent application of noradrenaline. The histogram shows the average percentage inhibition of I_{sAHP} (mean ± SEM). 200 nM kainate inhibited I_{sAHP} in control slices (white bar). Prior bath application of NEM (50 μM) blocked the effect of kainate (hatched bar) ($p < 0.0006$, unpaired t test). In calphostin C (1 μM) treated slices (black bars) kainate-induced inhibition was significantly reduced ($p < 0.002$), subsequent application of 10 μM noradrenaline blocked I_{sAHP}.

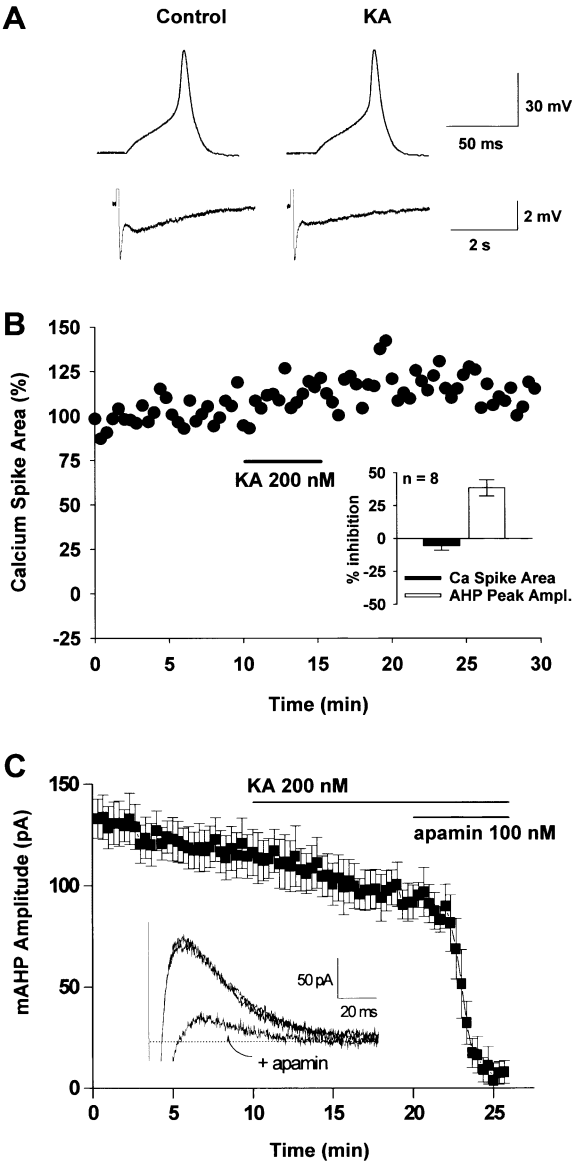

Figure 5. Kainate Does Not Affect Ca^{2+} Influx

(A) 200 nM kainate application reduced I_{sAHP} (bottom traces) without changing the profiles of calcium action potentials (top traces), which were generated in response to 50 ms depolarizing voltage steps. All traces were obtained in the presence of 1 μM TTX and 5 mM TEA.

(B) Normalized time course of calcium spike areas for the data illustrated above. The inset shows average histogram for eight experiments (mean ± SEM) where the effects of 200 nM kainate on I_{sAHP} and Ca^{2+} spikes are compared.

(C) Summary time course for the peak amplitude of the medium duration (apamin-sensitive) AHP current, which shows no sensitivity to kainate. Inset, three traces show control, kainite, and apamin sensitivity of mAHP tail currents following a depolarization.

kainate application although the sAHP showed a reduction of 38% ± 6% at this time. If kainate action on I_{sAHP} was secondary to a reduction of Ca^{2+} influx, it should also inhibit other Ca^{2+}-dependent K^+ currents. This hypothesis was tested on the medium duration AHP (mAHP) in CA1 pyramidal cells, which is generated by apamin-sensitive SK channels (Köhler et al., 1996; Stocker et al., 1999) and is distinct from the current underlying the sAHP. In these experiments, the mAHP was studied in isolation following block of I_{sAHP} by inclusion of 100 μM 8Br-cAMP in the electrode. The mAHP current was subject to a gentle rundown; however, this was unaffected by a 10 min application of 200 nM kainate ($n = 5$, Figure 5C). This evidence suggests that inhibition of voltage-dependent Ca^{2+} channels is not responsible for the kainate-induced reduction of I_{sAHP}.

Finally, we resolved the consequences of kainate action on excitability by studying the increase in action potential firing caused by 200 nM kainate. Spike frequency is the product of a number of factors including persistent Na current and M current in addition to I_{sAHP}, so the slight drift after kainate application may result from altered balance between other ionic currents. Nonetheless, 200 nM kainate, which reliably causes a 30%–40% reduction of I_{sAHP} (previous figures), resulted in increased spiking in response to 800 ms depolarizing current steps (Figure 6A). The membrane potential was maintained constant for the duration of the experiment. The summary data time course (Figure 6B) shows that spike number was stable during the 10 min control period and then increased significantly from 5.4 ± 0.4 spikes before kainate to 8.3 ± 0.8 spikes 10 min after kainate addition ($p < 0.006$), an average 53% increase in this measure of excitability.

Discussion

Our first concern in this study was to test whether the actions of kainate on the sAHP were indirect. There is particularly strong expression of mRNA for KAR subunits in the CA3 pyramidal layer (Bureau et al., 1999). This raises the question: does bath-applied kainate act indirectly by driving CA3 pyramidal cells? Several lines of evidence exclude this interpretation. The relation of I_{sAHP} inhibition to kainate concentration had an IC_{50} of 15 nM and plateau at ~100 nM. These concentrations are not associated with inward currents in CA1 pyramidal cells (this paper; Robinson and Deadwyler, 1981; Castillo et al., 1997; Schmitz et al., 2000) but are comparable to the reported binding affinities (reviewed in Lerma et al., 2001). Inhibition of I_{sAHP} by kainate was identical in the presence of either TTX or a cocktail of receptor antagonists; this excludes any role for action potential-dependent release or secondary activation of other receptor types. These results are strong evidence that I_{sAHP} can be inhibited by direct activation of kainate receptors on CA1 pyramidal neurons, leading to increased excitability of these cells.

Ionotropic responses mediated by KARs in CA1 pyramidal cells are absent in GluR6-deficient mice (Bureau et al., 1999). In concert with these observations, our experiments excluded any role for AMPAR or GluR5 subunits in kainate inhibition of I_{sAHP}. However, the characteristics of the putative GluR6 response reported here indicated a requirement for a second messenger. Sensitivity to the reagent NEM suggests the involvement of a pertussis toxin-sensitive G protein, although the nature of the coupling between KARs and G proteins is unclear. Calphostin C blocked the action of kainate without affecting the ability of noradrenaline to inhibit I_{sAHP}. This

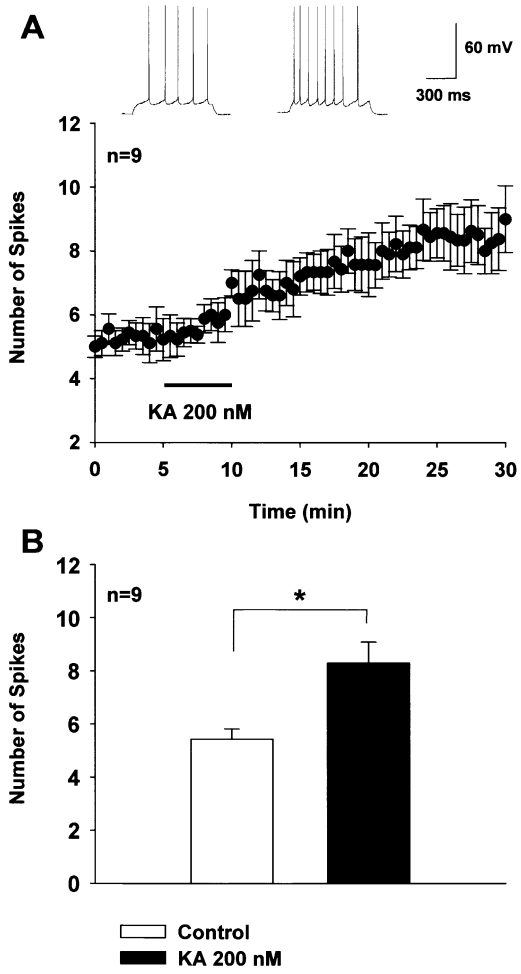

Figure 6. Kainate Increases Intrinsic Excitability of CA1 Pyramidal Cells

(A) Sample traces evoked by 800 ms depolarizing pulse were obtained in one neuron before and after kainate application. The summary time course shows the change in average spike number (mean ± SEM) caused by bath application of 200 nM kainate.

(B) Histogram shows averaged data for nine experiments (mean ± SEM), indicating that there is a significant increase in excitability ($p < 0.005$).

indicates a requirement for PKC in a transduction mechanism, which is distinct from the PKA-mediated action of noradrenaline (Pedarzani and Storm, 1993). Such a role for PKC is similar to the previously reported metabotropic function of KARs involved in presynaptic modulation of GABA release (Rodríguez-Moreno and Lerma, 1998). Since independent PKC activation also blocks the sAHP (Malenka et al., 1986), we suggest that PKC is not just a constitutive requirement, but that KARs activate a PKC-based signaling pathway. When inhibition of the sAHP by PKC was first reported (Malenka et al., 1986), the common supposition was that muscarinic receptors might be the trigger for this action. This possibility has been comprehensively invalidated (Müller et al., 1992; Pedarzani and Storm, 1996; Engisch et al., 1996), and the data presented here fills this gap by suggesting that KARs are coupled to inhibition of the sAHP by PKC.

Kainate has been reported to decrease the Ca^{2+} current in hippocampal neurons (Nistri and Cherubini, 1991), which might explain the reduction of a Ca^{2+}-dependent event like the sAHP. We found that inhibition of the sAHP was not associated with alteration in Ca^{2+} spikes (observed also for PKC activation) (Malenka et al., 1986), nor was there any action of kainate on the apamin-sensitive SK current present in these cells which also requires Ca^{2+} entry (Stocker et al., 1999). These two independent findings demonstrate that the reduction of I_{sAHP} was not secondary to reduced Ca^{2+} influx. This observation is comparable to sAHP inhibition evoked by other neurotransmitters, like noradrenaline (Madison and Nicoll, 1986; Gray and Johnston, 1987, Lancaster and Batchelor, 2000), and acetylcholine (Cole and Nicoll, 1984; Knöpfel et al., 1990), which also appear to inhibit the sAHP independently of Ca^{2+} influx.

Kainate receptors which mediate presynaptic actions can be activated by synaptically evoked glutamate release (Min et al., 1999; Schmitz et al., 2000). On CA1 pyramidal cells, however, there is no KAR ionotropic component to synaptic responses (Frerking and Nicoll, 2000), and it remains an open question whether the metabotropic action of KAR described here can be recruited by synaptic activation. Alternatively, it is conceivable that activation might result only from the excessive glutamate release that occurs during ischaemia or seizures. While low levels of agonist activate a number of KARs, it is the metabotropic nature of this change in intrinsic membrane properties that would help maintain increased pyramidal cell excitability through any transient increase of interneurone activity or GABA-release (Cossart et al., 1998; Semyanov and Kullmann, 2001). Higher levels of glutamate could set up a positive feedback system in which GABA-mediated inhibition is decreased (Fisher and Alger, 1984; Clarke et al., 1997; Rodríguez-Moreno et al., 1997; Min et al., 1999), leading to burst activity which is exacerbated by the reduction in the sAHP which normally follows the burst. Further glutamate release could additionally activate metabotropic glutamate receptors, which have also been reported to block sAHP (Charpak et al., 1990; Abdul-Ghani et al., 1996). These indirect actions may contribute to the more complete block of I_{sAHP} observed with high (3–10 μM) kainate concentrations which depolarize neurons. The end result of these KAR-dependent convergent mechanisms may be the long-term loss of the sAHP and chronic hyperexcitability observed in the hippocampus following kainate lesions (Ashwood et al., 1986).

Experimental Procedures

Hippocampi were obtained from 14- to 19-day-old rats after decapitation. Transverse slices (400 μm) were cut using a Vibratome and incubated in an interface chamber at room temperature before transfer to a submerged recording chamber. The storage and perfusion solution contained 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 11 mM glucose and was gassed with 95% O_2/5% CO_2. Recordings were made at 28°C–30°C with recording pipettes containing 150 mM potassium methylsulphate, 10 mM KCl, 10 mM Hepes, 4 mM NaCl, 4 mM Mg_2ATP, and 0.4 mM Na_4GTP. Osmolarity was adjusted to 280–290 mosmol l^{-1} and pH to 7.35–7.4 with KOH. The methylsulphate anion was used because it has been shown to preserve I_{sAHP} in a form similar to that observed with sharp electrode recordings (Lancaster

and Adams, 1986; Zhang et al., 1994). Chemicals were purchased from Sigma (Poole, UK), with the following exceptions: kainic acid (Ocean Products International, Shelburne, Nova Scotia, Canada; or Sigma), NaCl (Fisher Chemicals, Loughborough, UK), potassium methylsulphate (ICN Biomedicals, Aurora, OH), Calphostin C (Calbiochem, Nottingham, UK), CNQX, domoic acid, ATPA, (S)-5-fluorowillardiine, DL-AP5, (S)-MCPG, MSOP, 2-OH-saclofen, DPCPX, AM 251, naloxone, and tetrodotoxin were obtained from Tocris Cookson (Bristol, UK).

Whole-cell patch-clamp recordings were made from stratum pyramidale without visual identification. Open pipette resistance was 2–4 MΩ, and access resistance during recordings was <20 MΩ. I_{sAHP} was recorded (Axopatch 200B or 1D amplifier, Axon Instruments) in neurons voltage clamped at holding potentials from −50 to −65 mV following 80 ms voltage steps to −10 mV applied every 20 s. The records were filtered at 1 kHz and sampled at 2 kHz. Current-clamp recordings were made at membrane potentials from −50 to −65 mV (AxoClamp 2B, Axon Instruments). Long (800 ms) and brief (50–80 ms) depolarizing current steps (0.03–1.0 nA) were used to evoke action potentials and slow afterhyperpolarizations. Data are given as means ± SEM, and the significance was assessed with Student's t test.

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