Presynaptic Inactivation of Action Potentials and Postsynaptic Inhibition of GABA_A Currents Contribute to KA-Induced Disinhibition in CA1 Pyramidal Neurons

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1Department of Cell Biology and Anatomy, New York Medical College, Valhalla 10595; 2The Center for Neurobiology and Behavior, Columbia University, New York 10032; and 3Center for Aging and Developmental Biology, Rochester University Medical Center, Rochester, New York 14642

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Kang, Ning, Li Jiang, Wei He, Jun Xu, Maiken Nedergaard, and Jian Kang. Presynaptic inactivation of action potentials and postsynaptic inhibition of GABA_A currents contribute to KA-induced disinhibition in CA1 pyramidal neurons. J Neurophysiol 92: 873–882, 2004. First published March 3, 2004; 10.1152/jn.01231.2003. Kainate-type glutamate ionotropic receptors (KAR) mediate either depression or potentiation of inhibitory transmission. The mechanisms underlying the depressant effect of KAR agonists have been controversial. Under dual patch-clamp recording techniques in synaptically coupled pairs of CA1 interneurons and pyramidal neurons in hippocampal slices, micromolar concentrations of KAR agonists, kainic acid (KA, 10 μM) and ATPA (10 μM), induced inactivation of action potentials (APs) in 58 and 50% of presynaptic interneurons, respectively. Inactivation of interneuronal APs might have significantly contributed to KA-induced decreases in evoked inhibitory postsynaptic currents (eIPSCs) that are obtained by stimulating the stratum radiatum. With controlled interneuronal APs, KAR agonists induced a decrease in the potency (mean amplitude of successful events) and mean amplitude (including failures) of unitary inhibitory postsynaptic currents (uIPSCs) without significantly changing the success rate (P_s) at perisomatic high-P_s synapses. In contrast, KAR agonists induced a decrease in both the P_s and potency of uIPSCs at dendritic high-P_s synapses. KAR agonists induced an inhibition of GABA_A currents by activating postsynaptic KARs in pyramidal neurons; this was more prominent at dendrites than at soma. Both the exogenous GABA-induced current and the amplitude of miniature IPSCs (mIPSCs) were attenuated by KAR agonists. Thus the postsynaptic KAR-mediated inhibition of GABA_A currents may contribute to the KAR agonist-induced decrease in the potency of uIPSCs and KA-induced disinhibition.

INTRODUCTION

Glutamate ionotropic kainate receptors (KARs) have been reported to mediate excitatory synaptic currents in a certain area of neurons (Castillo et al. 1997; Cossart et al. 1998; DeVries 2000; DeVries and Schwartz 1999; Frerking et al. 1998; Kidd and Isaac 1999; Li et al. 1999; Vignes and Collingridge 1997) and to modulate either excitatory (Chittajallu et al. 1996; Kamiya and Ozawa 2000; Schmitz et al. 2000) or synapse-released glutamate coupled CA1 interneurons and pyramidal neurons reveals that submicromolar KAR agonists or synapse-released glutamate increases the efficacy of low release probability GABAergic synapses (Jiang et al. 2001). GABAergic synapses are heterogeneous in their release probability (Jiang et al. 2000), location (Buhl et al. 1994; Maccaferri et al. 2000; Miles et al. 1996), and function (Cossart et al. 2001a; Lambert et al. 1991). In neuronal circuits, a
perisomatic GABAergic synapse controls action potential firing (Melnek and Muller 1996; Miles and Wong 1987; Moore et al. 1983) while a dendritic GABAergic synapse modulates synaptic strength (Davies et al. 1991; Miles et al. 1996). KARs may differentially modulate these perisomatic and dendritic GABAergic synapses to play different roles in modulating dendritic inputs and controlling AP production. To investigate the mechanisms underlying KA-induced disinhibition, we tested the effects of KAR agonists KA (10 μM) and ATPA (10 μM) on interneuronal firing, mIPSCs, and uIPSCs at perisomatic or dendritic GABAergic synapses in hippocampal slices. We found that 10 μM KA and 10 μM ATPA caused inactivation of APs in 58 and 50% of interneurons, respectively. With control interneuronal APs, ATPA and KA induced a decrease in potency of uIPSCs at perisomatic high-Ps synapses but induced a more prominent decrease in both potency and Ps at dendritic high-Ps GABAergic synapses. Postsynaptic KAR-mediated inhibition of GABA _A_ currents contributed to this decrease in the potency of uIPSCs.

METHODS

Preparation of slices

Brain slices were prepared as described previously (Jiang et al. 2001; Kang et al. 1998). Briefly, 14- to 20-day-old (P14–P20) Sprague-Dawley rats were anesthetized with pentobarbitone sodium (55 mg/kg) and decapitated. Brains were removed rapidly and glued along a plane 18 mm away from the recorded pyramidal neurons. Three to 5 psi pressure was applied to the puffed pipette through a picospitzer III (Parker Hannifin General Valve Operation).

Electrophysiology

The recording chamber was placed on the stage of an Olympus BX51 upright microscope (Olympus Optical) equipped with DIC (differential interference contrast) optics, and cells were visualized with a ×63 water-immersion lens. Two electrical manipulators (TS Products, Arleta, CA) were mounted on the stage in opposing positions and moved along a plane 18° to the horizontal. Patch electrodes with a resistance of 4–7 MΩ were pulled from KG-33 glass capillaries (1.0 mm ID, 1.5 mm OD, Garner Glass, Claremont, CA) using a P-97 electrode puller (Sutter Instrument, Novato, CA). A seal resistance <5 GΩ was rejected. Dual patch-clamp recordings were performed in pairs of interneurons whose somata were located in the stratum radiatum and pyramidal neurons in the CA1 pyramidal layer. Interneurons were patched in the whole cell current-clamp configuration (Hamill et al. 1981) using a MultiClamp 700 amplifier (Axon Instruments, Burlingame, CA). The pipette solution contained (in mM) 120 K-glutamate, 10 KCl, 1 MgCl₂, 10 HEPES, 0.1 EGTA, 0.025 CaCl₂, 1 ATP, 0.2 GTP, and 4 glucose (pH adjusted to 7.2 with KOH). Pyramidal neurons were patched in the voltage-clamp configuration and recorded at a holding potential of −60 mV using an Axopatch 200B amplifier (Axon Instruments). The pipette solution contained (in mM) 120 CsCl, 10 KCl, 1 MgCl₂, 10 HEPES, 0.1 EGTA, 0.025 CaCl₂, 1 ATP, 0.2 GTP, 5 QX-314, and 4 glucose (pH adjusted to 7.2 with KOH). Experiments with a holding current > −200 pA were rejected. It is important for comparing the amplitude of uIPSCs to obtain relative constant access resistance during experiments. We started experiments after access resistance getting relative constant (5–10 min), and recordings with changes in series resistance >10% of control in pyramidal neurons were rejected.

Identification of synaptically connected pairs

Cell pairs that included an interneuron in the s. radiatum and a pyramidal neuron in the pyramidal layer in the hippocampal CA1 region were identified under DIC microscopy and patched in the whole cell configuration (interneuron: I clamp and pyramidal neuron: V clamp). Synaptically connected pairs were identified by a train of three depolarization pulses that evoked three interneuronal APs with an interval of 20 ms and a duration of 15 ms. A successful uIPSC event was defined as a downward current with decay >20 ms and amplitude >5 pA starting within the mean latency (for a given pair) ±2 SD (fast: 0.2 ms, slow: 1.1 ms).

Data analysis

Recording signals were filtered through an 8-pole Bessel low-pass filter with a 2-kHz cut-off frequency and sampled by a PCLAMP 8.1 program (Axon Instruments) with an interval of 50 or 200 μs. Data were further processed with Origin 5.1 (Microcal Software, Northampton, MA) and CorelDraw 9.0 (Corel) programs. Statistical data are presented as means ± SE if not indicated. Data from experiments with GYKI 53655 and SYM 2206 were pooled for analysis.

Local application of the GABA _A_ agonists

In the presence of 1 μM TTX, 50 μM SYM 2206, and 50 μM APV, whole cell GABA _A_ currents were induced by pressure application of GABA or muscimol (50 μM) through another pipette, which contained the slice solution supplemented with GABA or muscimol. Under DIC optics, the pipette was placed on the top of or 100–150 μm away from the recorded pyramidal neurons. Three to 5 psi pressure was applied to the puffed pipette through a picospitzer III (Parker Hannifin General Valve Operation).

Biocytin-staining

Neurons in pairs were patched with a biocytin-containing pipette (0.3%). After finishing experiments, slices were perfused with 4% paraformaldehyde +0.2% picric acid +0.1% glutaraldehyde (pH = 7.4) for 10 min. Slices were postfixed overnight at 4°C. After several washes with 0.1 M phosphate-buffered saline (PBS), slices were treated with 0.3% H₂O₂ for 10 min at room temperature. Then slices were incubated in 0.2% Triton X-100 and 0.2% albumin for 1 h at 4°C, and stained with the ABC kit (1:500, Pierce 32054) for 1 h at room temperature. Last, slices were incubated in 0.025% DAB plus 0.003% H₂O₂ Tris buffer solution for 15 min and mounted in Permount. Morphological subtypes of interneurons were identified with a criteria previously reported by Buhl et al. (1994).

RESULTS

Micromolar KAR agonists inactivate interneuronal APs

In our previous study, we found that submicromolar KAR agonists (KA or ATPA, 300 nM) increased the strength of low-P, GABAergic synapses but did not induce a significant change in the Ps and amplitude of uIPSCs at high-Ps synapses (Jiang et al. 2001). In this study, we examined the effects of micromolar concentrations (5 or 10 μM) of KAR agonists on uIPSCs. Evoked IPSCs (eIPSCs) in CA1 pyramidal neurons were patched in the voltage-clamp configuration (interneuron: I clamp and pyramidal neuron: V clamp). Synaptically connected pairs were identified by a train of three depolarization pulses that evoked three interneuronal APs with an interval of 20 ms and a duration of 15 ms. A successful uIPSC event was defined as a downward current with decay >20 ms and amplitude >5 pA starting within the mean latency (for a given pair) ±2 SD (fast: 0.2 ms, slow: 1.1 ms).

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are obtained by stimulating the s. radiatum. However, micro-
molar KA may induce changes in the 
ring ability of stimu-
lated fibers and affect recording of eIPSCs. Therefore we 
first examined the effects of KAR agonists on interneuronal 
ring using pair recordings. KA (10 μM) or ATPA (10 μM) was 
ferred to the recording chamber in the presence of the N-
methyl-D-aspartate (NMDA) receptor antagonist APV (50 μM) 
and the AMPA receptor antagonist GYKI 53655 (50 μM) or 
SYM 2206 (50 μM). During application of KA, the patched 
terneuron, which lacked holding currents, depolarized (ΔV = 
28.5 ± 3.3 mV) and fired a high-frequency of spontaneous 
ions in a short time period (Fig. 1A, Int, sAP). Meanwhile, the 
pyramidal neuron showed an inward current with a high 
frequency of spontaneous IPSCs (Fig. 1A, Pyr). The amplitude 
of current injection-evoked APs (Fig. 1A, Int, eAP) decreased 
gradually. After 2 min, evoked interneuronal APs disappeared, 
and only the current injection depolarization remained (Fig. 1, 
B and C, 4 and 5), indicating that the interneurons lost the 
ability to fire APs. Fifty-eight (11/19) and 50% (5/10) of 
terneurons temporarily lost firing ability during application 
of KA and ATPA, respectively. A transient decrease in the 
input resistance was also noted (Fig. 1B, 2), reflecting in-
creased channel conductance in the interneuron. Thus inacti-
vation of interneuronal APs significantly contributes to KA-
induced depression of eIPSCs. When interneuronal APs were 
fully inactivated, a strong depolarization pulse (600–800 pA) 
could not successfully trigger uIPSCs (16 pairs), suggesting 
that AP regeneration is required for triggering synaptic release 
of GABA. Furthermore, the attenuated APs evoked uIPSCs 
with a similar efficiency as control APs (Fig. 1, B and C, 3 and 
6), suggesting that attenuated APs could still regenerate along 
the axon. To assess AP width during KA application, we 
measured the AP half-widths (Fig. 1, B and C, 2 and 3, arrows). 
The mean half-width of APs after KA application (Fig. 1D, 
KA) was significantly longer than that during control (Fig. 1D, 
CON, P < 0.01). Increased AP width might increase AP-
driven GABA release due to increased opening time of volt-
age-gated calcium channels (VGCC), which could counteract 
the depressant effects of decreased AP amplitude on GABA 
release.

**KAR agonists induce a decrease in the potency and 
amplitude of uIPSCs at perisomatic high-P<sub>s</sub> synapses**

To study KAR-mediated depression of uIPSCs at periso-
matic and dendritic GABAergic synapses, we located...
GABAergic synapses in pyramidal neurons by analyzing the rising time and amplitude of uIPSCs (Jiang et al. 2000; Maccasferri et al. 2000). To confirm the locations of the GABAergic synapses, some pairs were recorded with patch pipettes containing 0.3% biocyn followed by immunostaining of biocyn. Similar to findings by Maccasferri et al. (2000), biocyn-staining showed that axons of the presynaptic interneurons (basket cells) in three pairs with large, fast uIPSCs (potency range: 67–292 pA; potency, mean amplitude of nonfailure events) were distributed around the pyramidal layer and made contacts on somata of postsynaptic cells (Fig. 2A, ▲). All interneurons in five pairs with small, slow uIPSCs (potency range: 9.9–32.7 pA) had axonal distribution in the s. radiatum and had axonal contacts on dendrites of pyramidal neurons (Fig. 3A, ▲).

GABAergic synapses are heterogeneous in their release probability and can be divided into high-Ps (Ps ≥ 0.5) and low-Ps (Ps < 0.5) synapses (Jiang et al. 2001). The activities of high-Ps synapses contribute to the majority of eIPSCs, whereas low-Ps synapses contribute much less to eIPSCs under normal conditions due to their very low release probability. Therefore we examined KA- or ATPA-induced changes in uIPSCs at high-Ps synapses in the presence of 50 µM SYM 2206 and 50 µM APV. To prevent interneurons from over-depolarization-induced inactivation of APs, we maintained interneuronal membrane potential at less than −55 mV (a value that did not affect spontaneous firing) by injecting hyperpolarizing currents during application of ATPA (10 µM) or KA (10 µM).

At perisomatic high-Ps synapses (potency >50 pA, T1/2 <2 ms), ATPA did not reduce the number of successful events (Fig. 2, B and C, ATPA) and did not induce a significant decrease in the Ps of uIPSCs (Fig. 2D, Ps = 0.135). The potency and amplitude of uIPSCs were significantly attenuated by ATPA (Fig. 2D, Pot: CON, 103.6 ± 20.1 pA; ATPA, 69.2 ± 15.9 pA; Amp: CON, 82.0 ± 21.6 pA; ATPA, 53.4 ± 17.1 pA, P < 0.05). A decrease of 34.7 ± 3.0% in the control potency of uIPSCs was induced by ATPA application. KA also induced a significant decrease in the potency and amplitude of uIPSCs (Fig. 2E, Pot: CON, 93.0 ± 4.1 pA; KA, 46.0 ± 14.6 pA; Amp: CON, 84.3 ± 9.1 pA; ATPA, 39.7 ± 15.1 pA, P < 0.05) but induced only a marginal decrease in the Ps (Fig. 2E, Ps, P = 0.07). KA induced a decrease of 51.5 ± 15.3% in the control potency of uIPSCs. These results suggest that at perisomatic high-Ps, GABAergic synapses, micromolar amounts of KAR agonists induce a significant decrease in the potency of uIPSCs and a minor effect on the release probability. The decrease in the potency of perisomatic uIPSCs implies a postsynaptic mechanism.

**FIG. 2.** KAR agonists induce a decrease in both the Ps and the potency of dendritic high-Ps, uIPSCs

Because the amplitude of dendritic uIPSCs was small, a successful uIPSC event was defined as a downward current with decay >20 ms and amplitude >5 pA starting within the mean latency (for a given pair) ± 2 SD (1.1 ms). In dendritic synapses (potency <40 pA, T1/2 ≥ 2 ms), ATPA reduced successful events of uIPSCs at dendritic high-Ps synapses (Fig. 3, B and C, ATPA). The Ps of uIPSCs during application of ATPA (Fig. 3D, Ps, ATPA, P < 0.01) or KA (Fig. 3E, Ps, KA, P < 0.01) was significantly lower than during the control, suggesting that activation of KARs induces a decrease in the release probability at dendritic GABAergic synapses. The potency and amplitude of uIPSCs was also significantly decreased during application of ATPA (Fig. 3D, Pot: CON, 25.4 ± 4.7 pA; ATPA, 18.0 ± 3.4 pA; Amp: CON, 16.9 ± 3.0 pA; ATPA, 5.1 ± 1.3 pA, P < 0.01) or KA (Fig. 3E, Pot: CON, 20.5 ± 4.7 pA; KA, 13.0 ± 4.5 pA; Amp: CON, 14.9 ± 3.2 pA; ATPA, 2.4 ± 0.9 pA, P < 0.01), again implying the involvement of postsynaptic mechanisms.
Micromolar ATPA facilitates low-\(P_s\) GABAergic synapses

In previous studies, we found that submicromolar KAR agonists increase the efficacy of low-\(P_s\) GABAergic synapses (Jiang et al. 2001). To test whether this facilitating effect is limited to submicromolar concentrations of KAR agonists, we tested the effects of 5\(\mu\)M ATPA on uIPSCs in low-\(P_s\) pairs. At perisomatic low-\(P_s\) synapses with large fast uIPSCs, ATPA induced a significant increase in the \(P_s\) of uIPSCs (Fig. 4, A and B; ATPA, \(P_s, P < 0.05\)). The mean amplitude of uIPSCs were also increased by ATPA (Fig. 4, A and B; ATPA, \(P < 0.05\) for both Pot and Amp), suggesting that micromolar concentrations of KAR agonists have facilitating effects on perisomatic GABAergic synapses that are similar to those of submicromolar quantities (Jiang et al. 2001). In three of six dendritic low-\(P_s\) pairs, ATPA induced biphasic responses: a transient increase (\(-1 \text{ min}\)) in successful events of uIPSCs followed by a subsequent decrease. Statistical data from all six dendritic low-\(P_s\) pairs showed no significant changes in the \(P_s\), potency, or mean amplitude (Fig. 4C; ATPA: \(P = 0.35, 0.78, \text{ and } 0.41\), respectively).

KAR agonists induce a postsynaptic inhibition of GABA\(_A\) currents

Application of ATPA or KA induced a decrease in the potency of perisomatic (Fig. 2, D and E, Pot) and dendritic (Fig. 3, D and E, Pot) uIPSCs, possibly by a postsynaptic
mechanism. To define the postsynaptic mechanism, we recorded the GABA_A-mediated Cl^− current in pyramidal neurons by locally applying the GABA_A receptor agonists GABA (50 μM) or muscimol (50 μM). Hippocampal slices were perfused with TTX (1 μM), SYM 2206 (50 μM), and APV (50 μM) during application of KAR agonists. Pyramidal neurons were first tested by whole cell patch-clamp recording at the soma, and GABA_A agonists were applied to the area around the soma. Application of ATPA (10 μM) induced a slow inward current (I_KAR) and a decrease in the amplitude of GABA_A currents (Fig. 5B, P < 0.01, 1-way ANOVA). The mean amplitude of I_KAR in the presence of TTX (Fig. 5, B and C, TTX) was similar to that in the absence of TTX (Fig. 5, A and C, TTX). The decrease in the amplitude of GABA_A currents was also similar in the presence or absence of TTX (Fig. 5D, P = 0.89, 2-way ANOVA). These results suggest that activation of postsynaptic KARs attenuates GABA_A currents, contributing to KAR agonist-induced decreases in the potency of perisomatic uIPSCs.

To investigate the postsynaptic mechanisms underlying ATPA- or KA-induced decrease in the amplitude of dendritic uIPSCs, we performed whole cell patch-clamp recording at a dendritic position 60–80 μm from the soma of pyramidal neurons (Fig. 6C, arrow). Application of ATPA (10 μM) induced I_KAR and a simultaneous decrease in the amplitude of dendritic GABA_A currents (Fig. 6, A and E). KA (10 μM) induced a larger I_KAR (Fig. 6, B and D, KA, 206.6 ± 41.9 pA) than ATPA (Fig. 6, A and D, ATPA, 109.4 ± 39.7 pA, P < 0.05, paired t-test). In 2 of 10 tested cells, the ATPA-induced I_KAR was undetectable, but the KA-induced I_KAR was noticeable. The reversal potential of I_KAR was +43.3 ± 5.1 mV (n = 6 cells), implying that KAR channels in pyramidal neurons may be Ca^{2+}-permeable. KA also induced a greater inhibition of dendritic GABA_A currents (Fig. 6, B and E, KA) than ATPA (Fig. 6, A and E, ATPA). The maximal inhibition by KA (59 ± 7% of control) was significantly larger than that by ATPA (44 ± 9% of control, P < 0.05, paired t-test). The I_KAR and inhibition of GABA_A currents were blocked by the AMPA/KAR antagonist CNQX (20 μM), confirming that I_KAR and current and inhibition are mediated by KAR activation (Fig. 6, D and E, KA/CNQX). The preceding results suggest that postsynaptic KAR-mediated inhibition of dendritic GABA_A currents contributes to the ATPA- or KA-induced decrease in the potency of dendritic uIPSCs (Fig. 3).

To test whether the GABA_A current activated by synaptic release of GABA is similarly inhibited by KAR agonists, we examined the effects of ATPA or KA on dendritic mIPSCs while monitoring exogenous GABA-induced currents. Frequency and amplitude of mIPSCs during a 50-s period were calculated for control, ATPA, or KA application and washout. The amplitude of mIPSCs was significantly attenuated by ATPA (Fig. 7, A and B, Amp), but the frequency was not significantly altered (P = 0.50). The decrease in the amplitude of mIPSCs occurred simultaneously with and correlated with inhibition of the exogenous GABA-induced current (Fig. 7C, I_{GABA}; R = 0.84, P < 0.01), suggesting that the decrease in the mIPSC amplitude is due to the postsynaptic KAR-mediated inhibition of the GABA_A current. The effect of KA on mIPSCs was similar to that of ATPA. The amplitude of mIPSCs was attenuated by KA (Fig. 7D, Amp, P < 0.01) while the frequency was reduced without statistical significance (P = 0.11). The results suggest that dendritic KARs in pyramidal neurons mediate an inhibition of the GABA_A current.

**DISCUSSION**

Many studies on KA-induced depression of inhibitory transmission have focused on eIPSCs. However, a serious disadvantage is that the firing ability of stimulated fibers is not well controlled. In this study, we have shown that 58% of patched
interneurons were unable to fire APs during application of 10 μM KA (Fig. 1). This inactivation of interneuronal APs may be due to depolarization-induced inactivation of voltage-gated Na⁺ channels and may contribute significantly to KA-induced depression of eIPSCs. Thus firing ability of stimulated fibers should be well controlled when eIPSCs are used to study the depressant effects of KAR agonists. During KA application, attenuated spikes could still induce uIPSCs (Figs. 1B, 2, 3, and 6) while strong depolarization pulses could not trigger uIPSCs successfully when interneuronal spikes were fully inactivated. This suggests that attenuated spikes could still regenerate along the axon. The attenuation of spikes is due to partial inactivation of Na⁺ channels. The initiation of action potential may occur at axons 30–60 μm from soma (Colbert and Johnston 1996; Colbert and Pan 2002), and the distance between the axonal initiation and terminals may be of the same scale as that between the axonal initiation and somata in interneurons. Thus when we recorded an attenuated spike from the soma, a comparable AP should also propagate to the terminals. When no spike was recorded from soma, the AP at the initiation position was either nonexistent or was too small to regenerate.

With controlled interneuronal APs, we found that micromolar KAR agonists differentially modulated individual GABA synapses. ATPA and KA induced a significant decrease in the potency and amplitude of uIPSCs without significantly changing the Pₛ at perisomatic high-Pₛ synapses. Thus ATPA and KA may not significantly affect the presynaptic release of GABA at perisomatic high-Pₛ synapses. In contrast, at dendritic high-Pₛ synapses, ATPA and KA induced a decrease in both the Pₛ and potency of uIPSCs (Fig. 3). The results suggest that KAR-

FIG. 6. ATPA induces dendritic I_{KAR} and inhibition of dendritic GABA_A currents. A: top: ATPA induces dendritic I_{KAR} and inhibition of dendritic GABA_A currents from indicated period. B: KA-induced dendritic I_{KAR} and inhibition of dendritic GABA_A currents. C: a DIC image showing a patch pipette ( ) on an apical dendrite of a pyramidal neuron and a puff pipette ( ) used to apply GABA locally. Bar represents 10 μm. D: the average amplitude of the I_{KAR} induced by ATPA and KA. KA-induced I_{KAR} is blocked by CNQX (KA/CNQX). E: normalized amplitude of dendritic GABA_A currents (I_{GABA}) plotted against time. Neither ATPA (○) nor KA ( ) significantly decreases the amplitude of dendritic GABA_A currents (P > 0.01 for both, 1-way ANOVA). The decrease is blocked by CNQX (KA/CNQX). F: relationship between the change in reversal potential of GABA_A currents (V_{rev}) and the decrease in dendritic GABA_A currents. R = 0.74, P < 0.05.

FIG. 7. A decrease in the amplitude of miniature IPSCs (mIPSCs) simultaneously occurs with the KAR-mediated inhibition of dendritic GABA_A currents. A: recording traces from a representative pyramidal neuron showing that the amplitude of mIPSCs is attenuated by ATPA. B: pooled data showing that the amplitude of mIPSCs is decreased by ATPA (Amp, ). **, P < 0.01, compared with control, paired t-test, n = 10 cells. C: the change in the amplitude of mIPSCs in each cell is linearly related to the inhibition of GABA_A currents. R = 0.84, P < 0.01. D: KA-induced dendritic inhibition of mIPSCs. The mean amplitude and frequency of mIPSCs during control ( ● ) and after KA application ( ● ). **, P < 0.01, paired t-test, n = 7 cells.
mediated presynaptic inhibition of GABA release at perisomatic GABAergic synapses is weaker than that at dendritic GABAergic synapses. The significant decrease in potency of perisomatic high-P, uIPSCs during application of ATPA and KA (Fig. 3) indicates a postsynaptic mechanism. ATPA induced a decrease in somatic GABA_A currents (Fig. 5, A, B, and D) both in the presence or absence of TTX (Fig. 5D), suggesting that the decrease is not related to the ATPA-induced increase in sIPSCs and that it contributes to a part of the ATPA-induced decrease in the potency of uIPSCs.

At dendritic high-P, uIPSCs, ATPA and KA induced a decrease in both the P_i and potency of uIPSCs (Fig. 3). Because the amplitude of dendritic uIPSCs is small during control, the further decrease in the amplitude of dendritic uIPSCs could cause postsynaptic failures (i.e., synaptic events become smaller than background levels). Besides previously reported activation of presynaptic GABA_A autoreceptors (Ferking et al. 1999; Ferking and Nicoll 2000) or direct inhibition of presynaptic release through the PKC pathway (Rodriguez-Moreno and Lerma 1998; Rodriguez-Moreno et al. 2000), postsynaptic failures due to inhibition of dendritic IPSCs might also have contributed to the KAR agonist-induced decrease in the P_i of dendritic uIPSCs. Moreover, KAR agonist-induced postsynaptic depolarization could trigger release of other transmitters or modulators such as endocannabinoids (Carlson et al. 2002; Hoffman et al. 2003; Wilson et al. 2001) or adenosine (Oliet and Poullain 1999; Scanziani et al. 1992). These transmitters/modulators may inhibit presynaptic GABA release and should be examined in further studies.

One postsynaptic mechanisms underlying the KAR agonist-induced decrease in the potency of uIPSCs (Fig. 3C) is the KAR agonist-induced decrease in postsynaptic dendritic GABA_A currents, which we demonstrated using dendritic whole cell recordings (Fig. 6). The time course of ATPA-induced decreases in GABA_A currents (Fig. 6, A and B, 3–4 min) is similar to that in the potency of uIPSCs (3–4 min). The experimental results in this study suggest that postsynaptic KAR-mediated decreases in GABA_A currents contribute to the ATPA- and KA-induced decreases in the potency of dendritic uIPSCs.

The mechanisms underlying KAR-mediated inhibition of GABA_A currents are not fully understood. Several mechanisms may contribute to this inhibition. First, KAR activation may trigger a shift of the reversal potential for GABA_A currents by modifying intracellular Cl^- and HCO_3^- concentrations (Alger and Nicoll 1979, 1982; Connors et al. 1988; Grover et al. 1993; Gullidge and Stuart 2003; Kaila et al. 1993, 1997; Lambert et al. 1991; Michelson and Wong 1991; Newberry and Nicoll 1985; Perkins 1999; Staley and Proctor 1999; Staley and Smith 2001; Staley et al. 1995; Taira et al. 1997; Van den Pol 1996; Wong and Watkins 1982). Intracellular Cl^- concentrations in CNS neurons can be bidirectionally regulated by the K^+-Cl^- co-transporter KCC2 and by the Na^+-K^+^-2Cl^- cotransporter NKCC1 (Beck et al. 2003; DeFazio et al. 2000; Rivera et al. 1999; Sung et al. 2000; Vu et al. 2000). Activity-dependent modulation of KCC2 and NKCC1 has been reported by several groups (Ganguly et al. 2001; Schomberg et al. 2003; Woodin et al. 2003). Second, ATPA and KA application resulted in a large current at somata and dendrites (Figs. 5 and 6). The ATPA- and KA-induced opening of KAR channels decreased the membrane resistance and caused a shunt effect on GABA_A currents. The average peak current of ATPA- and KA-induced I_{KAR} at a holding potential (V_h) of -60 mV was 109 and 207 pA, respectively (Fig. 6D).

The experimental data from recordings of dendritic mIPSCs (Fig. 7) reveal the effects of ATPA and KA on dendritic mIPSCs. The amplitude of dendritic mIPSCs decreased during application of ATPA (Fig. 7, A and B) and KA (D). This decrease is due to the KAR-mediated inhibition of GABA_A currents because the decrease in the amplitude of mIPSCs correlates with the inhibition of GABA_A currents (Fig. 7C). Moreover, the decrease in mIPSCs occurred simultaneously with the inhibition of GABA_A currents. Therefore the ATPA- and KA-induced decrease of dendritic mIPSCs results from a postsynaptic inhibition of GABA_A currents rather than from presynaptic inhibition of GABA release (Rodriguez-Moreno et al. 1997, 1998).

In this study, we demonstrated that micromolar concentrations of the KAR agonists KA and ATPA over-depolarized presynaptic interneurons and inactivated spikes, which may have significantly contributed to KA-induced depression of eIPSCs. With controlled interneuronal AP, ATPA (10 μM) and KA (10 μM) decreased the potency of both perisomatic and dendritic high-P, uIPSCs but only reduced the release probability at dendritic high-P, GABAergic synapses. Postsynaptic KAR receptors mediate an inhibition of GABA_A currents that contributes to the decrease in the potency of uIPSCs.

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