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Activation of presynaptic kainate receptors suppresses GABAergic synaptic transmission in the rat globus pallidus

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Abstract

The globus pallidus (GP) plays a central integrative role in the basal ganglia circuitry. It receives strong GABAergic inputs from the striatum and significant glutamatergic afferents from the subthalamic nucleus (STN). The change in firing rate and pattern of GP neurons is a cardinal feature of Parkinson's disease pathophysiology. Kainate receptor GluR6/7 subunits immunoreactivity is expressed presynaptically in GABAergic striatopallidal terminals (Kane-Jackson and Smith 2003; Jin et al., 2006), which provides a substrate for regulation of GABAergic transmission in GP. To test this hypothesis, we recorded GABA_A-mediated inhibitory postsynaptic currents (IPSCs) in the GP following electrical stimulation of the striatum. Following blockade of AMPA and NMDA receptors with selective antagonists, bath application of kainate (KA) (0.3–3 μ M) reduced significantly the amplitude of evoked IPSCs. This inhibition was associated with a significant increase in paired-pulse facilitation ratio and a reduction of the frequency, but not amplitude, of miniature IPSCs (mIPSCs), suggesting a presynaptic site of KA action. The KA effects on striatopallidal GABAergic transmission were blocked by the G-protein inhibitor, N-ethylmaleimide (NEM), or protein kinase C (PKC) inhibitor calphostin C. Our results demonstrate that KAR activation inhibits GABAergic transmission through a presynaptic G protein-coupled, PKC-dependent metabotropic mechanism in the rat GP. These findings open up the possibility for the development of kainate-mediated pharmacotherapies aim at decreasing the excessive and abnormally regulated inhibition of GP neurons in Parkinson's disease.

Keywords

IPSC; patch-clamp; striatum; PKC; paired-pulse facilitation

Kainate receptors (KARs) are one of the three subtypes of ionotropic glutamate receptors in the CNS. The family of KARs is composed of five different genes that encode for the subunits GluR5, GluR6, GluR7, KA1 and KA2 subunits (Hollmann and Heinemann, 1994; Bettler and Mulle, 1995). These receptors are widely expressed pre- and post-synaptically throughout the brain (Charara et al., 1999; Lerma et al., 2001; Kieval et al., 2001; Kullmann, 2001; Huettner, 2003; Lerma, 2003; Jin et al., 2006), but their role remains poorly understood compared to other ionotropic glutamate receptors. However, recent years have witnessed significant

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developments in the understanding of KARs physiology, largely based on data gathered from the hippocampus (Huettnner, 2003; Lerma, 2003). These studies have highlighted the unique properties of KARs as pre-synaptic auto- or hetero-receptors that mediate their effects through ion channel or G protein-coupled mechanisms (Rodríguez-Moreno and Lerma, 1998; Rodríguez-Moreno et al., 2000; Cunha et al., 2000; Jiang et al., 2001; Kang et al., 2004; Jin et al., 2006).

Despite the widespread distribution of KARs subunits through the basal ganglia, very little is known about the physiology and mechanisms of action through which these receptors regulate synaptic transmission in these nuclei. KARs are expressed in striatal glutamatergic, but not GABAergic, axons terminals in the monkey striatum (Charara et al., 1996; Kieval et al., 2001). KAR activation regulates inhibitory post-synaptic currents (IPSCs) evoked locally in the rat striatum (Chergui et al., 2000) and substantia nigra pars compacta (SNc) (Nakamura et al., 2003). Recent findings from our laboratory showed that KARs also play a significant role in regulating glutamatergic transmission in the rat GP (Jin et al., 2006). Although it has long been considered as a mere relay center, it is now well established that the globus pallidus (GP) (external globus pallidus, GPe, in primate) plays a **central** integrative role in the basal ganglia circuitry (Plenz and Kitai, 1999; Bevan et al., 2002). It receives massive GABAergic inputs from the striatum (Str) and a significant glutamatergic innervation from the subthalamic nucleus (STN). In turn, it sends GABAergic projections back to the STN and other basal ganglia nuclei. In contrast to most neurons in the CNS, more than 80% of synaptic inputs to GP neurons originate from GABAergic striatal projections (Shink and Smith, 1995; Smith et al., 1998). The loss of striatal dopamine in Parkinson's disease (PD) has opposite effects on the so-called "direct and indirect" striatofugal systems of the basal ganglia circuitry (DeLong, 1990). Increased GABAergic activity from the striatum and abnormal bursting pattern of the pallido-subthalamopallidal loop are cardinal features of Parkinson's disease pathophysiology (DeLong, 1990; Wichmann and DeLong, 1998). The introduction of novel drug therapy that may help regulate the abnormally increased striatal GABAergic influences towards GP neurons, therefore, represents a potential avenue for future pharmacotherapeutic development in Parkinson's disease. Recent findings from our laboratory have demonstrated GluR6/7 immunoreactivity in putative striatal GABAergic terminals in the monkey and rat GP (Kane-Jackson and Smith, 2003; Jin et al., 2006). Activation of these presynaptic KARs may, therefore, modulate striatopallidal GABAergic synaptic transmission. To further address this important issue, we employed whole cell patch-clamp recording techniques to examine the presynaptic function of KAR on striato-pallidal synapses in the rat GP.

Findings of this study have been presented on abstract form (Jin and Smith 2005, 2006).

EXPERIMENTAL PROCEDURES

Slice preparation

All electrophysiological experiments were performed on slices from 13 to 17-d old Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). After decapitation, brains were removed and quickly submerged in the ice-cold oxygenated sucrose buffer containing (in mM): 233.4 sucrose, 20 glucose, 47.3 NaHCO₃, 3 KCl, 1.9 MgSO₄, 1.2 KH₂PO₄, 2 CaCl₂ (Poisik et al. 2003; Jin et al. 2006). Parasagittal slices (300µm in thickness) were made on a Vibratome 3000 (The Vibratome Company, St. Louis, MO) in ice-cold oxygenated sucrose buffer. This plane of section was chosen based on previous anatomical data showing that striatal axons to the globus pallidus travel in the parasagittal plane from their origin to their pallidal targets in both rats and monkeys (Kawaguchi et al., 1990; Parent et al., 1995). Slices were stored at room temperature in a chamber containing artificial cerebrospinal fluid (ACSF) (in mM): 124 NaCl, 2.5 KCl, 1.3 MgSO₄, 1.0 NaH₂PO₄, 2.0 CaCl₂, 20 glucose, 26 NaHCO₃, at pH 7.3–7.4 with 95% O₂, 5% CO₂ bubbling through it. The osmolarity of the ACSF was ~ 310 mOsm.

Whole cell patch-clamp recordings

Whole cell patch-clamp recordings were performed as described previously (Poisik et al., 2003; Jin et al., 2006). During the recording, the slice was maintained fully submerged in the recording chamber and perfused with oxygenated ACSF (~3ml/min). GP neurons were visualized by IR-differential interference contrast microscopy (BX51WI) using a 40X water immersion objective (Olympus, Pittsburgh, PA). Whole cell patch electrodes were pulled from borosilicate glass on a vertical patch pipette puller (Narishige, Tokyo, Japan) to have resistance in the range of 3–5 M Ω when filled with an intracellular patch solution. All experiments were performed at room temperature. Tight-seal (>1G Ω) whole-cell recording was obtained from the cell body of GP neurons. Series resistance was regularly monitored during recording, and cells were rejected if the series resistance changed by > 20%.

For whole cell voltage-clamp experiments, patch pipettes were filled with the internal solution containing (in mM): 124 Cs-methanesulfonate, 11 KCl, 2 MgCl₂, 10 HEPES, 2 Na₂ATP, 0.3 GTP, 5 N-(2,6-Dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX314) and 0.5% biocytin (pH 7.4 (and 300–310 mOsm). Neurons were voltage-clamped at a holding potential of –10 mV and whole-cell membrane currents were recorded with a Patch-Clamp PC-501A (Warner Instruments, Hamden, CT). To isolate GABA_A receptor mediated-IPSCs, 100 μ M of the AMPA receptor antagonist, 4-(8-methyl-9H-1,3-dioxolo[4,5 h] {2,3} benzodiazepine-5-yl)-benzenamine hydrochloride (GYKI 52466) and 50 μ M of the NMDA receptor antagonist, D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5), were added to the ACSF. Three IPSCs were averaged and their peak amplitude was measured before, during and after KA application.

To record miniature IPSCs (mIPSCs), pipettes contained the following (in mM): 125 KCl, 10 NaCl, 1 CaCl₂, 2 MgCl₂, 10BAPTA, 10 HEPES, 2 Na₂-ATP, 0.3 GTP and 0.5% biocytin (pH 7.4 and 300–310 mOsm). The mIPSCs were recorded at a holding potential of –60 mV in the presence of 1 μ M tetrodotoxin (TTX), 100 μ M GYKI and 50 μ M D-AP5. Data were collected over continuous 10 – 20 min periods.

In some experiments, N-ethylmaleimide (NEM) (200 μ M), a pertussis toxin-sensitive G-proteins inhibitor, calphostin C (1 μ M), a selective protein kinase C (PKC) inhibitor, staurosporine (0.5 μ M), a broad spectrum inhibitor for protein kinases, or (H-89) (0.5 μ M), a selective protein kinase A inhibitor (PKA) were applied. Kainate, AMPA, D-AP5, CNQX, GYKI 52466, TTX and staurosporine were purchased from Tocris Cookson (Ellisville MO). Calphostin C and H-89 were purchased from Sigma (St Louis MO). All compounds were made in a 1000x and diluted into the ACSF immediately before use. Compounds were aliquoted and stored at –20°C. To assess their exact location, recorded GP neurons were filled with biocytin and their location within the GP was confirmed at the light microscopic level.

Electrical stimulation

Bipolar matrix stimulating electrodes (FHC, Bowdoinham, ME) were placed in the striatum close to the GP. Evoked inhibitory postsynaptic currents (IPSCs) in GP neurons were recorded by stimulation of striatum with single pulses that ranged from 3 to 10 V, 150–200 μ sec, delivered once every 20 sec. The paired-pulse facilitation (PPF) of evoked IPSCs was performed as follows: two stimuli of the striatum were paired with an interstimulus interval of 40–50ms. The ratio of peak 2/peak1 was calculated.

Biocytin histochemistry

Following overnight fixation in 10% neutral formalin, the slices were rinsed in PBS for 30 min and incubated in PBS containing 1% sodium borohydride for 20 min. They were then incubated in an ABC solution overnight at room temperature. After two 10 min washes in PBS and one

10 min wash in TRIS buffer (0.05 M, pH 7.6), the immunostaining was revealed by incubation for 10 min in a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.01 M imidazole (Fisher Scientific, Atlanta, GA), and 0.006% hydrogen peroxide (H₂O₂). The reaction was stopped by repeated washes in PBS. The biocytin-labeled neurons were then viewed with a Leica DMRB microscope, equipped with Leica digital Imaging system software.

Data analysis

Signals were filtered at 5 kHz and digitized with a Digidata 1200 analog-to-digital converter (Axon Instruments, Foster City, CA). Data were analyzed off-line using pClamp 6 (Axon Instruments). The mIPSCs were detected and analyzed using Mini Analysis software (Synaptosoft, Fort Lee, NJ). The cumulative probability distributions were compared by the Kolmogorov-Smirnov test. All group data were expressed as means $\pm$ SEM. Statistical significance was assessed by Student's *t*-test.

RESULTS

Activation of KAR depresses GABAergic synaptic transmission in GP

Previous studies have demonstrated that KARs modulate GABAergic transmission in the striatum and substantia nigra pars compacta (SNc) (Chergui et al., 2000; Nakamura et al., 2003). Our recent findings have shown GluR6/7 immunoreactivity in putative striatal GABAergic terminals in the monkey and rat GP (Jin et al., 2006; Kane-Jackson and Smith, 2003). We, therefore, examined whether bath application of KA modulates GABAergic transmission in slices of rat GP. GABA_A receptor-mediated inhibitory post synaptic currents (IPSCs) were evoked every 20 seconds in GP neurons at holding potential of -10 mV in the presence of 50 μ M D-AP5 and 100 μ M GYKI 52466. Bath application of KA (0.3 – 3 μ M) reversibly decreased IPSC amplitude in all cells tested (Fig. 1, A and B). On average, the IPSC amplitude was $76.5 \pm 6.4\%$ ($n = 8$, $p < 0.001$), $45.8 \pm 4.76\%$ ($n = 13$, $p < 0.001$) and 29.1 ± 3 ($n = 7$, $p < 0.001$) of control when perfused with 0.3 , 1 and 3 μ M KA, respectively (Fig. 1B). We did not detect any effect of KA on IPSC amplitude with KA concentrations below 0.3 μ M (Fig. 1B). To confirm that the KA-induced IPSC inhibition was due to KAR activation, we tested the effect of 1 μ M KA on IPSC amplitude in the presence of 50 μ M CNQX, an AMPA/KA receptor antagonist. As demonstrated in figure 1B, we found no significant effect of KA on IPSC amplitude under this condition ($91.2 \pm 4.6\%$ of control, $n = 5$, $p > 0.05$). The evoked inhibitory synaptic currents by striatal stimulation were GABA_A receptor-mediated because they could be completely and reversibly blocked by bicuculline (20 μ M) ($n = 9$) (Fig. 1, C and D), a selective GABA_A receptor antagonist. All biocytin-filled recorded neurons were confined to the GP and displayed morphological features consistent with those described in our previous study (Jin et al., 2006).

KAR-mediated depression of GABAergic transmission in GP involves presynaptic mechanisms

Although 1 μ M KA GluR6/7 induces small postsynaptic currents in GP neurons, these effects are small and almost assuredly do not account for a majority of the effect on glutamatergic transmission in rat GP (Jin et al. 2006). Therefore, one would predict that the effects KA (0.3 – 1 μ M) on IPSCs evoked from the striatum are likely mediated by activation of presynaptic KARs. To test this hypothesis, we examined the effect of KA on PPF of evoked IPSCs. To record paired IPSCs, two stimuli of the striatum close to the GP were paired with an interstimulus interval of 40 – 50 ms. We then calculated ratio of *peak2/peak1* in the presence or absence of KA (1 μ M). The ratio of *peak2/peak1* was significantly increased in the presence of KA compared to control (1.91 ± 0.1 and 1.25 ± 0.15 , respectively. $n = 6$, $p < 0.01$) (Fig. 2A, B and C), indicating a presynaptic effect.

To provide further evidence, we recorded mIPSCs from GP neurons using a high-Cl internal solution at holding potential -60 mV in the presence of 1 μ M TTX, 100 μ M GYKI 52466 and 50 μ M D-AP5. Figure 3A shows that 1 μ M KA application induced a significant reduction of the frequency of mIPSC. On average, the IPSC frequency was $79.4 \pm 4\%$ ($n = 8$, $p < 0.005$) and $63 \pm 5.2\%$ ($n = 7$, $p < 0.005$) of controls when perfused with 0.3 or 1 μ M KA respectively (Fig. 3E). This inhibitory effect of KA on mIPSCs frequency was blocked by 50 μ M CNQX ($101 \pm 7.4\%$, $p > 0.5$, $n = 5$) (Fig. 3E). The inter-mIPSC intervals were significantly increased following 1 μ M KA application ($P < 0.01$, Kolmogorov-Smirnov test, Fig. 3C). In contrast, KA (0.3 and 1 μ M) had no significant effect on their mean amplitude ($96 \pm 5\%$, $p > 0.5$, $n = 8$; $99.8 \pm 11.75\%$, $p > 0.05$, $n = 7$, Fig. 3F) or the amplitude distribution (Fig. 3D) of mIPSCs. We confirmed that mIPSCs were GABA_A receptor-mediated events since they were completely blocked by 20 μ M bicuculline (Fig. 3B, E and F). Taken together, these data strongly support the hypothesis that KA-induced inhibition of GABAergic transmission at striatopallidal synapses is mediated by presynaptic mechanisms.

KA- induced modulation of GABAergic transmission in GP requires activation of NEM toxin-sensitive G-protein

Data obtained so far indicate that KARs mediate their effects through metabotropic and/or ionotropic mechanisms (Rodriguez-Moreno and Lerma, 1998). We have recently shown that KAR-mediated inhibitory effect on glutamatergic transmission in the rat GP involves a G-protein-dependent signal transduction pathway (Jin et al., 2006). Therefore, we tested whether the KA-induced depression of GABAergic transmission was also due to a G-protein-coupled transduction cascade. First, we studied the effect of the G-protein inhibitor NEM (200 μ M) on the KA induced presynaptic inhibition of evoked IPSCs. As reported in several brain regions and spinal cord, bath application of NEM alone increased synaptic transmission (Frerking et al., 2001; Kubota et al., 2003; Rozas et al., 2003; Jin et al., 2006). The amplitude of IPSC was $132 \pm 5.3\%$ of control in the presence of NEM ($n = 7$, $P < 0.001$) (Fig. 4A and B). After 15 min of NEM perfusion, application of KA had no effect on IPSC amplitude. The IPSC amplitude in the presence of KA together with NEM was $122.6 \pm 4\%$ of control, which was not significantly different from the amplitude of IPSC recorded with NEM alone ($132 \pm 5.3\%$, $P > 0.5$, $n = 7$) (Fig. 4B).

Since several studies have reported that KA-induced inhibition of GABAergic transmission may involve G-protein-coupled GABA_B and adenosine receptors (Frerking et al., 1999; Chergui et al., 2000; Nakamura et al., 2003), we performed experiments to test whether the KA-induced inhibition of evoked IPSCs in the GP was due to the secondary activation of G-protein-coupled receptors. Slices were pretreated with a metabotropic glutamate receptor antagonist cocktail (1 mM MCPG and 100 μ M CPPG), a GABA_B receptor antagonist (20 μ M SCH 50911), a dopamine receptor antagonist cocktail (10 μ M sulpiride and 10 μ M SCH 23390) or a non-selective adenosine receptor antagonist (10 μ M CGS 15943) for 10 min prior to bath application of KA. Pretreatment with any of these antagonists had no significant effect on KA-induced inhibition of evoked IPSCs (Fig. 4C).

To test whether activation of G-protein was also required for KA-induced regulation of spontaneous GABA release, we examined the effect of NEM (200 μ M) on KA-induced inhibition of mIPSCs. As expected, NEM increased the mIPSC frequency (Fig. 5A, D). In the cumulative distributions of the inter-mIPSC intervals, 200 μ M significantly shift the curve towards shorter inter-event intervals ($P < 0.01$, Kolmogorov-Smirnov test, Fig. 5B). On average, the frequency of mIPSCs was $279 \pm 44\%$ ($P < 0.05$, $n = 6$) of control after NEM application (Fig. 5D). However, NEM did not affect either the amplitude distribution (Fig. 5C) or mean amplitude ($111 \pm 7\%$, $P > 0.5$, $n = 6$, Fig. 5D) of mIPSCs. After 15 min of NEM perfusion, KA had no significant effect on mIPSC frequency and amplitude. The mIPSC

frequency and amplitude in the presence of KA together with NEM was $269 \pm 40\%$ and $102 \pm 7.1\%$ of control respectively, which was not significantly different from the frequency and amplitude of mIPSC recorded with NEM alone ($279 \pm 44\%$, $P > 0.5$, $111 \pm 7\%$, $P > 0.5$, $n = 6$) (Fig. 5D and E). Taken together, these results suggest that the inhibitory effect of KA on evoked IPSC and mIPSC is modulated through NEM-sensitive G protein.

KA- induced inhibition of GABAergic transmission requires activation of PKC

To examine whether protein kinase activation downstream of the G protein activity is needed for the effect of KA on GABAergic transmission in the rat GP, we performed the following experiments. First, we tested the effect of KA on evoked IPSC amplitude after pretreatment of slices with staurosporine ($0.5 \mu\text{M}$, 2–4 h), a broad-spectrum inhibitor of protein kinase. As shown in figure 6B, staurosporine significantly prevented the inhibitory effect of KA on evoked IPSC amplitude ($100.3 \pm 3\%$ of control, $n = 6$, $P > 0.5$). We then performed further experiments to address which protein kinase might be responsible for the effect of staurosporine on KA-induced inhibition on IPSC amplitude. Pretreatment of slices with H-89 ($0.5 \mu\text{M}$, 2–4 h), a selective PKA inhibitor, did not prevent the effect of KA on IPSC amplitude ($49.2 \pm 2.7\%$ of control, $n = 5$, $P < 0.005$) (Fig. 6B). However, the effect of KA on evoked IPSC amplitude was significantly blocked ($98 \pm 3.1\%$ of control, $n = 6$, $P > 0.05$) (Fig. 6A and B) after slices were incubated with calphostin C ($1 \mu\text{M}$, 2–4 h), a specific PKC inhibitor. We also found that pretreatment of slices with staurosporine or calphostin C but not H-89 prevented the effect of KA on mIPSC frequency (Fig. 7A–D). The mean mIPSC frequency was $95 \pm 5\%$, $91.4 \pm 4.7\%$ and $62.4 \pm 6.5\%$ of control in the presence of staurosporine, calphostin and H-89 respectively ($n = 6$, $P > 0.05$, $n = 7$, $P > 0.05$ and $n = 7$, $P < 0.05$ respectively, Fig. 7E). These results demonstrate that activation of PKC, but not PKA, is involved in KA-induced inhibition of GABAergic transmission in rat GP.

DISCUSSION

The findings presented in this study demonstrate that activation of presynaptic KARs suppresses GABAergic transmission in the GP through a G-protein-dependent mechanism that involves PKC. Together with our recent data showing that KA also regulates glutamatergic transmission (Jin et al., 2006), these findings indicate that KARs are located to subserve subtle, but widespread, regulatory influences towards excitatory and inhibitory synaptic transmission in the GP.

Presynaptic KAR modulation of GABAergic transmission

It has been reported that inhibition of GABAergic synaptic transmission by KA is mediated, at least in part, by activation of presynaptic KARs in the hippocampus (Clarke et al., 1997; Rodriguez-Moreno et al., 1997; Rodriguez-Moreno et al., 1998; Maingret et al., 2005) and SNc (Nakamura et al., 2003). Similarly, our results show that KA ($0.3\text{--}1 \mu\text{M}$)-induced inhibition of GABAergic transmission is associated with a significant increase in PPF ratio of evoked IPSC and decrease in the frequency, but not amplitude of mIPSCs in the rat GP. These results are consistent with our previous electron microscopic immunocytochemical data showing that GluR6/7 immunoreactivity is expressed in GABAergic axon terminals in rat GP (Jin et al., 2006). This effect is unlikely due to changes in the membrane properties of GP neuron since $1 \mu\text{M}$ KA does not induce significant whole-cell membrane current or depolarization (Jin et al., 2006). However, $1 \mu\text{M}$ KA could induce dendritic conductance changes that are unlikely to be monitored by somatic recordings. Thus, we cannot rule out that such dendritic effects may contribute to some of the KA-induced inhibition of evoked and miniature IPSCs.

Since some of the GP neurons project back to the striatum and have local axon collaterals in the GP (Kita and Kitai, 1991; Bevan et al., 1998; Smith et al., 1998; Kita et al., 1999; Kita and

Kita, 2001; Sadek et al., 2007), electrical stimulation of the striatum could activate both striatopallidal and pallidostriatal fibers. Because it is technically not possible to clearly discriminate striatopallidal from pallidostriatal IPSCs in our preparation, we cannot rule out that both sets of afferents could potentially contribute to the evoked IPSCs recorded in the present study. However, our electron microscopic immunocytochemical data showing that GluR6/7 immunoreactivity is expressed in GABAergic terminals morphologically similar to striatal, but not pallidal boutons (Smith et al., 1998; Jin et al., 2006), strongly suggest that striatopallidal axon terminals are likely to be the main targets of KAR-mediated effect on IPSCs in the GP.

KAR activation can modulate GABA release in a bidirectional manner in the hippocampus (Jiang et al., 2001) and basolateral amygdala (Braga et al., 2003). For instance, bath application of a low dose of KA (0.3 μ M) and ATPA (0.3 μ M) facilitates GABA release, whereas a high dose of KA (5 μ M) or ATPA (10 μ M) inhibits GABAergic transmission (Jiang et al., 2001; Braga et al., 2003). In the present study, the amplitude of evoked IPSCs or the frequency of miniature IPSCs were constantly reduced by bath application of different concentrations (0.3–1 μ M) of KA. We did not find any effect of KA on either evoked or mIPSC when concentrations lower than 0.3 μ M KA were applied, indicating that presynaptic KAR activation does not modulate GABA release in a bidirectional manner in the rat GP. It is not clear why we could not find the facilitatory effects of low KA concentration (<300nM) on GABAergic transmission in the GP. One possibility is that the GluR5 subunit is not part of KARs in GP, while it is a constitutive subunit of KARs in the amygdala and hippocampus (Bischoff et al., 1997; Braga et al., 2003). It is also possible that activation of axonal KARs mediates this facilitatory effect in the hippocampus and amygdala, while in the GP the KARs-induced regulation of GABAergic transmission may be largely mediated by pre-synaptic KARs in GABAergic terminals.

Taking into consideration that axo-axonic synapse are rare in the GP, extrasynaptic glutamate spillover and/or glial release of glutamate are the two most likely sources of transmitter to activate these presynaptic heteroreceptors. The GP receives its main glutamatergic innervation from STN though modest inputs have also been described from the thalamus and brainstem (Kincaid et al., 1991; Naito and Kita, 1994; Shink and Smith, 1995; Mouroux et al., 1997). In contrast to many brain regions, glutamate transporter 1 (GLT-1)-immunoreactive astrocytic processes do not reach the synaptic cleft of glutamatergic synapse in the GP (Charara et al., 2002). They rather tightly ensheat axo-dendritic complexes (Charara et al., 2002), which suggests that synaptically released glutamate could spill over from glutamatergic synapse to activate KARs in neighboring GABAergic terminals. The close spatial arrangement of glutamatergic and GABAergic terminals onto individual GP dendrites favors this possibility (Shink and Smith, 1995; Smith et al., 1998). A similar mechanism has been described for mGluR-mediated inhibition of GABAergic transmission in other brain regions (Mitchell and Silver, 2000; Semyanov and Kullmann, 2000; Valenti et al., 2003). In addition to synaptic glutamate spillover, non-synaptic glial release of glutamate should also be considered as a potential source of activating transmitter for these receptors (Bezzi et al., 2004; Volterra and Steinhauser, 2004; Volterra and Meldolesi, 2005; Jourdain et al., 2007). Future studies are needed to determine the physiological or pathological conditions necessary for synaptically released glutamate to activate presynaptic KARs on GABAergic terminals in the GP.

Mechanism(s) underlying KAR-mediated presynaptic regulation of GABAergic transmission

Several hypotheses have been proposed to explain the possible mechanism (s) underlying the presynaptic effects of KARs on GABAergic synaptic transmission (for reviews, see Huettner, 2003; Lerma, 2003). For example, a direct depolarization of GABAergic axon terminals through Ca^{2+} permeable GluR5-containing KARs (Braga et al., 2003) or activation of axonal

KARs to bring the axon close to firing threshold, could account for KA-mediated increase in GABA release (Semyanov and Kullmann, 2001). In the present study we show that presynaptic activation of KARs reduces the amplitude of evoked IPSCs and decreases the frequency of mIPSCs. As mentioned above, differences in subunit composition may account for this differential degree of kainate sensitivity between GP and hippocampal KARs (see above).

Another alternative is that KA activates somatodendritic KARs on local neurons to cause the release of neurotransmitters, such as adenosine or GABA, which in turn, could activate GABA_B and A_{2A} receptors heterosynaptically on neighboring boutons to modulate the release of GABA (Frerking et al., 1999; Chergui et al., 2000; Nakamura et al., 2003). This is unlikely to be the case in GP, since blockade of GABA_B, mGluRs, adenosine and dopamine receptors did not have any significant effects on KAR-mediated inhibition of GABAergic transmission, which is consistent with data from other brain regions (Rodríguez-Moreno and Lerma, 1998; Rodríguez-Moreno et al., 2000; Braga et al., 2003; Maingret et al., 2005).

There is a growing body of evidence suggesting that presynaptic KAR activation could modulate GABAergic and glutamatergic transmission via a direct, presynaptic metabotropic action (Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000; Rodríguez-Moreno et al., 2000; Frerking et al., 2001; Lauri et al., 2005; Jin et al., 2006). In these studies, the presynaptic inhibitory effects of KARs were abolished by G-protein inhibitors (NEM) or pertussis toxin (PTx) (Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000; Frerking et al., 2001; Jin et al., 2006), but not by KA-mediated depolarization (Rodríguez-Moreno and Lerma, 1998; Rodríguez-Moreno et al., 2000). Furthermore, recent studies indicated that PKC activation downstream of G-protein activity is essential for KAR-mediated pre and post-synaptic effects. For instance, KA-induced presynaptic inhibition of GABAergic or glutamatergic transmission and KA-mediated postsynaptic inhibition of slow afterhyperpolarization currents (I_{sAHP}) were blocked by PKC inhibitor (Calphostin C) but not by PKA inhibitor (H-89) (Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000; Rodríguez-Moreno et al., 2000; Frerking et al., 2001; Melyan et al., 2002, 2004; Lauri et al., 2005; Jin et al., 2006). In line with these findings, we showed that presynaptic KAR-mediated effects on evoked and mIPSCs were blocked by NEM and Calphostin but not by H-89 in the rat GP. These results therefore, demonstrate that KAR-induced depression of GABAergic synaptic transmission in the rat GP requires G-protein and PKC activation but does not rely on the secondary activation of G protein-coupled receptors. Thus, together with our recent study showing KAR-mediated regulation of glutamatergic transmission (Jin et al., 2006), these findings demonstrate that KARs mediate their presynaptic effects on both GABAergic and glutamatergic transmission in the GP through a metabotropic mode of action.

Functional implications

The KAR-induced presynaptic inhibition of evoked and mIPSCs suggests KARs can function as heteroreceptors to modulate GABAergic transmission at the striatopallidal synapse. Knowing that increased GABAergic transmission from striatum to GP is one of the cardinal features of Parkinson's disease (PD) pathophysiology (DeLong, 1990), KAR activation may have beneficial effects in PD through attenuation of the overactive "indirect" GABAergic outflow from the striatum. It is noteworthy that other ionotropic or metabotropic glutamate receptor antagonists have proven to be successful in alleviating parkinsonian symptoms in both rat and primate models of PD (Brotchie et al., 1991; Klockgether et al. 1991; Greenamyre et al., 1994; Conn et al., 2005). However, the use of AMPA/NMDA receptor antagonists in humans is severely hampered by the widespread distribution, fast excitatory effects and need of these receptors for normal brain functioning. In contrast, KARs mediate slower modulatory effects that resemble those induced by mGluRs activation. It is noteworthy that blockade of mGluR5 receptors or activation of group III mGluRs in the GP alleviates akinesia by

normalizing activity of selective basal ganglia structures in parkinsonian rats (Breyse et al., 2003; Marino et al., 2003; Valenti et al., 2003; Lopez et al., 2007). Through the possible regulation of overactive striatopallidal GABAergic synapses and glutamatergic subthalamopallidal transmission (Jin et al., 2006), KARs represent an additional target of interest for the development of novel pharmacotherapeutic approaches in Parkinson's disease.

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Abbreviations

ABC	avidin-biotin-peroxidase complex
ACSF	artificial cerebrospinal fluid
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid
ATPA	(<i>RS</i>)-2-amino-3-(3-hydroxy-5- <i>tert</i> -butylisoxazol-4-yl)propanoic acid
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
CPPG	(<i>RS</i>)- α -cyclopropyl-4-phosphonophenylglycine
DAB	3,3-diaminobenzidine tetrahydrochloride
D-AP5	D-(-)-2-amino-5-phosphonopentanoic acid
IPSCs	inhibitory post synaptic currents
GLT-1	glutamate transporter 1
GP	globus pallidus
GYKI 52466	4-(8-Methyl-9H-1,3-dioxolo[4,5 h]{2,3}benzodiazepine-5-yl)-benzenamine hydrochloride
KARs	kainate receptors
MCPG	α -Methyl-4-carboxyphenylglycine
NEM	

	N-ethylmaleimide
NMDA	N-methyl-D-aspartate
PD	Parkinson's disease
PBS	phosphate-buffered saline
PKA	protein kinase A
PKC	protein kinase C
PPF	paired-pulse facilitation
QX314	N-(2,6-Dimethylphenylcarbamoylmethyl) triethylammonium bromide
SNe	substantia nigra parts
STN	subthalamic nucleus
TTX	tetrodotoxin

Fig. 1.

Kainate receptor (KAR) activation depressed GABAergic synaptic transmission in the GP. **A:** time course of the effect of 1 μ M KA on GABA_A-mediated inhibitory postsynaptic current (IPSC) amplitude (pA) in the presence of 100 μ M GYKI 52466 and 50 μ M D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5). Three IPSCs are averaged in each trace at the time indicated by the corresponding letters in the graph. **B:** a summary bar graph showing that KA (0.3–3 μ M) significantly reduced the IPSC amplitude and this effect was blocked when 1 μ M KA was applied together with 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) (50 μ M). **C:** time course of the effect of bicuculline (20 μ M) on IPSC amplitude in the presence of D-AP5 and CNQX. Three IPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. **D:** a summary bar graph shows that 20 μ M bicuculline completely blocked IPSC amplitude recorded from nine neurons. There was a significant difference from control: * P < 0.001. In this and subsequent figures, “NS” indicates non-significant differences and ‘n’ indicates the number of cells tested under each condition. All averaged data except figure 2, are presented as percent of control $\pm$ SEM.

Fig. 2.

Kainate receptor (KAR) activation increased paired-pulse facilitation (PPF) at GABAergic synapse in the GP. *A*: paired IPSCs were recorded before (left trace) and during (middle) 1 μ M KA application. The right trace shows the KA-induced effect after scaling to the peak of the first IPSC. *B*: the same neuron presented in (*A*) shows the time course of increased paired-pulse facilitation ratio (PPFR) of IPSCs in response to 1 μ M KA application. *C*: a bar graph shows that the PPFR expressed as a mean ratio of $P2/P1 \pm \text{SEM}$ was significantly increased in the presence of 1 μ M KA (* $P < 0.01$).

Fig. 3.

Kainate receptor (KAR) activation reduced the frequency but not the amplitude of miniature IPSCs (mIPSCs). *A*: Sample traces show mIPSCs before, during and after 1 μ M KA application. These mIPSCs were recorded in the presence of 100 μ M GYKI 52466, 50 μ M D-AP5 and 1 μ M TTX. *B*: Sample traces show mIPSCs before, during and after 20 μ M bicuculline application. After three mins application of bicuculline, mIPSCs were completely abolished (bottom trace). *C* and *D*: The cumulative distributions of the inter-mIPSC intervals and cumulative amplitude distributions of mIPSCs obtained from the same neuron as in panel *A*. KA (1 μ M) significantly shifted the inter event interval distribution curve to the right (left, $P < 0.05$), but had no significant effect on the distribution of mIPSCs amplitude (right, $P > 0.5$). *E*: A summary bar graph shows KA (0.3–1 μ M) significantly reduced the frequency of mIPSCs, which was blocked in the presence of 50 μ M CNQX. *F*: A summary bar graph shows that neither KA nor KA together with CNQX affected the amplitude of mIPSCs. Both the frequency and amplitude of mIPSCs were significantly blocked by 20 μ M bicuculline (* $P < 0.005$).

Fig. 4.

Application of G-protein inhibitor, N-ethylmaleimide (NEM) but not G protein coupled receptors antagonist blocked the KAR activation-induced inhibition on IPSCs. **A:** The time course of the effect of 1 μ M on IPSC amplitude in the presence of 200 μ M NEM. Three IPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. **B:** A summary bar graph shows that the inhibitory effect of KA on IPSC amplitude was blocked in the presence of NEM. **C:** Bar graph shows that 1 μ M KA significantly reduced the IPSCs amplitude in the presence of mGluR antagonist (1 mM MCPG and 100 μ M CPPG), the GABAB antagonist (20 μ M SCH 50911), the adenosine receptor antagonist (10 μ M CGS 15943), or dopamine receptor antagonists (10 μ M SCH 23390 and 10 μ M sulpiride).

Fig. 5.

Application of G-protein inhibitor (NEM) blocked the KAR activation-induced inhibition on mIPSCs frequency. **A**: Sample traces show mIPSCs in control condition (left trace), in the presence of 200 μ M NEM (middle trace) or after the application of NEM together with 1 μ M KA (right trace). **B** and **C**: The cumulative distributions of the inter-mIPSC intervals and cumulative amplitude distributions of mIPSCs obtained from the same neuron as in **A**. KA (1 μ M) did not show any significant effect on either the inter event interval (**B**) or the amplitude distribution (**C**) curves of mIPSCs in the presence of NEM ($P > 0.5$). **D** and **E**: A summary bar graph shows that KA had no effect on either mIPSCs frequency or amplitude in the presence of NEM.

Fig. 6.

Pretreatment with protein kinase C inhibitor (calphostin C) but not protein kinase A inhibitor (H-89) prevents KAR activation-induced inhibition on IPSCs. **A:** The time course of the effect 1 μ M KA on IPSC amplitude in the presence of 1 μ M calphostin C. Three IPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. **B:** A bar graph shows that the KAR activation-induced inhibition on IPSCs was blocked by 0.5 μ M staurosporine, a broad-spectrum inhibitor for protein kinase and calphostin C but not H-89. For comparison, the effect of 1 μ M KA on IPSCs in control conditions from Fig. 1B is shown. There was a significant difference from control, * $P < 0.01$.

Fig. 7.

Pretreatment with protein kinase C inhibitor (calphostin C) but not protein kinase A inhibitor (H-89) prevents KAR activation-induced inhibition of mIPSCs. **A:** mIPSCs were recorded in the presence of 1 μ M calphostin C and calphostin C together with 1 μ M KA. **B:** mIPSCs were recorded in the presence of 0.5 μ M H-89 and H-89 together with 1 μ M KA. **C and D:** the cumulative distributions of the inter-mIPSC intervals and amplitude distributions of mIPSCs obtained from the same neuron as in A. KA had no effect on inter-event interval (left) and mIPSCs amplitude (right) distribution curves in the presence of calphostin C ($P > 0.5$). **E:** summary bar graph shows that KA had no effect on mIPSCs frequency in the presence of 0.5 μ M staurosporine and calphostin C but not in the presence of H-89. **F:** summary bar graph shows that KA had no effect on mIPSCs amplitude in the presence of staurosporine, calphostin C and H-89. * $P < 0.05$.