Kappa Opioid Receptor Activation in the Nucleus Accumbens Inhibits Glutamate and GABA Release Through Different Mechanisms

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Hjelmstad, Gregory O. and Howard L. Fields. Kappa opioid receptor activation in the nucleus accumbens inhibits glutamate and GABA release through different mechanisms. J Neurophysiol 89: 2389–2395, 2003; 10.1152/jn.01115.2002. Through their actions in the nucleus accumbens (NAc), kappa opioid (KOP) receptors and their endogenous ligand, dynorphin, modify behaviors associated with the administration of drugs of abuse and are regulated by exposure to such drugs. Despite their demonstrated behavioral significance, the synaptic actions of KOP receptor ligands in the NAc are not clearly understood. Using whole-cell voltage-clamp recordings of NAc medium spiny neurons, we have found that, in addition to suppressing glutamate release, the KOP receptor agonist U69593 also inhibits GABA release. Interestingly, the mechanism of inhibition of the release of glutamate differs from that controlling GABA. U69593 reduces the frequency of Ca2+-independent miniature excitatory postsynaptic currents, but not miniature inhibitory postsynaptic currents. Furthermore, while the U69593 inhibition of GABAergic transmission is blocked by the N-type Ca2+ channel blocker ω-CgTx, the inhibition of excitatory glutamatergic transmission by U69593 is unaffected by N-type Ca2+ channel blockade. These results indicate that KOP receptor activation inhibits GABA release by reducing Ca2+ influx, but inhibits glutamate release at a step downstream of Ca2+ entry.

INTRODUCTION

The nucleus accumbens (NAc) is critically involved in a variety of goal-directed behaviors, including several that are strongly reinforced by drugs of abuse (Evertt and Wolf 2002). The major neuronal class in the NAc is the GABAergic medium spiny neuron, which receives excitatory glutamatergic inputs from a number of brain regions, including the hippocampus, amygdala, and prefrontal cortex (Pennartz et al. 1993). These cells also receive several inhibitory inputs, primarily from a small population of interneurons (Kawaguchi et al. 1995) but also through feed-forward inhibition (Pennartz and Kitai 1991; Van Bockstaele and Pickel 1995) and presumably through recurrent axon collaterals of local medium spiny neurons (Chang and Kitai 1985).

Kappa opioid (KOP) receptor agonists can powerfully modify behaviors associated with drugs of abuse. Specifically, U69593, a KOP receptor agonist, reduces cocaine self-administration (Schenk et al. 1999) and inhibits sensitization to cocaine and amphetamine (Chefer et al. 1999; Gray et al. 1999; Heidbreder et al. 1993; Heidbreder and Shippenberg 1994). Microinjection of a KOP receptor agonist directly into the NAc shell produces conditioned place aversion (Bals-Kubik et al. 1993). Moreover, the KOP receptor system itself is clearly altered by exposure to drugs of abuse. KOP receptor levels in the NAc are also altered following acute or chronic exposure to psychostimulants (Turchan et al. 1998; Unterwald et al. 1994). In addition to receptor changes, preprodynorphin mRNA in the NAc is up-regulated following exposure to drugs of abuse (Kreek 1996; Steiner and Gerfen 1998). These changes in KOP receptor and dynorphin levels may be linked to the behavioral changes related to addiction (Carlezon et al. 1998). Thus the KOP system may present a potential avenue for the development of treatments for addiction.

It has been proposed that KOP receptors on the terminals of dopamine (DA) afferents are responsible for the behavioral effects of KOPs (Di Chiara and Imperato 1988; Spanagel et al. 1992). However, KOP receptors are also present on presynaptic terminals of presumed excitatory and inhibitory synapses in the NAc (Meshul and McGInty 2000; Svingos et al. 1999). Consistent with the former finding, we recently reported that KOP receptor activation presynaptically inhibits glutamatergic excitatory postsynaptic currents (EPSCs) in the NAc shell in vitro (Hjelmstad and Fields 2001).

Here, we report the effects of KOP receptor activation on inhibitory transmission in the NAc shell. We find that U69593 also produces an inhibition of GABAergic transmission. Furthermore, the mechanisms underlying the inhibition of excitatory and inhibitory release are not the same. Specifically, at inhibitory synapses, KOP receptor activation inhibits an N-type Ca2+ channel, while, at excitatory synapses, KOP receptor activation modulates transmitter release at some step downstream of Ca2+ entry.

METHODS

Two- to 4-week-old male Sprague–Dawley rats were anesthetized with isoflurane and decapitated, and the brain was removed and placed into ice-cold Ringer solution (approximately 3°C) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO4, 1.0 NaH2PO4, 2.5 CaCl2, 26.2 NaHCO3, and 11 glucose saturated with 95% O2-5% CO2. Coronal slices (350 μm thick) containing the NAc were cut using a vibratome (Leica Instruments, Germany). Slices were submerged in Ringer solution and allowed to recover for >1 h at room temperature.

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Individual slices were transferred to a poly-d-lysine–coated coverslip and visualized under an Olympus upright microscope with differential interference contrast (DIC) optics and infrared illumination. Extracellular field or whole cell patch-clamp recordings were made at room temperature. This improves slice viability; however, it should be noted that some second messenger systems are temperature sensitive. Field recordings were made by placing a 3- to 5-MΩ electrode filled with Ringer solution into the medial shell of the NAc, which can be visually distinguished from the neighboring core region in a coronal slice. Whole cell voltage-clamp recordings from medium spiny neurons were made using 2.5- to 4-MΩ pipettes containing (in mM) 120 Cs-glucuronate, 10 HEPEs, 0.2 EGTA, 8 NaCl, 2 MgATP, and 0.3 Na₂GTP (pH 7.2, osmolarity adjusted to 280). Cells were identified as medium spiny neurons by their appearance and by their relatively hyperpolarized resting potential (Uchimura et al. 1989). Excitatory postsynaptic field potentials (EPSPs) and inhibitory postsynaptic currents (IPSCs) were evoked (0.06–0.1 Hz) with a bipolar stimulating electrode placed along the dorsal edge of the NAc.

Recordings were made using an Axopatch 1-D (Axon Instruments) amplifier and were filtered at 2 kHz and collected at 5 kHz using Igor Pro (Wavemetrics, Lake Oswego, OR). Series resistance was monitored on-line by measuring the peak of the capacitance transient in response to a –4 mV voltage step applied prior to each stimulus. Amplitudes were calculated by comparing a 2-ms period at the peak of the response and a similar period just prior to the stimulus artifact. Miniature spontaneous activity, recorded in the presence of 1 μM TTX and 100 μM cadmium, was analyzed in 3- to 5-min epochs for each pharmacological condition. Events were detected automatically if the smoothed first derivative of the current exceeded a set threshold and were visually verified. Amplitudes were calculated by comparing a 1-ms period at the peak of the response to a 1-ms period immediately prior to the onset of the mini. Asynchronous mIPSCs (asIPSCs), recorded in 4 mM strontium were similarly detected for the period from 200 to 800 ms following stimulation. To account for the falling phase of the IPSC as well as the increased likelihood of temporally correlated asIPSCs (see Fig. 2), an exponential curve was fit to the baseline prior to the mini (20-ms period prior to onset of event or from the peak of previous event). The amplitude of the event was then calculated as the value of the extrapolated curve at that same time. In each experiment, the time constant of the baseline fit was constrained to the time constant of the falling phase of the averaged asIPSC.

All drugs were applied by bath perfusion. Stock solutions were made and diluted in Ringer solution immediately prior to application. U69593 was diluted in 50% EtOH to a concentration of 10 mM; nor-Binaltorphimine (10 mM), d-2-amino-5-phosphonovaleric acid (100 mM), ω-conotoxin-GVIA (ω-Ctx, 500 mM), and ω-agatoxin-IVA (ω-Aga, 250 nM) were diluted in H₂O and 6-cyano-7-nitroquinoline-2,3 dione (10 mM), 4-aminopyridine (4-AP, 100 mM), and picrotoxin (100 mM) were mixed in DMSO. Chemicals were obtained from Sigma Chemical (St. Louis, MO) or Tocris (Ballwin, MO). Unless otherwise noted, statistical analyses were performed using the Student’s t-test, and significance was defined at P < 0.05. Results are presented as means ± SE.

RESULTS

**Kappa opioids inhibit GABA release**

Whole cell voltage-clamp recordings were made from medium spiny neurons in the medial shell of the NAc. Neurons were held at 0 mV and electrically evoked monosynaptic IPSCs were pharmacologically isolated using d-APV (100 μM) and DNQX (10 μM). This IPSC was completely blocked by application of 100 μM picrotoxin (Fig. 1A), confirming that it is mediated by the GABAₐ receptor. Bath application of the KOP receptor agonist U69593 (1 μM) caused an inhibition of the IPSC (74.9 ± 7.3% of baseline, n = 11, P < 0.01; Fig. 1). This inhibition was blocked by preapplying 100 nM of the selective KOP receptor antagonist nor-BNI (104.25 ± 9.88%, n = 3), confirming that the inhibition is mediated by the KOP receptor.

KOP receptors are found at presynaptic GABA terminals, however, there is also some dendritic localization (Svingos et al. 1999). To determine whether KOP receptor activation acts presynaptically to reduce the release of GABA, we measured the coefficient of variation (CV), a measure that changes inversely with the probability of release (del Castillo and Katz 1954; Faber and Korn 1991; Manabe et al. 1993). Consistent with a presynaptic locus for the KOP receptor action, the CV increased following U69593 application (31.0 ± 15% increase; Fig. 1C). Although this overall change did not reach statistical significance (P = 0.06), the change in the CV was highly correlated with the degree of inhibition produced by U69593 for each experiment (r² = 0.75; P < 0.01, n = 11; Fig. 1D).

To further confirm that U69593 acts at the terminals of GABAergic neurons, we monitored the asynchronous release...
of GABA by replacing extracellular Ca$^{2+}$ with 4 mM strontium (Sr$^{2+}$). Sr$^{2+}$ can substitute for Ca$^{2+}$ in the exocytotic process but desynchronizes the neurotransmitter release (Dodge et al. 1969; Meiri and Rahamimoff 1971; Miledi 1966). This allows the individual quantal components, or asIPSCs, to be counted. Analyzing a 600-ms window following the evoked IPSC, we found that U69593 produced a significant reduction in the frequency of asIPSCs (73.4 vs. 2.31 Hz) with no change in the amplitude (94.7 vs. 3.2%; n.s.; Fig. 2). Moreover, this reduction in frequency was comparable in magnitude to the overall decrease in the mean amplitude of asIPSCs (67.3 ± 4.8%). Together these data indicate that U69593 actions are predominantly presynaptic, presumably by reducing the probability of GABA release, although these data could also be accounted for by a KOP receptor–mediated decrease in the number of release sites.

Mechanisms of kappa opioid inhibition

Typical opioid receptor actions include the activation of a potassium conductance or inhibition of a Ca$^{2+}$ conductance. While either of these mechanisms acting at the nerve terminal could inhibit neurotransmitter release, it is also possible that KOPs can influence transmitter release downstream of calcium entry, for example, by affecting the release machinery itself (Thompson et al. 1993). Since U69593 inhibits release of both glutamate and GABA in the NAc, we performed a series of experiments to determine which of these mechanisms are responsible for these effects.

Initially, we tested whether KOP-mediated inhibition occurs up- or downstream of calcium entry by monitoring miniature EPSCs (mEPSCs) in the presence of cadmium, a nonselective blocker of Ca$^{2+}$ channels. U69593 caused a significant reduction in the frequency of Cd$^{2+}$-resistant mEPSCs (56.9 ± 12.1% of baseline; P < 0.01, n = 5) but had only a small, nonsignificant effect on the amplitude (88.2 ± 5.5%; n.s.) (Fig. 3).

The same experiment was performed monitoring Cd$^{2+}$-resistant mIPSCs. Consistent with previous results (Hoffman and Lupica 2001), the baseline frequency of mIPSCs in medium spiny neurons is much lower than the frequency of mEPSCs. However, neither the frequency (106.3 ± 14.7% of baseline; n.s., n = 5) nor the amplitude (105.6 ± 3.5%; n.s.) of mIPSCs were altered by application of U69593 (Fig. 4). This indicates that KOP receptors modulate Ca$^{2+}$ entry at GABA terminals but act downstream of Ca$^{2+}$ entry at glutamate terminals.

Next, we tested the role of specific presynaptic voltage-dependent calcium channel subtypes by using the irreversible Ca$^{2+}$ channel inhibitors ω-Ctx, which blocks N-type channels, and ω-Aga, which is selective for P/Q-type channels. Because U69593 has a much more consistent effect on field recordings than on EPSCs, presumably because with field recordings we are sampling a large population of cells, we chose to look at the
The effect of these blockers on fEPSPs. N- or P/Q-type channels were blocked by a 10-min application of \( \omega \)-Ctx or \( \omega \)-Aga. We found that the inhibition of fEPSPs was somewhat greater for \( \omega \)-Ctx than for \( \omega \)-Aga (33.9 \pm 8.9 and 50.3 \pm 11.3\% of baseline, respectively; Fig. 5). After the effect of the Ca\(^{2+} \) channel blocker stabilized, U69593 was applied. Consistent with the results from the Cd\(^{2+} \) experiments, neither \( \omega \)-Ctx (500 nm) nor \( \omega \)-Aga (250 nm) inhibited the U69593 effect on glutamate release (77.9 \pm 2.3 and 73.6 \pm 9.0\% of baseline, respectively; \( n = 3 \) for each, Fig. 5).

We next examined the role of \( \omega \)-Ctx and \( \omega \)-Aga on IPSCs. Here, the two antagonists had similar effects on IPSCs (45.2 \pm 4.9 and 43.3 \pm 9.1\%). However, there was a differential effect on the KOP receptor–mediated inhibition of IPSCs. In the presence of \( \omega \)-Ctx, the inhibition of GABA release was eliminated (97.6 \pm 3.1\% of baseline; n.s., \( n = 4 \)), while the U69593 inhibition persisted in \( \omega \)-Aga (86.5 \pm 4.8\% of baseline; \( P < 0.05, n = 5 \); Fig. 6). This indicates that the inhibition of GABA release involves the modulation of N-type Ca\(^{2+} \) channels.

K\(^+ \) channels have been implicated in the presynaptic inhibition of transmitter release at a number of synapses (Robbe et al. 2001; Simmons and Chavkin 1996; Vaughan et al. 1997). Therefore we tested whether 4-AP (100 \( \mu \)M), which blocks the I\( _{\alpha} \) current, has any effect on the KOP receptor–mediated inhibition of neurotransmitter release. Because 4-AP dramatically enhances the probability of neurotransmitter release (Llinas et al. 1976), the Ca\(^{2+} \)/Mg\(^{2+} \) ratio was lowered (from 2.5/1.3 to 1.3/1.1).

**FIG. 4.** Cadmium-resistant miniature IPSCs (mIPSCs) are unaffected by U69593. A: 5-s portion of epoch recorded at baseline (top) and during application of 1 \( \mu \)M U69593. B and C: cumulative frequency plots of inter-event interval (B) and mIPSC amplitude (C) for the same cell shows no change in frequency (0.20 versus 0.197 Hz) or amplitude (7.99 vs. 7.72 pA) D: average of 5 experiments shows no change in frequency or amplitude during application of U69593.

**FIG. 5.** A: inhibition of field EPSPs (fEPSPs) by U69593 persists following blockade of N-type channels by \( \omega \)-conotoxin (\( n = 3 \)). B: inhibition of fEPSPs by U69593 persists despite blockade of P/Q-type channels by \( \omega \)-agatoxin (\( n = 3 \)). At the arrow, time base was expanded and data were renormalized to 100% for presentation purposes.

**FIG. 6.** A: inhibition of IPSCs by U69593 is blocked following application of \( \omega \)-conotoxin (\( n = 4 \)). B: inhibition of IPSCs by U69593 persists following application of \( \omega \)-agatoxin (\( n = 5 \)). There was no statistical difference between the inhibition recorded in \( \omega \)-agatoxin and under control conditions. At the arrow, time base was expanded and data were renormalized to 100% for presentation purposes.
In this study we have shown that, in addition to its effects at glutamate terminals, KOP receptor activation also inhibits GABA-mediated IPSCs in the shell region of the NAc. Similar to its actions at glutamate terminals (Hjelmstad and Fields 2001), KOP receptor agonists also act at GABA terminals, presumably by reducing the probability of release (pr), although our data do not rule out the possibility that U69593 is reducing the number of release sites (n). We found that the inhibition of GABA was highly variable, similar to our previous observations for EPSCs (Hjelmstad and Fields 2001) and similar to the delta opioid receptor–mediated inhibition of IPSCs in the NAc (Hoffman and Lupica 2001). Given that there are multiple subtypes of medium spiny neurons with distinct projection patterns and each receives distinct afferent populations (Heimer et al. 1997; Joel and Weiner 2000), it is plausible that KOP receptors regulate GABA release at a subset of these neurons.

This inhibition of GABA release appears to involve the modulation of N-type Ca2+ channels. Specifically, the N-type Ca2+ channel blocker, ω-Ctx, blocked the actions of U69593. Moreover, the frequency of Ca2+-independent mIPSCs recorded in the presence of Cd2+ was not affected by the KOP receptor agonist, while the frequency of asIPSCs, which are dependent on strontium entry through Ca2+ channels, was inhibited by U69593. Both N- and P-type Ca2+ channels can be regulated by G protein–coupled receptors; however, N-type channels tend to be more strongly modulated (Currie and Fox 1995), possibly explaining the effects observed in the NAc. Alternatively, KOP receptors may be restricted to a subset of inhibitory terminals that only, or predominantly, express N-type channels. Such segregation has been observed in the hippocampus (Ohno-Shosaku et al. 1994; Poncer et al. 1997). In the NAc, inhibitory inputs to the medium spiny neurons are derived from a number of sources, including a subpopulation of interneurons (Kawaguchi et al. 1995), extrinsic GABAergic inputs from hippocampus (Pennartz and Kitai 1991), as well as the ventral tegmental area (Van Bockstaele and Pickel 1995), and presumably from the axon collaterals of the medium spiny neurons themselves (Chang and Kitai 1985, but see Koos and Tepper 1999). Thus it would not be surprising to find a different proportion of N- and P/Q-type channels at these different terminals.

While the results for IPSCs are most consistent with a direct action onto N-type channels, it is also possible that an action upstream of the Ca2+ channel, such as modulation of a potassium conductance, might preferentially affect N-type–mediated release. This might occur through different voltage sensitivities of the two classes of channels or through the segregation of channels to separate terminals. 4-AP has no effect on the inhibition of IPSCs produced by U69593; however, it remains possible that the KOP effect on the IPSC occurs secondary to some other upstream effect, thus indirectly modulating Ca2+ entry specifically through N-type channels.

The KOP inhibition of glutamate release, on the other hand, occurs downstream of Ca2+ entry. While it is not clear where this interaction between the KOP receptor and release takes place, there are a number of proteins involved in the exocytotic pathway whose activity could be altered by a G protein–coupled receptor (Lin and Scheller 2000). KOP receptors reduce glutamate release at the hippocampal mossy fiber synapse by modulating K+ channel activity (Simmons and Chavkin 1996). Similarly, cannabinoids reduce glutamate release in the NAc by modulating K+ channel activity in a Ca2+-channel–independent manner (Robbe et al. 2001). This proposal does not explain the KOP receptor effect on glutamate release, since we see the inhibition in the presence of 4-AP.

On the basis of our results, we cannot rule out the possibility that, in addition to their action on neurotransmitter release, KOPs may also inhibit Ca2+ entry at glutamatergic synapses. In the cerebellar granule cell–Purkinje cell synapse, the GABAβ receptor agonist baclofen modulates both Ca2+-channels and also affects the downstream release process (Dittman and Regehr 1996). However, the fact that the reduction in Ca2+-resistant mEPSCs is as strong as the change in evoked EPSCs, as well as the lack of a differential effect using selective Ca2+ channel antagonists, argues against this possibility.

It is curious that the KOP receptor uses different mechanisms for reducing GABA and glutamate release at terminals in the NAc. Differential mechanisms for the modulation of presynaptic release have also been observed in other brain regions. For example, in the hippocampus, while GABAβ receptors inhibit both glutamate and GABA release, they reduce the frequency of Ca2+-resistant mEPSCs but not mIPSCs (Scanziani et al. 1992). Similarly, dopamine in the NAc also appears to utilize different mechanisms for inhibiting EPSCs and IPSCs. Specifically, the frequency of Ca2+-resistant mEPSCs is reduced by dopamine, whereas the frequency of mIPSCs is only reduced if the minis are made calcium dependent, by increasing extracellular potassium (Nicola and Malenka 1997). However, this does not appear to be a universal phenomenon. For example, in both the periaqueductal gray (PAG), where met-enkephalin inhibits both EPSCs and IPSCs, as well as in the basal forebrain, where EPSCs and IPSCs are inhibited by dopamine, the reduction in mIPSC frequency persists in Cd2+ (Momiyama and Sim 1996; Momiyama et al. 1996; Vaughan and Christie 1997; Vaughan et al. 1997).

What are the potential consequences of having different...
mechanisms for reducing neurotransmitter release at GABA and glutamate terminals? First, these different mechanisms may differentially alter the frequency-response function for neurotransmitter release. For example, N- and P/Q-type Ca²⁺ channels have different inactivation rates (Usowicz et al. 1992). Thus inhibiting N-type channels will increase the role of P/Q-type channels, which inactivate less, changing the profile of Ca²⁺ entry during bursts of action potentials. Second, the different mechanisms may differentially alter the corelease of peptides. While fast neurotransmitters are packaged in clear vesicles, peptides are located in dense core vesicles. The release processes for the two types of vesicles depend on Ca²⁺ entry but utilize different release machinery (Langley and Grant 1997). Thus the action of KOP receptor agonists at glutamate terminals may be specific to the fast release process of clear vesicles, leaving the release of dense core vesicles intact. Finally, the different mechanisms of inhibition raises the intriguing possibility that the KOP receptor inhibition of glutamate and GABA release could be modulated differentially in response to chronic exposure to drugs of abuse. In general, the prodynorphin/KOP receptor system is highly plastic. Dynorphin mRNA and protein in the NAc is up-regulated following exposure to drugs of abuse, such as cocaine (Kreek 1996; Steiner and Gerfen 1998). Furthermore, KOP receptor levels are altered following exposure to cocaine or amphetamine (Turchan et al. 1998; Unterwald et al. 1994). These changes may be critical to the behavioral responses to drugs of abuse. The differential regulation of KOP-mediated inhibition at glutamate versus GABA terminals may have profound impact on signal processing within the NAc.

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