Kappa Opioid Receptor Inhibition of Glutamatergic Transmission in the Nucleus Accumbens Shell

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Hjelmstad, Gregory O. and Howard L. Fields. Kappa opioid receptor inhibition of glutamatergic transmission in the nucleus accumbens shell. J Neurophysiol 85: 1153–1158, 2001. Microinjection of κ-opioid receptor agonists into the nucleus accumbens produces conditioned place aversion. While attention has focused primarily on the inhibition of dopamine release by κ-receptor agonists as the synaptic mechanism underlying this effect, recent anatomical studies have raised the possibility that regulation of noncatecholamnergic transmission also contribute. We have investigated the effects of κ-receptor activation on fast excitatory synaptic transmission in an in vitro slice preparation using whole cell voltage-clamp or extracellular field recordings in the shell region of the nucleus accumbens. The κ-receptor agonist U69593 produces a pronounced, dose-dependent inhibition of glutamatergic excitatory postsynaptic currents (EPSCs) that can be reversed by 100 nM nor-BNI. Furthermore, U69593 causes an increase in the paired-pulse ratio as well as a decrease in the frequency of spontaneous miniature events, suggesting a presynaptic site of action. Despite anatomical evidence for κ-receptor localization on dendritic spines of nucleus accumbens neurons, no electrophysiological evidence of a postsynaptic effect was found. This presynaptic inhibition of excitatory synaptic transmission in the nucleus accumbens shell provides a novel mechanism that may contribute to the κ-receptor–mediated aversion observed in intact animals.

INTRODUCTION

The endogenous opioid peptides, which act through three types of opioid receptors, μ, κ, and δ (MOP, KOP, and DOP), are found throughout the CNS and influence a wide variety of behaviors (Aki1 et al. 1997; Dhawan et al. 1996). While the direct cellular actions mediated by different opioid receptors are often similar, μ-receptor and κ-receptor ligands microinjected into the same CNS sites often have opposing behavioral effects due to the localization of the receptor on different cell populations (Pan 1998).

The shell region of the nucleus accumbens (NAc) has been implicated in both the appetitive and aversive effects of opioid agonists. The NAc is involved in morphine reward (Wise 1996), and microinjection of κ-receptor agonists into the NAc produces conditioned place aversion (Bals-Kubik et al. 1993). κ-opioids also reduce sensitization to cocaine and amphetamine (Chefer et al. 1999; Gray et al. 1999; Heidbreder and Shippenberg 1994; Heidbreder et al. 1993).

While considerable work has been done to elucidate the actions of μ-opioids in the NAc (Chien and Williams 1998; Jiang and North 1992; Martin et al. 1997; Yuan et al. 1992), the synaptic mechanisms underlying κ-opioid actions in this nucleus are not well understood. Most studies to date have focused on the regulation of dopamine (DA) release. There is evidence suggesting that κ-receptor agonists inhibit DA release, and that this inhibition plays a significant role in κ-receptor–mediated behaviors. Recent electron microscopy studies have provided evidence for the presence of κ-receptors on presumed catecholamine terminals and axons in the NAc shell (Meshul and McGinty 2000; Svingos et al. 1999). In addition, the κ-receptor agonist U50488 injected systemically (Di Chiara and Imperato 1988) or directly into the NAc (Spanagel et al. 1992) reduces extracellular DA levels, presumably through a direct action at the terminals of DA containing afferents (Spanagel et al. 1992). Furthermore, D1 receptor antagonists can also produce conditioned place aversion (Shippenberg et al. 1993), consistent with the idea that κ-receptor–mediated aversion is due to the inhibition of DA release in the NAc.

Although this is an attractive hypothesis, there are reasons to believe that it may not sufficiently account for the effects of κ-receptor agonists in the NAc. In fact, the theory that an increase in NAc DA produces reward has been recently called into question (Bardo 1998; Horvitz 2000). For instance, many aversive events, such as foot shock or tail pinch, are associated with an elevation, as opposed to a decrease, in DA (Horvitz 2000). Furthermore, some behavioral effects of κ-receptor agonists are inconsistent with their being caused by a simple decrease in DA. For example, the κ-receptor agonist U69593 decreases cocaine self-administration, while DA antagonists typically increase self-administration (Schenk et al. 1999). These data suggest that inhibition of DA release may not be the only κ-receptor action in the NAc relevant to its aversive action.

In addition to their dopaminergic input from the ventral tegmental area (VTA), the GABAergic medium spiny neurons of the NAc shell receive a significant glutamatergic input from the prefrontal cortex, amygdala, and hippocampus (Pennartz et al. 1994). Interestingly, Svingos et al. (1999) demonstrated that κ-receptors are present on the presynaptic terminals of presumed excitatory synapses as well as on the dendrites of the medium spiny neurons. This anatomical arrangement raises the...
possibility that the behavioral effects of k-receptor actions in the NAc are due, in part, to the regulation of glutamatergic excitatory transmission. We have addressed the issue in the present study by investigating the effects of k-receptor activation on glutamatergic synaptic transmission in the NAc shell. Specifically, we show that U69593 inhibits glutamatergic transmission through an action at the presynaptic terminal.

METHO DS

Two- to 3-wk old male Sprague-Dawley rats were anesthetized with halothane and the brain removed and placed into a Ringer solution (–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, Nussloch, Germany). Slices were submerged in Ringer solution and allowed to recover for >1 h at room temperature.

Individual slices were transferred to a poly-lysine-coated coverslip and visualized under an Olympus upright microscope with differential interference contrast optics and infrared illumination. Extracellular field or whole cell patch-clamp recordings were made at room temperature. Field recordings were made by placing a 3–5 MΩ electrode filled with Ringer solution into the medial shell of the NAc, which can be visually distinguished from the neighboring core region by their appearance and by their relatively hyperpolarized resting potential (Uchimura et al. 1989). Extracellular field potentials (fEPSPs) and excitatory postsynaptic currents (EPSCs) were evoked (0.06–0.1 Hz) with a bipolar stimulating electrode placed along the dorsal edge of the NAc. In all experiments, inhibitory transmission was blocked with 100 μM picrotoxin.

Recordings were made using an Axopatch 1-D (Axon Instruments, Foster City, CA) 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 EPSC. 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. The paired-pulse ratio in response to two stimuli (50-ms interval) was calculated by estimating the tail of the first EPSC with an exponential and subtracting off any residual current from the second EPSC. Spontaneous activity was analyzed in 5-min epochs for each EPSC. Amplitudes were calculated automatically if the smoothed first derivative of the data trace exceeded a set threshold, and were visually verified. 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.

All drugs were applied by bath perfusion. Stock solutions were 0.3 Na₃GTP (pH 7.2, osmolarity adjusted to 280) except for the experiments measuring N-methyl-D-aspartate (NMDA) currents, where the K-gluconate was replaced with Cs-gluconate to improve recordings at depolarized potentials. 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 (fEPSPs) and excitatory postsynaptic currents (EPSCs) were evoked (0.06–0.1 Hz) with a bipolar stimulating electrode placed along the dorsal edge of the NAc. In all experiments, inhibitory transmission was blocked with 100 μM picrotoxin.
made and diluted in Ringer immediately prior to application. U69593 stock was diluted in 50% EtOH to a concentration of 10 mM; nor-Binaltorphimine (nor-BNI; 10 mM), [D-Ala², N-Me-Phe⁴, Gly⁵]-Enkephalin (DAMGO; 1 mM), SCH-23390 (10 mM), and D-2-amino-5-phosphonovaleric acid (D-APV; 100 mM) were diluted in H₂O; sulpiride (10 mM) was diluted in EtOH, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 mM) and picrotoxin (100 mM) were mixed in DMSO. Chemicals were obtained from Sigma Chemical (St. Louis, MO) or Tocris (Ballwin, MO).

RESULTS

In an initial set of experiments, we looked at the effects of the k-receptor–selective agonist U69593 on the amplitude of evoked field responses in the shell region of the NAc. U69593 produced a dose-dependent inhibition of the field EPSP amplitude (Fig. 1, A and B). At doses <0.5 μM, the U69593 inhibition was completely blocked by application of 100 nM of the k-receptor antagonist nor-BNI (Fig. 1B, ○). Furthermore, nor-BNI was capable of completely reversing the U69593-mediated inhibition (Fig. 1C). Finally, this action on excitatory transmission does not appear to be due to a modulation of endogenous dopamine release, since a combination of the D1-selective antagonist, SCH-23390 (10 μM), and the D2-selective antagonist, sulpiride (10 μM), had no effect on the magnitude of inhibition (Fig. 1D; n = 5).

While excitatory field responses in the NAc are driven by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and completely blocked by the AMPA receptor antagonist CNQX (Nicola et al. 1996), it is not clear that the amplitude of the field response is due entirely to the AMPA receptor current. That is, voltage-dependent currents may contaminate the field response. Therefore to determine whether the U69593 effect was modulating synaptic transmission, we monitored EPSCs from medium spiny neurons voltage clamped at −75 mV. Application of 1 μM U69593 caused a reduction of the EPSC to 78 ± 4% of baseline (P < 0.01; Fig. 2A). Interestingly, there was a wide variability in the magnitude of the U69593 effect in our whole cell recordings with a number of recordings showing no response at all to the drug application (Fig. 2B).

Since k-receptors have been localized to both presynaptic excitatory terminals as well as to dendritic spines (Svingos et al. 1999), we undertook a number of experiments to determine whether the U69593 inhibition was due to a change in glutamate receptor responsiveness or a change in glutamate release. Changes in the paired-pulse ratio (PPR) have been inversely correlated to changes in probability of release (Manabe et al. 1993), such that a decrease in the probability of release causes either an enhancement of paired-pulse facilitation or a decrease in the amount of paired-pulse depression. Under baseline conditions, we observed, on average, a PPR of 1.10 ± 0.05 using FIG. 3. Paired-pulse ratio is altered by U69593. Scatter plot of the amount of inhibition vs. change in the paired-pulse ratio. The open circle shows the average of all experiments. The dashed line is the linear regression through all of the data (r² = 0.47, P < 0.01). Inset: average (20 consecutive sweeps) EPSCs from an individual experiment before (left) and during (right) application of U69593.

FIG. 4. Frequency of spontaneous EPSCs is reduced by U69593. A: 5-s portion of 5-min epochs recorded at baseline (top), during application of 1 μM U69593 (middle), and following application of 100 nM nor-BNI from a single experiment illustrates the reduction in frequency of spontaneous EPSCs. Average (n = 30) evoked EPSCs for each epoch are shown at the right (scale bars: 20 pA/200 ms for sEPSCs; 100 pA/20 ms for evoked). B: cumulative frequency plots of sEPSC amplitude (left) and inter-event interval (right) for the same cell illustrates the decrease in sEPSC frequency (5.59 vs. 1.75 Hz) and the reversal by nor-BNI (6.08 Hz). The mean sEPSC amplitude in this cell increased slightly from 11.0 ± 3.3 pA (n = 1,706) to 11.9 ± 3.3 pA (n = 530). C: average of 6 experiments (white bars) shows decrease in frequency (P < 0.01) but no change in amplitude of spontaneous EPSCs. This effect was reversed by 100 nM nor-BNI (shaded bars, n = 4).
an interstimulus interval of 50 ms, although in many cases we observed paired-pulse depression. In the presence of U69593, this ratio increased to 1.28 ± 0.11 (P < 0.05). Furthermore, the change in the PPR was significantly correlated with the magnitude of the U69593 inhibition (R² = 0.47, P < 0.01; Fig. 3).

To confirm that the κ-receptor agonist effect was on the glutamatergic terminal, we concomitantly measured spontaneous EPSC (sEPSC) activity in a number of cells. U69593 caused a decrease of the evoked EPSCs by 25.7%. In these same cells, U69593 caused a marked reduction in the frequency of spontaneous events (2.4 vs. 4.0 Hz, P < 0.01; n = 6) but had no effect on their amplitude (Fig. 4). Moreover, this reduction in sEPSC frequency was completely reversed following the application of 100 nM nor-BNI.

If the effect of U69593 on glutamatergic transmission is presynaptic, what is the role of the anatomically demonstrated postsynaptic κ-receptors? We did not observe any changes consistent with a postsynaptic κ-receptor action. Specifically, there was no change in input resistance (n = 18), nor, in a separate set of current-clamp recordings, was there a change in resting potential or action potential shape (n = 3, data not shown). NMDA receptor responses to exogenously applied NMDA are enhanced by μ-receptor agonists (Martin et al. 1997). We sought to determine whether NMDA receptors are modulated by U69593. While this experiment is confounded by the presynaptic inhibition of glutamate release, we were able to circumvent this by exploiting the different time courses of the AMPA- and NMDA-mediated EPSCs. While voltage clamping a neuron at +40 mV, the early portion of the dual-component EPSC is primarily AMPA mediated. It is relatively unaffected by 100 μM D-APV, but is completely blocked by 10 μM CNQX (Fig. 5A). The late, NMDA-mediated portion, on the other hand, is completely blocked by 100 μM D-APV. If NMDA receptors were enhanced by U69593, we would expect to see a smaller inhibition in the late portion of the dual-component EPSC. Instead, we observed no difference in the amount of inhibition between the early and late component of the EPSC (Fig. 5; 25.1 ± 5.9% vs. 26.9 ± 7.6%, n = 5), indicating that synaptic NMDA receptors are not affected by U69593.

Because of the evidence that there are sub-classes of medium spiny neurons in the NAc, as well as a number of different sources for their excitatory afferents (Pennartz et al. 1994), we studied the variability of the U69593 effect. Although there was a wide range in the magnitude of the effect, the recorded distribution was not significantly different from a normal distribution with the same mean and standard deviation using a Kolmogorov-Smirnov test, although it should be noted that this negative finding may be due to a relatively small sample size. Also, there was no correlation between the magnitude of the U69593 effect and baseline PPR, input resistance, or resting membrane potential.

We tested whether cells could be segregated on the basis of their response to the μ-receptor agonist DAMGO. Figure 6A shows that, on average, both U69593 and DAMGO produce an inhibition of EPSCs. Importantly, there was no correlation between the magnitude of the U69593 effect and baseline PPR, input resistance, or resting membrane potential.

**DISCUSSION**

This study illustrates that the κ-receptor agonist U69593 decreases neurotransmitter release at glutamate terminals in the shell region of the NAc. A similar inhibition has been observed...
at both the mossy fiber–CA3 pyramidal cell synapse (Weisskopf et al. 1993) as well as in the perforant path–granule cell synapse of the hippocampus (Wagner et al. 1992). \(\kappa\)-receptors have been shown to either reduce a \(\text{Ca}^{2+}\) conductance (Gross and Macdonald 1987) or increase a \(\text{K}^{+}\) conductance (Grudt and Williams 1993; Moore et al. 1994), either of which can reduce the probability of transmitter release.

Our results in the NAc are consistent with experiments examining calcium-dependent release of glutamate from striatal synaptosomes (Rawls et al. 1999) and in vivo experiments measuring the effect of \(\kappa\)-receptor agonists on extracellular levels of glutamate (Rawls and McGinty 1998). There has been one report showing an inhibition of excitatory transmission by 1 \(\mu\)M U50488h in the NAc core. Although U50388 is relatively \(\kappa\)-receptor selective, this inhibition was not blocked by the antagonist naloxone (Yuan et al. 1992) and thus was not confirmed as opioid-receptor mediated. Furthermore, nonopioid-receptor–mediated effects of high doses of U50488h and U69593 have been observed in the hippocampus (Alzheimer and ten Bruggencate 1990). The present results clearly demonstrate that, in the shell region of the NAc, the effects of U69593 are \(\kappa\)-receptor mediated. First, the effect is blocked by the selective antagonist nor-BNI. Second, the effects of \(\kappa\)-receptor and \(\mu\)-receptor agonists were clearly dissociable in many neurons, and \(\mu\)-receptor inhibition occurred in the presence of nor-BNI, further establishing the specificity of the U69593 effect. Finally, the doses required to produce nonopioid actions in the hippocampus were 50-fold larger than those used in this study. While the doses required in this study (EC\(_{50} \approx 0.65 \ \mu\text{M}\)) are somewhat higher than those in other studies from our lab (Pan et al. 1997), they are within an order of magnitude.

Consistent with the observed lack of \(\kappa\)-receptor on cell bodies in the NAc (Meskel and McGinty 2000; Svingos et al. 1999), we saw no evidence for a postsynaptic effect such as the increase in \(\text{K}^{+}\) conductance observed in a subset of cells in the medullary nucleus raphe magnus (Pan et al. 1997). However, the role of \(\kappa\)-receptors on postsynaptic spines remains unknown. \(\mu\)-Receptor agonists have been shown to enhance responses to exogenous application of NMDA but not to synaptically released glutamate in the NAc (Martin et al. 1997). We could not detect a difference between AMPA and NMDA receptor–mediated currents in response to U69593, implying that \(\kappa\)-receptors do not directly interact with NMDA receptors. The possibility remains, however, that \(\kappa\)-receptors interact with second-messenger systems otherwise involved in synaptic transmission or synaptic plasticity.

It is important to note the wide variability in the magnitude of the \(\kappa\)-receptor–mediated inhibition. This cannot be simply attributed to cell health or to access of the agonist to the synapse, since some cells that did not respond to U69593 showed a clear depression in response to DAMGO. Three possibilities exist to explain this variance. First, it may be related to the class of medium spiny neuron. As discussed below, there are two classes of medium spiny neurons in the NAc. There is evidence that afferents into the NAc segregate such that different classes of neurons receive specific inputs (Heimer et al. 1997). Furthermore, it has been shown in the hippocampus that function of the presynaptic terminal can be determined by the postsynaptic cell, even when multiple terminals arise from the same presynaptic cell (Scazzion et al. 1998). Therefore, although the \(\kappa\)-receptor–mediated inhibition is presynaptic, the variance in the effect may be controlled by the postsynaptic cell. A second possibility is that the variability is independent of the postsynaptic cell, but rather is determined by the source of the stimulated fibers. With our recording conditions, we cannot determine which afferents are being stimulated, and it is probable that we are stimulating a mixed population. It is possible that only those afferents arising from one projection have functional \(\kappa\)-receptors on their terminals. On the other hand, \(\kappa\)-receptor mRNA is expressed in neurons of each of the nuclei that send excitatory projections to the NAc (Mansour et al. 1995). This issue could be resolved by recording in vivo, where different afferent populations can be independently stimulated. Finally, \(\kappa\)-receptors may be present on every afferent, but the magnitude of the effect at a given terminal is regulated. Consistent with this model is the observation that \(\kappa\)-receptors are associated with synaptic vesicles in the NAc (Meskel and McGinty 2000), as well as dense core vesicles in the hippocampus (Drake et al. 1996) and neurosecretory vesicles in hypothalamic magnocellular neurosecretory neurons (Shuster et al. 1999). Furthermore, \(\kappa\)-receptors can be translocated to the plasma membrane in a stimulus-dependent manner (Shuster et al. 1999). Thus the amount of \(\kappa\)-receptor–mediated inhibition may be a function of prior activity at that synapse.

Both \(\kappa\) and \(\mu\)-receptor agonists inhibit glutamatergic transmission in the NAc, an effect also produced by DA (Nicola et al. 2000). It is perhaps surprising that a \(\kappa\)-receptor agonist, which produces aversion when microinjected into the NAc, has a cellular mechanism similar to that of \(\mu\) and DA receptor agonists, which are rewarding. There are at least two possibilities to explain this apparent paradox. First, the inhibition of glutamate may be unrelated to the rewarding or aversive properties of the drugs, but rather may underlie a behavioral process that is common to each (e.g., arousal, attention). In this case, the hedonic sign of the specific agonist would have to be governed by some other cellular mechanism. For the \(\kappa\)-receptor–selective agonist U69593, this might be through the local inhibition of DA release. However, in this case, the decrease in DA would increase glutamate release (since DA-mediated inhibition of release would be reduced), which would be expected to counteract the direct effect of U69593 on the excitatory terminals.

A more parsimonious model is that \(\kappa\) and \(\mu\)-receptor agonists presynaptically modulate the inputs of different populations of NAc neurons. As noted above, there is clear evidence for multiple classes of medium spiny neurons in the NAc. One class contains enkephalin and predominantly expresses mRNA for the D2 DA receptor, while the other class contains dynorphin and substance P and expresses the D1 DA receptor (Lu et al. 1998). In addition, there is evidence that these two cell types have different projection patterns (Joel and Weiner 2000). While the substance P/dynorphin–containing cells appear to project directly to the VTA to synapse onto DA cells, the enkephalin-containing neurons make an indirect connection with the DA cells, either via the ventral pallidum or through the GABAergic interneurons of the VTA. Thus activation of the cells in the NAc shell could either inhibit or excite (i.e., disinhibit) DA cell firing in the VTA. In our experiments, we did not observe an anti-correlation between the \(\kappa\) and \(\mu\)-receptor–mediated inhibition of excitatory transmission. Never-
however, there were a number of cells that responded preferentially to one opioid or the other. Future experiments using in vivo electrophysiology may be required to resolve this issue.

The present study demonstrates that κ-receptor agonists exert a robust, presynaptic inhibition of glutamatergic transmission in a subset of neurons in the NAc shell. Previous investigators have focused on the local inhibition of DA release in the NAc by κ-receptor agonists to explain their aversive action. However, the present results raise the possibility that the behavioral actions of κ-receptor agonists in the NAc shell are at least partially dependent on κ-receptor modulation of glutamatergic inputs to NAc medium spiny neurons.

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