Both Kappa and Mu Opioid Agonists Inhibit Glutamatergic Input to Ventral Tegmental Area Neurons

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Margolis, Elyssa B., Gregory O. Hjelmstad, Antonello Bonci, and Howard L. Fields. Both kappa and mu opioid agonists inhibit glutamatergic input to ventral tegmental area neurons. J Neurophysiol 93: 3086–3093, 2005. First published December 22, 2004; doi:10.1152/jn.00855.2004. The ventral tegmental area (VTA) contributes to the motivational actions of natural rewards and a variety of drugs including opioid agonists. Both kappa opioid receptors (KOP-R) (Arvidsson et al. 1995; Mansour et al. 1996) and mu opioid receptors (MOP-R) (Garzon and Pickel 2001; Svingos et al. 2001) are present in significant density in the VTA. Furthermore, microinjections of both KOP-R and MOP-R agonists directly into the VTA produce robust behavioral responses. The KOP-R agonist U50488H produces conditioned place aversion (Bals-Kubik et al. 1993). In contrast, the MOP-R agonist [d-Ala2, N-Me-Phe4, Gly-ol5]-enkephalin (DAMGO) produces conditioned place preference (Bals-Kubik et al. 1993; Nader and van der Kooy 1997; Phillips and LePiane 1980).

Glutamate transmission within the VTA is required for the motivational properties of opioids (e.g., Cornish et al. 2001; Harris and Aston-Jones 2003; Xi and Stein 2002). In the VTA, glutamatergic inputs are derived from neurons in the medial prefrontal cortex (mPFC), subthalamic nucleus (STN), and the pedunculopontine nucleus (PPN) (Charara et al. 1996; Christie et al. 1985; Groenewegen and Berendse 1990; Sesack and Pickel 1992). There is also indirect evidence that lateral hypothalamic projections to the VTA contain glutamate (Chou et al. 2001; Rosin et al. 2003). Under physiological conditions, glutamate can induce phasic firing in dopaminergic neurons through the activation of N-methyl-D-aspartate (NMDA) receptors (Chergui et al. 1993; Johnson et al. 1992; Overton and Clark 1997), and this effect is facilitated by group 1 metabotropic glutamate receptor activation (Zheng and Johnson 2002). There is also evidence that glutamate activation of 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA) receptors in the VTA can increase extracellular dopamine (DA) in the nucleus accumbens (NAc) (Karreman et al. 1996; Schilstrom et al. 1998).

Essential to determining how KOP and MOP receptor agonists in the VTA produce their behavioral actions is elucidating their synaptic effects at the cellular level in all VTA cell types. VTA neurons are classified as principal, secondary, or tertiary according to their electrophysiological and pharmacological properties (Cameron et al. 1997). Both principal and tertiary neurons exhibit the hyperpolarization-activated nonspecific cation current (Ih) and have relatively long duration action potentials. Secondary cells lack an Ih and have shorter duration action potentials. They are directly hyperpolarized by MOP-R agonists and are GABA neurons. Secondary cell inhibition by MOP-R agonists has been proposed to disinhibit principal neurons through local circuitry (Johnson and North 1992; Margolis et al. 2003). Tertiary neurons differ from principal cells in that they are directly hyperpolarized by MOP-R agonists and serotonin. While most principal neurons are dopaminergic (~80%), <40% of tertiary neurons are dopaminergic. KOP-R agonists postsynaptically inhibit a subset of both principal and tertiary neurons, an effect limited to dopaminergic neurons of each class (Margolis et al. 2003).

Despite the evidence that VTA glutamatergic transmission is critical for reward and motivation, our understanding of presynaptic control of glutamate release by opioids is incomplete. We therefore examined the effects of both KOP and MOP receptor agonists on glutamate release onto each VTA cell type. We directly compared KOP and MOP effects within and across individual neurons and neuron types. Because both KOP and MOP effects were observed, we addressed the issue of...
whether KOP and MOP receptor agonists act on the same terminals by testing whether the effects of the two agonists occluded.

METHODS

Twenty- to 36-day-old male Sprague-Dawley rats were anesthetized with isofluorane, and their brains were removed. Horizontal slices (200 µm thick) containing the VTA were prepared using a vibratome (Leica Instruments). Slices were submerged in artificial cerebrospinal fluid (ACSF) solution 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 and allowed to recover at 32°C for ≥1 h.

Individual slices were visualized under a Zeiss Axioskop with differential interference contrast optics and infrared illumination. Whole cell patch-clamp recordings were made at 31°C using 2.5- to 4-MΩ pipettes containing (in mM) 123 K-glucuronate, 10 HEPS, 0.2 EGTA, 8 NaCl, 2 MgATP, and 0.3 NaGTP (pH 7.2, osmolarity adjusted to 275). Recordings were made using an Axopatch 1-D, filtered at 2 kHz, and collected at 5 kHz using IGOR Pro (Wavematicks, Lake Oswego, OR). $I_h$ was recorded by voltage clamping cells and stepping from −60 to −120 mV. Series resistance and input resistance were sampled throughout voltage-clamp experiments with 4-mV, 200-ms depolarizing steps once every 10 s. For purposes of classification, every neuron was tested for postsynaptic actions of either the MOP-R selective agonist DAMGO (3 µM) or the 5-HT1 agonist 5-carboxamidotryptamine (5-CT; 500 nM) in current clamp following the voltage-clamp experiment. Cells were recorded in voltage-clamp mode ($V = −70$ mV) while measuring excitatory postsynaptic currents (EPSCs). All EPSCs were measured in the presence of picrotoxin (100 µM). Stimulating electrodes were placed 60–150 µm rostral to the patched cell. In neurons where paired pulses were administered, two pulses 50 ms apart were delivered once every 10 s. The EPSC amplitude was calculated by comparing a 2-ms period around the peak to a 2-ms interval just before stimulation. The paired-pulse ratio (PPR) was calculated by dividing the amplitude of the second EPSC by that of the first, trial by trial, and averaging across trials. Spontaneous events were identified in a subset of experiments by searching the smoothed first derivative of the data trace for values that exceeded a set threshold, and these events were visually confirmed. Experiments with baseline sEPSC frequencies that exceeded a set threshold, and these events were visually confirmed. Results are presented as means ± SE where appropriate. Summary comparisons were made between the average of the 4 min of baseline just preceding each respective drug application to 4 min of stable drug effect. The significance of drug effects was tested across all VTA neurons and within individual cell types using the two-way repeated-measures ANOVA followed by the Student-Newman-Keuls (SNK) method for multiple comparisons. Differences in effect sizes between measures ANOVA followed by the Student-Newman-Keuls (SNK) method for multiple comparisons. Differences in effect sizes between

RESULTS

Whole cell voltage-clamp recordings were made from neurons in the VTA. Pharmacologically isolated (100 µM picrotoxin) EPSCs were electrically evoked, and we confirmed that this evoked current was due to AMPA receptor activation by blocking the response with the non-NMDA glutamate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 µM, n = 3).

To address the question of whether opioids differentially alter glutamatergic transmission onto different cell types in the VTA, we classified neurons by their electrophysiological and pharmacological properties as principal, secondary, or tertiary (Margolis et al. 2003). In most neurons, the changes in stimulated EPSC amplitude following bath application of both the KOP-R selective agonist U69593 (1 µM) and the MOP-R selective agonist DAMGO (3 µM) were measured (Fig. 1). U69593 produced a modest reduction in EPSC amplitude in principal neurons (14 ± 4%), significantly smaller than the DAMGO effect in the same neurons (42 ± 8%, n = 11, ANOVA: P < 0.01). In contrast, in secondary cells, EPSCs

![FIG. 1. Excitatory postsynaptic currents (EPSCs) in the ventral tegmental area (VTA) neurons are inhibited by both kappa opioid and mu opioid receptor (KOP-R and MOP-R) agonists. Evoked EPSCs in principal (n = 11), secondary (n = 10), and tertiary (n = 9) neurons are inhibited by the KOP-R agonist (trans)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl] benzencacetamide methane-sulfonate hydrate (U69593) diluted in 50% EtOH to a concentration of 1 mM; nor-binaltorphimine (nor-BNI; 1 mM), 5-CT (1 mM), CTAP (1 mM), and DAMGO (1 mM) were diluted in H2O. Picrotoxin was diluted in DMSO (100 mM). All chemicals were obtained from Sigma Chemical (St. Louis, MO) or Tocris (Ballwin, MO).](http://jn.physiology.org/)

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were inhibited to a similar degree by both U69593 (47 ± 10%) and DAMGO (45 ± 10%, n = 10). EPSCs in tertiary neurons were inhibited by both U69593 (33 ± 6%) and DAMGO (35 ± 6%, n = 9). Because inhibition by the KOP-R agonist U69593 persisted for ≥15 min after washout commenced (1 μM, n = 6, data not shown), we used the KOP-R selective antagonist nor-BNI (100 nM) to reverse the KOP-mediated inhibition. Application of nor-BNI before U69593 completely blocked the KOP-R agonist effect (2 ± 2%, n = 3, 1 cell of each type). Because DAMGO was applied a second time to identify the cell type while recording in current-clamp mode, in most experiments no antagonist was used to reverse the prolonged presynaptic MOP-R activation response. However, in a separate set of cells, the application of the MOP-R selective antagonist n-Phe-Cys-Tyr-d-Tryp-Lys-Thr-Pen-Thr-NH2 (CTAP; 500 nM) reversed the DAMGO (3 μM) effect (n = 4, data not shown).

Comparing cell types, the observed U69593 effect was significantly larger in secondary and tertiary neurons than in principal neurons (SNK: principal vs. secondary, P < 0.05; principal vs. tertiary, P < 0.05). The DAMGO EPSC inhibition was not significantly different among cell types. To provide evidence that the observed EPSC inhibitions were presynaptic, we examined changes in the PPR for each drug. A drug-induced decrease in the probability of release is typically correlated with an increase in the PPR (Manabe et al. 1993). We found no significant differences between the baseline PPRs of the different cell types (principal: 0.9 ± 0.1, n = 12; secondary: 1.2 ± 0.2, n = 9; tertiary: 0.93 ± 0.06 Hz, n = 8; ANOVA: P > 0.05).

Although opioid-induced changes in PPR for both U69593 and DAMGO varied greatly across cells and cell types, enhancement of PPR was observed for both receptor types. Figure 2A shows examples comparing baseline evoked EPSCs to those recorded in the presence of either U69593 or DAMGO and the time courses of the EPSC amplitude and PPR. This principal neuron exhibited a significant increase in PPR with both drugs. Overall, VTA neurons showed a significant facilitation of PPR in the presence of U69593 (n = 29, ANOVA: P < 0.05, Fig. 2B) and DAMGO (n = 28, ANOVA: P < 0.05, Fig. 2C). However, when broken down by cell type, the only significant effect observed was that produced by DAMGO in secondary neurons. Individually, less than one-third of all neurons (8/30) showed a significant increase in PPR with U69593 (Student’s t-test, P < 0.05), and these neurons were distributed across all three cell types. A slightly higher proportion of neurons showed a significant increase in PPR with DAMGO (11/29), and these also included neurons of each type. There was overall a significant linear correlation between the magnitude of EPSC inhibition and the change in PPR for both U69593 (Fig. 2D; principal, n = 12; secondary, n = 9; tertiary, n = 8; r² = 0.67, P < 0.05) and DAMGO (Fig. 2E; principal, n = 11; secondary, n = 8; tertiary, n = 8; r² = 0.22, P < 0.05), but these relationships did not hold for individual neuron types.

The relative insensitivity of PPR in VTA neurons to U69593 and DAMGO leaves open the possibility that the observed KOP and MOP effects on EPSC amplitude may not be due to presynaptic mechanisms. Therefore, as an alternative method to test for a presynaptic site of action of the observed effects, we monitored spontaneous EPSCs (sEPSCs) simultaneously with the evoked responses to test for correlations between changes in spontaneous and evoked EPSCs. Baseline sEPSC frequencies were variable across neurons, but there were no differences in baseline frequencies between cell types (principal: 1.3 ± 0.5 Hz, n = 8; secondary: 3.9 ± 1.4 Hz, n = 9; tertiary: 2.5 ± 0.5 Hz, n = 8, ANOVA: P > 0.05). U69593 was found to inhibit sEPSCs, but did not change their amplitude (Fig. 3). Pooled data show that the frequency of sEPSCs significantly decreased from baseline across all cell types in response to both U69593 (ANOVA: P < 0.005) and DAMGO (P < 0.005, Fig. 3D). These inhibitions were not accompanied by changes in sEPSC amplitude for either drug (ANOVA: U69593 P > 0.05, DAMGO P > 0.05, Fig. 3E). The presence of a decrease in sEPSC frequency and a lack of an effect on

FIG. 2. Paired-pulse ratio (PPR) in VTA neurons increases due to KOP and MOP receptor agonists. A: significant paired-pulse facilitation (PPF) in response to both U69593 (1 μM) and DAMGO (3 μM). Recording traces show example baseline (light) and drug (dark) evoked EPSCs. Time courses show both inhibition of evoked EPSC amplitude and facilitation of PPR during drug applications. B: there is a significant PPF in pooled VTA neurons in the presence of U69593; however, no cell type alone exhibits a significant change in PPR (principal, n = 12; secondary, n = 9; tertiary, n = 8; ANOVA: P < 0.05, SNK: P > 0.05 principal, secondary, and tertiary). C: in addition to the overall PPF observed in the pooled VTA neurons during DAMGO application, there was also a significant increase among secondary neurons (principal, n = 11; secondary, n = 8; tertiary, n = 8; ANOVA: P < 0.05 secondary, P > 0.05 principal and tertiary). D: there is a significant linear correlation (P < 0.05) between the magnitude of EPSC inhibition by U69593 and the change in the PPR caused by the drug in pooled VTA neurons. E: similarly, there is a significant linear correlation in pooled VTA neurons (P < 0.05) between DAMGO-induced EPSC inhibition and PPF. *P < 0.05.
sEPSC amplitude are consistent with an opioid-induced decrease in glutamate release probability. Furthermore, changes in sEPSC frequency were correlated with U69593-induced decreases in evoked EPSC amplitudes in each cell type (Fig. 3F). Although only principal neurons showed a significant correlation between evoked EPSC inhibition and sEPSC frequency in the presence of DAMGO, a trend toward a relationship is evident in secondary and tertiary neurons (Fig. 3G).

To ensure that the observed effects on sEPSC frequencies were not due to postsynaptic inhibitions of KOP- or MOP-sensitive spontaneously active glutamatergic neurons in the slice that synapse onto the neurons recorded in the preceding experiments, in separate experiments, we measured changes in miniature EPSCs (mEPSCs) in the presence of TTX (500 nM). The application of TTX did not change the frequency of observed spontaneous events in any cell type (principal: 1.8 ± 0.6 Hz baseline, 1.6 ± 0.6 Hz TTX, n = 7; secondary: 1.6 ± 0.4 Hz baseline, 1.5 ± 0.5 Hz TTX, n = 10; tertiary: 1.7 ± 0.3 Hz baseline, 1.4 ± 0.3 Hz TTX, n = 8, ANOVA: P > 0.05) or amplitude (principal: 14 ± 5 pA baseline, 13 ± 5 pA TTX, n = 7; secondary: 13 ± 1 pA baseline, 14 ± 1 pA TTX, n = 10; tertiary: 14 ± 2 pA baseline, 12.8 ± 0.6 pA TTX, n = 8, ANOVA: P > 0.05). The lack of an effect of TTX on either the frequency or the amplitude of spontaneous excitatory events in these neurons indicates that the previously observed sEPSCs are not action potential dependent.

In the presence of TTX, there was a significant inhibition of mEPSC frequency by U69593 (1 μM) among pooled VTA neurons (n = 10, ANOVA: P < 0.001), with the largest effect occurring in tertiary neurons (Fig. 4A). There was no change in mEPSC amplitude observed during U69593 application (n = 10, ANOVA: P < 0.05, Fig. 4B). In separate experiments, DAMGO (3 μM) also inhibited the frequency of mEPSCs in pooled VTA neurons (n = 15, ANOVA: P < 0.005, Fig. 4A). While as a group VTA neurons did show a significant decrease in mEPSC amplitude in the presence of DAMGO (n = 15, ANOVA: P < 0.05), no individual cell type showed a significant change (Fig. 4B). That the frequency of mEPSCs decreases with both U69593 and DAMGO further supports a presynaptic mechanism for the observed effects.

A subset of I_h-expressing neurons are postsynaptically inhibited by KOP-R agonists (Margolis et al. 2003). To confirm that this postsynaptic effect was not contributing to our evoked presynaptic observations, we compared the evoked presynaptic effects of U69593 in I_h expressing (principal and tertiary) neurons in which the KOP-R agonist induced a positive change in the holding current (n = 6) to those recorded in neurons that...
exhibited no change in holding current with U69593 (n = 13, Fig. 5A). There was no difference between the KOP-R agonist-mediated presynaptic inhibitions of EPSCs for these two groups (ANOVA: P > 0.05). Interestingly, there was a significant difference in the MOP-R agonist DAMGO inhibition of evoked EPSCs in these two groups: IF neurons that were postsynaptically hyperpolarized by the KOP-R agonist showed a significantly greater EPSC inhibition during a separate application of DAMGO (ANOVA: P < 0.01). Conversely, IF neurons that were postsynaptically inhibited by the MOP-R agonist DAMGO (i.e., tertiary neurons, n = 9) showed a greater EPSC inhibition by U69593 than those that were not (i.e., principal neurons, n = 12, ANOVA: P < 0.05, Fig. 5B). There was no difference in the presynaptic DAMGO effect for neurons postsynaptically inhibited by MOP-R agonists compared with those that were not (Fig. 5B).

For both MOP and KOP receptor agonists, the largest presynaptic EPSC inhibitions were observed in secondary neurons, and only secondary cells showed a significant correlation between KOP and MOP receptor agonist EPSC inhibi-

FIG. 4. Opioids decrease the frequency of miniature excitatory events. A: mEPSC frequency among pooled VTA neurons is significantly diminished by U69593 (1 μM, ANOVA: P < 0.05). Furthermore, tertiary neurons (n = 2), but not principal (n = 4) or secondary (n = 4) neurons, show a decrease in mEPSC frequency during U69593 application [Student-Newman-Keuls (SNK): P < 0.05 tertiary, P > 0.05 principal and secondary]. The MOP-R agonist DAMGO (3 μM) significantly decreased the frequency of mEPSC in those that were not postsynaptically hyperpolarized by U69593 than those that were not (Fig. 5B). B: U69593 did not significantly affect mEPSC amplitude in pooled data or any cell type. DAMGO significantly decreased mEPSC amplitude in the pooled data, but not in any individual neuron type. *P < 0.05.

FIG. 5. Presynaptic KOP and MOP actions in IF neurons partially segregate according to postsynaptic KOP and MOP actions. A: compared with IF neurons not postsynaptically inhibited by KOP-R agonist U69593 (1 μM, n = 13), those that are exhibit a greater EPSC inhibition by DAMGO (3 μM, n = 6). There is no difference in EPSC inhibition by U69593 (1 μM) between neurons postsynaptically hyperpolarized by U69593 and those with no postsynaptic sensitivity to U69593. B: neurons that were postsynaptically hyperpolarized by the MOP-R agonist DAMGO (3 μM, tertiary neurons, n = 9) showed a greater EPSC inhibition by U69593 than those that were not (principal neurons, n = 12). There was no difference in EPSC inhibition by DAMGO between those neurons that were postsynaptically inhibited by DAMGO and those that were not. *P < 0.05, **P < 0.01.

FIG. 6. EPSC inhibition by KOP and MOP in secondary neurons is correlated. There is no apparent relationship between the magnitude of EPSC inhibition by U69593 (1 μM) and DAMGO (3 μM) in principal (n = 12) or tertiary (n = 8) neurons. There is a significant linear correlation between EPSC inhibition by U69593 and DAMGO in secondary neurons (n = 9, P < 0.05). The greatest inhibitions by both U69593 and DAMGO were also observed in secondary neurons. There was no difference between the KOP-R agonist DAMGO inhibition of EPSCs in these two groups (ANOVA: P < 0.05). *P < 0.05.
DISCUSSION

Our results show that glutamate terminals onto each class of VTA neuron are inhibited by both KOP and MOP receptor agonists. KOP EPSC inhibition is larger in secondary and tertiary cells than it is in principal neurons. Among neurons with an $I_h$ (principal and tertiary), those that are postsynaptically hyperpolarized by KOP-R agonists show greater EPSC inhibition by MOP-R agonists. In those VTA neurons without an $I_h$ (secondary), there is a significant correlation between the magnitudes of the KOP- and MOP-induced EPSC inhibitions.

Our results confirm and extend previous findings on opioid modulation of glutamate release in the VTA. The results presented here are in agreement with earlier observations of presynaptic inhibition of glutamate release by MOP-R agonists in $I_h$ and non–$I_h$-expressing VTA neurons (Bonci and Malenka 1999; Manzoni and Williams 1999). However, the presynaptic KOP effect showed here was not observed in a previous investigation of glutamate release onto $I_h$-expressing neurons in the VTA (Manzoni and Williams 1999). Given the large variability reported for the seven neurons tested with U69593 in that study, an EPSC inhibition is likely to have occurred in a subset of those neurons. The variability of KOP inhibition across all $I_h$ neurons reported here may have led to an average effect that was not significantly different from zero in their study when principal and tertiary neurons were not distinguished. The small but significant U69593 effect we observed in principal neurons was not only confirmed by sEPSC and mEPSC measurements, but was reversed by nor-BNI, confirming its KOP-R selectivity.

We observed a previously unreported and significant relationship between the pre- and postsynaptic effects of KOP and MOP receptor agonists in $I_h$ neurons. The EPSC inhibition by KOP-R agonists was larger among neurons postsynaptically hyperpolarized by MOP-R agonists (i.e., tertiary neurons), and MOP-mediated EPSC inhibition was larger in neurons postsynaptically inhibited by KOP-R agonists. Interestingly, while about 70% of $I_h$-expressing neurons in the VTA are dopaminergic, all neurons hyperpolarized by KOP are dopaminergic (Margolis et al. 2003). Although there are also DA neurons that are not inhibited by KOP-R agonists, the data reported here suggest that glutamatergic inputs to DA neurons are more sensitive to MOP-R agonists than those onto non-DA, $I_h$-expressing neurons. This conclusion will need to be confirmed in future experiments.

The inhibition of excitatory input to DA neurons by MOP-R agonists seems counter to the observation that MOP-R agonists in the VTA excite DA neurons. However, it is possible that the function of MOP-R activation in the VTA is to increase firing rate without producing bursting in DA neurons. Glutamate input to DA neurons tends to shift the tonic, spontaneous activity of DA neurons to a bursting pattern, often without changing the overall firing rate of the neuron (Chergui et al. 1993; Connelly and Shepard 1997; Floresco et al. 2003; Johnson et al. 1992; Overton and Clark 1992). The combination of indirect disinhibition of DA neurons by MOP-R agonists (through the inhibition of local GABAergic neurons) with presynaptic EPSC inhibition could produce greater neuron activity without shifting the firing pattern to bursting. Such a change could increase a temporally broad DA signal, which is likely to carry very different information from the pulsed release associated with bursting of VTA DA neurons (Phillips et al. 2003).

Presynaptic inhibition of glutamate release in the VTA provides an important mechanism by which inputs to the VTA can be differentially controlled by KOP and MOP receptor agonists. The occlusion experiments reported here provide evidence that MOP-Rs and KOP-Rs differentially regulate glutamatergic inputs onto both principal and tertiary neurons. The lack of a difference among principal neurons in EPSC inhibition by U69593 applied in the presence of DAMGO compared with that observed in ACSF is consistent with KOP-Rs and MOP-Rs being segregated to separate terminals. However, secondary and tertiary neurons must have at least partial overlap of receptor expression on individual glutamatergic terminals. In tertiary neurons, this conclusion is supported by the finding that the KOP-R agonist mediated EPSC inhibition was diminished in the presence of the MOP-R agonist. In many secondary cells, U69593 and DAMGO each inhibited EPSC amplitude by >50%. Together with the correlation between U69593 and DAMGO EPSC inhibitions in these neurons, these data support the hypothesis that KOP-Rs and MOP-Rs are on the same glutamatergic terminals synapsing onto secondary neurons. Interestingly, glutamate excitation of secondary neurons in the VTA increases firing rates without causing bursting (Steffensen et al. 1998). Therefore not only does glutamate appear to have a very different postsynaptic function in secondary neurons compared with that in principal and tertiary cells, but the opioid regulation of these inputs seems to be fundamentally different.

Unlike the combinations of postsynaptic KOP-Rs and MOP-Rs that are differentially expressed in different VTA cell classes, presynaptic KOP and MOP receptor activation does inhibit glutamate release onto all VTA neurons. This provides a broad functional range of opioid modulation of VTA neuronal activity. Similar presynaptic KOP inhibition of glutamate release onto cell types having different postsynaptic opioid responses has also been observed in the nucleus raphe magnus (Bie and Pan 2003). Functional roles for these multiple opioid receptor sites in the VTA may be related to the synaptic location and timing of release of endogenous KOP and MOP receptor ligands. Projections to the VTA of neurons containing enkephalin, a MOP and 6 opioid receptor agonist peptide, arise from the ventral pallidum, and those immunoreactive for endomorphin, a MOP-R selective agonist peptide, arise from the hypothalamus (Greenwell et al. 2002; Kalivas et al. 1993). It is possible then that endogenous ligands acting at the MOP-R in the VTA could be released by different events and at different times from those that lead to the release of the endogenous KOP-R selective ligand dynorphin, which is released from terminals of neurons located in the nucleus accumbens, lateral hypothalamus, and amygdala (Chou et al. 2001; Fallon et al. 1985).

Postsynaptic inhibitions by both KOP-R and MOP-R agonists in the VTA have previously been examined (Cameron et al. 1997; Johnson and North 1992; Margolis et al. 2003). Postsynaptically, only subsets of dopaminergic principal and tertiary neurons are inhibited by KOP-R agonists, while, by definition, all secondary and tertiary cells are directly inhibited by MOP-R agonists. Thus concurrent KOP- or MOP-induced presynaptic inhibition of glutamate release and postsynaptic hyperpolarization would be synergistic in secondary and ter-
tary neurons. It is also important to point out that principal and
tertiary neurons fire in the absence of synaptic input. Therefore
opioids can modify the output of these VTA neurons in the
absence of an excitatory input, and this modulation may have
very different consequences from inhibiting excitatory inputs
to the same neuron. There may also be an anatomical differ-
ence between the different actions of opioids on these signals.
Endogenous opioids may have a limited radius of effect when
released and therefore depending on their precise location, pre-
and postsynaptic KOP-Rs and MOP-Rs could be activated
independently in vivo. Such mechanisms could account for the
seeming contradiction that MOP-R agonists both inhibit exci-
tatory input and indirectly disinhibit principal neurons.

The sources of the differences in the responses to opioids
reported here and their possible functional implications are
unclear. Since in most cases, especially in the principal neu-
rons, inhibition of glutamatergic inputs by KOP-R ligands was
only partial, it is likely that not all glutamatergic terminals bear
the KOP-R. Thus it is tempting to hypothesize that variation in
EPSC modulation by opioids across cell types depends on the
source of glutamatergic afferents. For instance, Carr and
Sesack (2000) showed that glutamate afferents from the mPFC
to the VTA synapse selectively on DA neurons that project
to the mPFC and GABAergic neurons that project to the
NAc, but not DA neurons that project to the NAc or GABA
neurons that project to the mPFC. Therefore some combination
of PPN, STN, and hypothalamus afferents to the VTA likely
provides major glutamate input to the nondopaminergic neu-
rons that comprise 60% of the VTA projection to the mPFC,
the dopaminergic neurons that comprise 80% of the VTA
projection to the NAc, and possibly the VTA neurons that
project to other targets such as the amygdala and hippocampus
(Swanson 1982). However, since in other brain regions a single
axon can give rise to multiple excitatory synapses with signif-
ically different properties (Maccalferri et al. 1998; Markram
et al. 1998; Scanziani et al. 1998), we cannot conclude that the
observed differences are due to differential anatomical origins
of the glutamatergic afferents. Further work needs to be done
to discern if there is indeed an anatomical correlate to the
effects observed here.

In conclusion, we show that KOP and MOP receptor ago-
nists inhibit glutamatergic input onto all neuron types in the
VTA. Presynaptic regulation of synaptic transmission by opi-
oids in the VTA provides a mechanism for selective control of
specific inputs to VTA neurons. How these presynaptic effects
interact with the postsynaptic inhibitions through MOP and
KOP receptor activation in the VTA not only depends on
whether the glutamatergic afferents are active when opioid
ligands are present, but may also depend on the site of origin
of the neuron giving rise to the glutamatergic terminal or the
projection target of the postsynaptic neuron. The modulation
of glutamate release by KOP and MOP receptor agonists reported
here provides important information for understanding signal
modulation in the VTA and is an important key to elucidating
the influence on motivation and reward of endogenous opioids
acting in the VTA.

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