0022-3565/07/3202-917–925
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
U.S. Government work not protected by U.S. copyright
JPET 320:917–925, 2007

Vol. 320, No. 2
112722/3174349
Printed in U.S.A.

Presynaptic δ Opioid Receptors Regulate Ethanol Actions in Central Amygdala

Maeng-Hee Kang-Park, Brigitte L. Kieffer, Amanda J. Roberts, George Robert Siggins, and Scott D. Moore

Department of Psychiatry, Duke University Medical Center, Durham, North Carolina (M.-H.K.-P., S.D.M.); Institut de Génétique et Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, Strasbourg, France (B.L.K.); Molecular and Integrative Neuroscience Department, The Scripps Research Institute and Alcohol Research Center, La Jolla, California (A.J.R., G.R.S.); and Research Service, Veterans Administration Medical Center, Durham, North Carolina (S.D.M.)

Received August 23, 2006; accepted November 16, 2006

ABSTRACT

Endogenous opioid systems are implicated in the reinforcing effects of ethanol consumption. For example, δ opioid receptor (DOR) knockout (KO) mice show greater ethanol consumption than wild-type (WT) mice (Roberts et al., 2001). To explore the neurobiological correlates underlying these behaviors, we examined effects of acute ethanol application in brain slices from DOR KO mice using whole-cell patch recording techniques. We examined the central nucleus of amygdala (CeA) because the CeA is implicated in alcohol reinforcement (Koob et al., 1998). We found that the acute ethanol effects on $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) were greater in DOR KO mice than in WT mice. Ethanol increased the frequency of miniature IPSCs (mIPSCs) significantly more in DOR KO mice than in WT mice. In CeA of WT mice, application of ICI 174864 [[allyl]2-Tyr-α-amino-isobutyric acid (Aib)-Aib-Phe-Leu-OH], a DOR inverse agonist, augmented ethanol actions on mIPSC frequency comparable with ethanol effects seen in DOR KO mice. Superfusion of the selective DOR agonist D-Pen^2,D-Pen^5-enkephalin decreased the mean frequency of mIPSCs; this effect was reversed by the DOR antagonist naltrindole. These findings suggest that endogenous opioids may reduce ethanol actions on IPSCs of CeA neurons in WT mice through DOR-mediated inhibition of GABA release and that the increased ethanol effect on IPSCs in CeA of DOR KO mice could be, at least in part, due to absence of DOR-mediated inhibition of GABA release. This result supports the hypothesis that endogenous opioid peptides modulate the ethanol-induced augmentation of $GABA_A$ receptor-dependent circuitry in CeA (Roberto et al., 2003).

Endogenous opioid systems regulate alcohol-seeking behavior because opiate receptor antagonists reduce ethanol consumption in human alcoholics and in a rat model of relapse (Froehlich, 1996; Herz, 1997). The opiate antagonist naltrexone is currently available as an adjunct medication for treatment of alcoholism. However, not all alcoholic patients respond to this medicine. In this context, the involvement of different opioid receptor subtypes on the ethanol reinforcement is under investigation. Although pharmacological studies using subtype-specific antagonists show the consistent involvement of μ opioid receptors (MORs) in ethanol reinforcement, the involvement of δ opioid receptors (DORs) is controversial (Matsuzawa et al., 1999; Mhatre et al., 2000; Ciccocioppo et al., 2002; Ingman et al., 2003). Recent studies using knockout (KO) mice suggest that MOR and DOR may act in an opposing manner to regulate alcohol consumption. MOR KO mice do not self-administer alcohol, whereas DOR KO mice consume more alcohol than wild-type (WT) controls (Roberts et al., 2000, 2001). In addition, DOR and MOR KO mice show differences in baseline emotional responses: DOR KO mice exhibit increased anxiety-like behavior relative to WT mice, whereas MOR KO mice exhibit less anxiety (Filliol et al., 2000; Roberts et al., 2001; Sasaki et al., 2002). Fur-

This work was supported by the National Institute on Alcohol Abuse and Alcoholism Integrative Neuroscience Initiative on Alcoholism (Grant UO1 AA013498 to G.R.S. and S.D.M.), by the National Institute on Drug Abuse (Grant R01 DA03665 to G.R.S.), and by a Veterans Administration Merit Review (to S.D.M.).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.106.112722.

ABBREVIATIONS: MOR, μ opioid receptor; DOR, δ opioid receptor; KO, knockout; WT, wild type; CeA, central nucleus of the amygdala; IPSC, inhibitory postsynaptic current; ACSF, artificial cerebrospinal fluid; RMP, resting membrane potential; eIPSC, evoked IPC; AP-5, D-2-amino-5-phosphonovalerate; DNQX, 6,7-dinitroquinoxaline-2,3-dione; CGP 55845, 3-*N*[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino-2-(*S*)-hydroxypropyl-*p*-benzyl-phosphinic acid; I/O, input/output; TTX, tetrodotoxin; mIPSC, miniature IPSC; ICI 174864, [allyl]2-Tyr-α-amino-isobutyric acid (Aib)-Aib-Phe-Leu-OH; DPDPE, D-Pen^2,D-Pen^5-enkephalin; CRF, corticotropin-releasing factor.

thermore, the anxiolytic effect of ethanol is greater in DOR KO mice than WT mice (Roberts et al., 2001). These behaviors, possibly reflecting innate responses to stress, may play a role in regulating ethanol consumption in DOR and MOR mice.

The amygdala formation plays a critical role in emotion and the response to stress (for review, see LeDoux, 2003). Because stress has long been considered to contribute to ethanol-seeking behavior in humans (Brown et al., 1995), the amygdala may have a significant role in regulating alcohol consumption. In addition, the central nucleus of the amygdala (CeA), as part of the extended amygdala, has been implicated in the positive reinforcing effects of ethanol in animal models (Koob et al., 1998). Microinjection of opioid antagonists into CeA produced significant reductions in ethanol self-administration, suggesting that the CeA is a critical site for opioid modulation of ethanol drinking (Heyser et al., 1999; Foster et al., 2004). In CeA, GABAergic neurons are the most abundant cell type (Sun and Cassell, 1993). Behavioral studies indicate that the CeA GABAergic system mediates the rewarding effect of ethanol (Hyytiä and Koob, 1995). A recent in vitro electrophysiological study demonstrated that ethanol enhances GABAergic inhibitory postsynaptic currents (IPSCs) in CeA at both pre- and postsynaptic sites (Roberto et al., 2003). Endogenous opioids are localized within GABAergic neurons in CeA (Veinante et al., 1997; Cassell et al., 1998), and acute ethanol increases c-Fos expression in enkephalin-containing GABAergic neurons in the CeA (Morales et al., 1998; Criado and Morales, 2000), suggesting an interaction between GABAergic and endogenous opioid systems on ethanol drinking. Therefore, we examined the ethanol modulation of $GABA_A$ receptor function in DOR KO mice using whole-cell patch recordings of CeA slices in vitro. Portions of this study have been reported in abstract form (Kang-Park et al., 2004; Park et al., 2004).

Materials and Methods

Generation of Knockout Mice. The methods for generation of the DOR KO mice have been described in detail in reports by Roberts et al. (2000, 2001). A total of 32 male homozygous δ-opioid receptor KO and 28 WT litter mate mice shipped from The Scripps Research Institute (La Jolla, CA) were used in this experiment. The genetic background of these mice was a hybrid C57BL/6Orl × 129/SV strain. We housed the mice one to three per cage in a temperature-controlled room in which the lights were on a 12-h light/dark cycle with lights off at 10:00 AM. All experimental procedures were approved by the Duke University Medical Center and the Durham Veterans Affairs Medical Center Institutional Animal Care and Use Committees, in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Whole-Cell Recordings. Brains were rapidly removed from 5- to 6-month old DOR KO or WT mice under halothane anesthesia. We immersed intact brains in ice-cold oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (ACSF). We cut coronal slices (300 μm, between bregma −1.0 to approximately −1.9 mm; Paxinos and Franklin, 2004) using a Vibraslice (model 752; Campden Instruments Ltd., Leicester, UK) and incubated them in ACSF containing 120 mM NaCl, 3.3 mM KCl, 1.23 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 0.9 mM $MgSO_4$, and 10 mM glucose that was continuously bubbled with 95% O_2-5% CO_2 at room temperature (for brain dissection and slicing only, ACSF $CaCl_2$ was reduced to 0.5 mM).

After a minimum of a 1-h incubation, we transferred a single slice to the recording chamber (volume, 0.5 ml) in which oxygenated ACSF was superfused over submerged slices at a rate of approximately 3 to 4 ml/min. We viewed individual cells with an upright fixed-stage microscope (Zeiss Axioskop; Carl Zeiss Inc., Thornwood, NY) equipped with a water immersion objective (40×, 0.75 numerical aperture, NA), IR filtered light, differential interference contrast optics, and a Hitachi (Tokyo, Japan) charge-coupled device camera. For recording, patch pipettes were pulled from borosilicate glass capillary tubing (1.5-mm o.d., 1.05-mm i.d.; World Precision Instruments, Sarasota, FL) using a Flaming-Brown horizontal microelectrode puller (model P-97; Sutter Instrument, Novato, CA). We filled the pipettes (input resistance, 2–5 MΩ) with the following recording solution: 75 mM potassium gluconate, 70 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, 4 mM Mg-ATP, and 0.3 mM Tris-GTP, pH 7.25 (285 mOsM). Liquid junction potentials were not measured or compensated.

We made whole-cell patch recordings in the medial ventral portion of CeA (Fig. 1A). IPSCs were recorded continuously using an Axopatch 200B (Axon Instruments, Foster City, CA), filtered at 2 KHz (−3 dB), and digitized by Lab PC+ (National Instruments, Austin, TX). The CeA neurons had resting membrane potentials (RMPs) at least as negative as −55 mV immediately after the cell membrane rupture and a minimum acceptable input resistance of 100 MΩ. We monitored series resistance (15–30 MΩ) online throughout the experiment using a digital oscilloscope, and we rejected cells if resistance changed by >20%. No series resistance compensation was used.

For all IPSC recordings, we voltage-clamped cells at −70 mV at room temperature. In some cases, cells were held at the RMP under current-clamp mode before switching to voltage clamp to examine cell membrane properties such as input resistance and firing properties. Under current-clamp mode, we injected hyperpolarizing and depolarizing current steps (10-pA incremental steps, 500-ms duration, Fig. 1B) to generate current-voltage curves and to evoke spikes. We evoked IPSCs (eIPSCs) in the presence of ionotropic glutamate receptor antagonists, (+)-2-amino-5-phosphonotentanoic acid (AP-5, 50 μM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM), and the $GABA_B$ receptor antagonist, CGP 55845 (1 μM), using a monopolar tungsten electrode positioned at the lateral division of CeA. Stimuli were square-wave current pulses (0.1-ms duration) delivered every 30 s. We adjusted the stimulus strength (range, 50–70 μA) to evoke 30 to 40% of the maximal amplitudes of the IPSCs determined in the input/output (I/O) relationship (Fig. 1E). In addition, we recorded spontaneous IPSCs during continuous 2- to 3-min recordings in the presence of tetrodotoxin (TTX; 1 μM) to block action potentials; these events are then referred to as miniature IPSCs (mIPSCs). The mIPSCs were studied using either 145 mM KCl in the patch electrodes to maximize the driving force for Cl^- or with replacement of potassium gluconate and KCl with cesium gluconate (130 mM) and CsCl (7 mM) in the electrode internal solution. All cells were held at −70 mV.

After a 10-min baseline recording period, we applied ethanol for 10 to 12 min and then observed effects of ethanol washout for 15 to 30 min. We used 40 mM ethanol, comparable with the concentration showing maximal effect on IPSCs in CeA (Roberto et al., 2003; Nie et al., 2004). Ethanol was diluted in gassed ACSF directly from sealed stock solutions of reagent grade 95% ethanol immediately before administration to avoid loss of ethanol by evaporation and then bath applied. For the ICI 174864 studies, a stock solution of ICI 174864 (1 mM) was prepared, aliquoted, and stored at −70°C until use. ICI 174864 (1 μM) was then diluted into ACSF immediately before administration and bath-applied after a 10-min baseline recording period. We examined the effect of ICI 174864 alone for 4 to 10 min, followed by ethanol application plus ICI 174864 for 10 to 12 min and washout for 20 to 30 min.

Data Acquisition and Analysis. We acquired eIPSCs at 10-KHz sample rate and analyzed them off-line using LabView (National Instruments) on an IBM-compatible computer. We measured IPSC peak amplitude and decay time constants and tested the ethanol

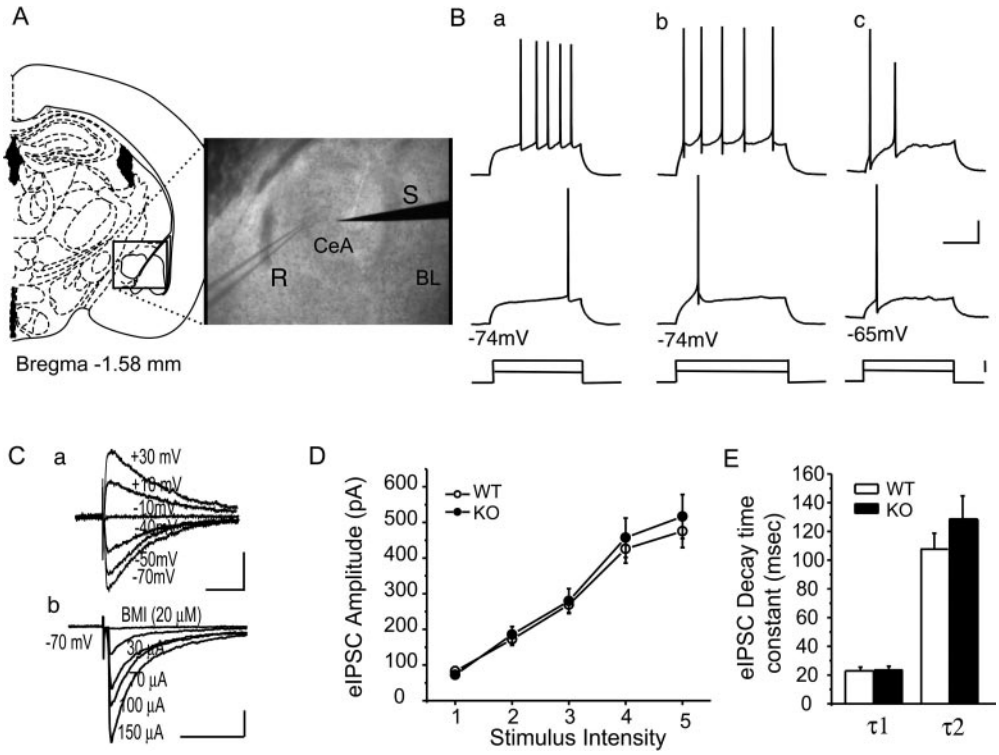

Fig. 1. Location of CeA and characterization of $GABA_A$ receptor-mediated IPSCs. A, schematic representation of a coronal slice illustrating the location of the CeA (Paxinos and Franklin, 2004) and photographic image of a slice containing CeA. The positions of the recording electrode (R) and stimulating electrode (S) are shown. B, cell types showing different firing patterns. Spikes were evoked by current injection (80 and 160 pA, 500–600-ms duration) at RMP. Cell types shown are: delayed firing (a), regular firing (b), and adapting cells (c). Scales, 20 mV and 200 ms for traces and 80 pA for current pulses. C, a, eIPSCs at different holding potentials (−70 to +30 mV), showing the reversal potential close to E_{Cl}^- (−10 mV) with a pipette containing 70 mM KCl. b, input/output relationship of eIPSCs at a holding potential of −70 mV. The eIPSCs were completely blocked by bath application of bicuculline (20 μM), indicating their mediation by $GABA_A$ receptors. Scales, 100 pA and 100 ms. D, input/output curves of eIPSC amplitudes at different stimulus intensities in slices from WT (open circles, $n = 21$) and KO (closed circles, $n = 14$) mice. There is no difference in the peak amplitude of eIPSCs between WT and KO mice. E, decay time constants of eIPSCs were also not different between cells from WT and KO mice at a stimulus strength adjusted to evoke 30 to 40% of maximum amplitude. Open column, results from WT mice ($n = 21$); closed column, results from KO mice ($n = 14$).

effect using paired Student's t test. To measure decay time constants, we fitted the 10 to 90% decay phase of eIPSCs to a double exponential time course using the equation: $y(t) = y_0 + A_1 \times \exp(-t/\tau_1) + A_2 \times \exp(-t/\tau_2)$, where τ_1 and τ_2 are fast and slow decay time constant, respectively. We acquired mIPSCs at a 5-KHz sample rate and 1-s/sweep using WINWCP (Strathclyde Whole Cell Program, courtesy of Dr. John Dempster). We analyzed mIPSCs using Mini Analysis (Synaptosoft, Leona, NJ). Only IPSCs that had amplitudes greater than 5 times baseline noise level (6–8 pA) were accepted for analysis. We re-examined all events visually for acceptance, plotted results as cumulative amplitude and frequency histograms, and tested the effects of drugs using the Kolmogorov-Smirnov statistical method. For both evoked and spontaneous IPSCs, we compared the results between groups using independent Student's t tests; we considered $p < 0.05$ to be statistically significant. We used SPSS (SPSS, Chicago, IL) for all statistical analysis and Origin (Origin Lab, Northampton, MA) for plotting figures. Results in the text and figures are presented as the mean ± S.E.M.

Drugs. Drugs used were AP-5, bicuculline methiodide, DNQX, CGP 55845, TTX, ICI 174864, naltrindole, and D-Pen^2,D-Pen^5-enkephalin (DPDPE). We purchased all drugs from Sigma (St. Louis, MO), except AP-5 was obtained from Acros Organics (Gell, Belgium).

Results

CeA Neuronal Properties. We recorded neurons in the medial ventral part of CeA (Fig. 1A), shown to receive enkephalin-containing GABAergic projections from the lateral subdivision of CeA (Cassell et al., 1998). In current-clamp mode, we observed three types of cells (Fig. 1B) similar to reported neurons previously classified as "late firing" (76%), "regular firing" (12%), and "accommodating" (with one or two spikes; 12%) (for review, see Sah et al., 2003). The late firing cells displayed a pronounced outward rectification in the depolarizing direction and did not show spike adaptation. There was no significant difference in ethanol sensitivity among these cells because all three types of cells showed an enhancement of eIPSC amplitude by ethanol. The most abundant cell type (i.e., delayed firing) showed a mean 27% increase in eIPSC amplitude, whereas regular firing and adapting cells showed 17 and 18% increases, respectively. In addition, there was no difference between DOR KO and WT mice in the relative number of the CeA cell types. There were no differences in the mean RMPs or mean input resistances between DOR KO and WT mice. The mean RMP (±S.E.M.) was −65 ± 1.4 ($n = 12$) and −67 ± 1 ($n = 21$) mV for DOR KO and WT mice, respectively ($p = 0.35$); the mean input resistance was 235 ± 28 ($n = 8$) and 226 ± 31 ($n = 11$) MΩ for DOR KO and WT mice ($p = 0.86$), respectively.

There were no differences in eIPSC properties such as peak amplitude and decay time constants between WT and DOR KO mice (Fig. 1, D and E). Stimulus intensities were normalized through I/O curves from threshold and maximal response intensity. There were no differences in peak amplitudes throughout the range of stimulus intensities used

between WT and DOR KO mice [$F(1,33) = 0.123$, $p = 0.73$] (Fig. 1D). In addition, there was no difference in the baseline peak amplitude at the test intensity, which was 30 to 40% of the maximal response. At test intensity, mean eIPSC amplitude was 237 ± 33 ($n = 21$) and 223 ± 21 ($n = 14$) pA from WT and DOR KO mice, respectively ($p = 0.37$). In baseline responses, there were no differences in the mean fast and slow decay time constants between WT and DOR KO mice: 23 ± 2.7 versus 23 ± 3 ms ($p = 0.98$) for fast decay time constants and 107 ± 11 versus 128 ± 16 ms ($p = 0.284$) for slow decay time constants (Fig. 1E).

Ethanol Augments Evoked $GABA_A$ IPSCs. Electrical stimulation in the lateral aspect of CeA (in the presence of the glutamate antagonists DNQX and APV) evoked an inward synaptic current at a holding potential of −70 mV. This inward current reversed at approximately −10 mV (Fig. 1C), close to the calculated chloride reversal potential (E_{cl} of −10 mV with 70 mM KCl in the pipette) and was blocked by the $GABA_A$ receptor antagonist bicuculline methiodide (Fig. 1C). Therefore, we considered this inward current to be a $GABA_A$ receptor-mediated IPSC. For baseline responses we used ~30 to 40% of the maximal IPSC amplitude as determined from the I/O relationship (Fig. 1D). Brief (10–12 min) superfusion of ethanol (at a near-maximal 40 mM concentration; Nie et al., 2004) reversibly augmented eIPSC peak amplitudes in CeA neurons from both WT and DOR KO mice (Fig. 2, A and B). Ethanol effects began 2 to 3 min after application, plateaued between 7 and 10 min, and then almost completely reversed 15 to 20 min after washout. However, the magnitude of mean IPSC augmentation was significantly larger ($p < 0.01$) in CeA neurons from DOR KO (35 ± 4%, $n = 11$, $p < 0.01$) than from WT mice (20 ± 2.9%, $n = 11$, $p < 0.01$; Fig. 2Ba). Ethanol did not significantly affect the decay time constants of eIPSCs in either WT or KO mice. We hypothesized that inhibitory effects mediated by DOR were absent in KO mice. Therefore, in a separate set of experiments with WT mice, we studied the ethanol effect in CeA slices after blocking DOR with the selective DOR antagonist naltrindole (1 μM). Consistent with our observations in DOR KO and WT mice, the ethanol enhancement of eIPSC amplitude was greater in the presence of naltrindole. The ethanol-induced increase in mean peak IPSC amplitude in the presence of naltrindole (29 ± 5.3%, $n = 10$) was significantly greater ($p = 0.038$) than in controls (14 ± 2.4%, $n = 14$; Fig. 2Da). In these experiments, we also examined the effect of ethanol on the paired-pulse ratio of eIPSCs in the presence of naltrindole (1

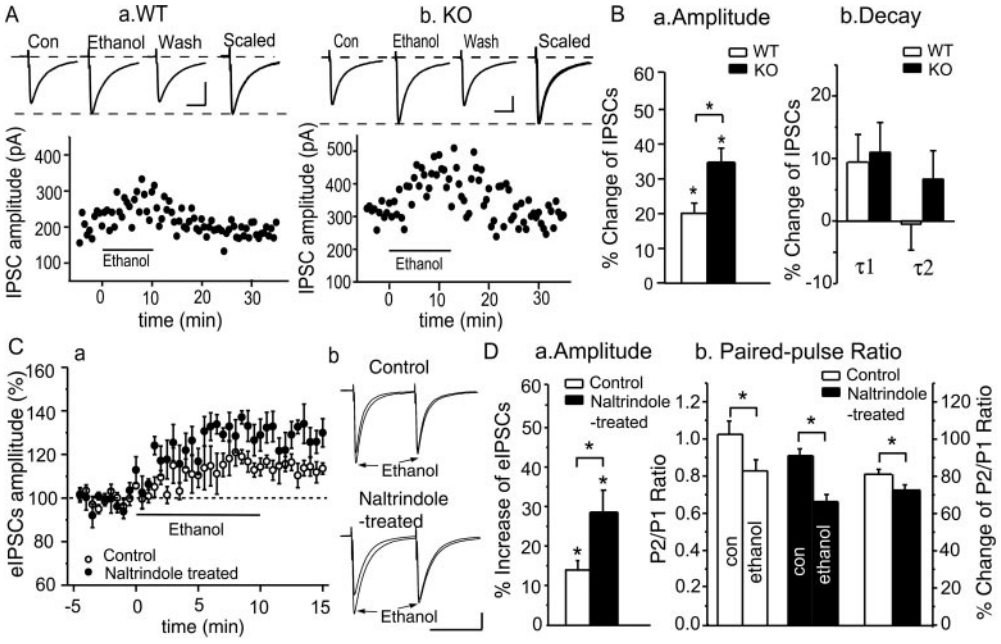

Fig. 2. Superfusion of ethanol (40 mM) increases the amplitude of eIPSCs in CeA to a greater extent in KO than WT mice. A, representative results from CeA neurons from WT (a) and KO (b) mice. Upper panels, averaged traces of eIPSCs before (con), during 40 mM ethanol superfusion (ethanol), and after washout of ethanol (wash). The traces before and during ethanol superfusion were scaled and superimposed (scaled). Ethanol (40 mM) increases the eIPSC amplitude without changing decay time, and the effect was reversed upon washout. Calibration scales, 100 pA and 100 ms. Lower panels, IPSC amplitudes are plotted as a function of time for CeA cells from WT (a) and KO (b) mice. Ethanol effects occurred 2 to 3 min after application, reached a plateau between 7 and 10 min, and then almost completely reversed 15 to 20 min after washout. Ethanol increased the amplitude of IPSCs to a greater extent in KO than in WT mice. B, averaged ethanol effect on eIPSCs in CeA neurons from WT and DOR KO mice. a, ethanol effects on mean eIPSC amplitude; b, mean ethanol effects on fast and slow decay time constants. Open and closed columns, results from WT and MOR KO mice, respectively. Ethanol significantly enhances the amplitude of eIPSCs without affecting decay time constants in WT and DOR KO mice. The magnitude of enhancement in eIPSC amplitudes by ethanol is significantly larger ($p < 0.01$) in DOR KO (35 ± 4.0%, $n = 11$, $p < 0.01$ compared with baseline) than in WT (20 ± 2.9%, $n = 11$, $p < 0.01$ compared with baseline) mice. C, effect of naltrindole, a DOR antagonist, on ethanol responses in WT mice. a, IPSC amplitudes are plotted as a function of time in control condition (open circles) and in the presence of naltrindole (closed circles). Superfusion of ethanol (40 mM) increases the amplitude of eIPSCs in CeA of WT mice to a greater extent in the presence of naltrindole (1 μM) than in the control condition. b, traces before and during ethanol superfusion in the control condition (upper panel) and in the presence of naltrindole (lower panel). Calibration scales, 100 pA and 100 ms. D, averaged increase in eIPSC amplitude after ethanol application in control condition (open column) and in the presence of naltrindole (closed column). a, ethanol effect in eIPSC amplitude. The amplitude increases in the presence of naltrindole (29% increase, $n = 10$) were significantly greater than in control (14% increase, $n = 14$) ($p = 0.038$). b, ethanol effects on the paired-pulse ratio of eIPSCs. Ethanol decreased the paired-pulse ratio, suggesting increases in presynaptic transmitter release. The magnitude of decrease in the paired-pulse ratio was also greater in the presence of naltrindole (27% decrease, from 0.91 to 0.66; $n = 8$) compared with control (19% decrease, from 1.02 to 0.83%; $n = 14$), suggesting presynaptic DOR-mediated mechanisms ($p = 0.04$ between groups).

μM). Ethanol significantly decreased the mean paired-pulse ratio of evoked IPSCs from 1.02 to 0.83 (19% decrease, $p < 0.01$, $n = 14$) in the control (Fig. 2Db). The paired-pulse ratio of evoked IPSCs was also significantly decreased from 0.91 to 0.66 in the presence of naltrindole (27% decrease, $p < 0.01$, $n = 8$). The magnitude of change in the paired-pulse ratio was significantly greater in the presence of naltrindole compared with the control condition ($p = 0.04$). Naltrindole alone also slightly increased eIPSC amplitude in five of 12 cells, yielding a 7.2 ± 2.5% mean enhancement (p = N.S.). This naltrindole effect was also reflected in paired-pulse ratios, in which the averaged paired-pulse ratio decreased from 0.94 to 0.88 by 6.6 ± 2.1% ($p < 0.01$). This result suggests the presence of small tonic endogenous effects mediated through DORs. However, naltrindole-sensitive and -insensitive cells showed a similar range of eIPSC amplitude enhancement (from 5–45%) by ethanol in the presence of naltrindole.

Ethanol Increases mIPSC Frequency. The presynaptic inhibition of neurotransmitter release is a well documented action of DORs in several brain regions (Jiang and North, 1992; Yuan et al., 1992; Lupica, 1995; Stanford and Cooper, 1999; Pan et al., 2002; Shen and Johnson, 2002), and in CeA, DORs are located primarily on axon terminals (Wilson et al., 2002). Therefore, we assessed the site of action of DORs in regulating the ethanol effect. In a separate set of CeA neurons, we used TTX (1 μM) to block action potential-dependent GABA release and examined ethanol effects on spontaneous mIPSCs. There was no significant difference in the baseline mIPSC frequency ($p = 0.61$) or amplitude ($p = 0.65$) between WT and KO mice. Baseline frequencies were 0.66 ± 0.09 (WT; $n = 7$) and 0.60 ± 0.08 (KO; $n = 7$) Hz. Baseline amplitudes were 31.7 ± 2.2 (WT) and 29.8 ± 3.6 (KO) pA. Ethanol (40 mM) increased the frequency of mIPSCs in both DOR KO mice and WT mice (Fig. 3A). The Kolmogorov-Smirnov test showed significant frequency increases in 10 of 14 cells (five of seven cells for each group), indicating a presynaptic site of ethanol action on vesicular GABA release. Across all cells in both groups, the magnitude of the ethanol-induced increase in the mean frequency of mIPSCs was markedly and significantly greater ($p < 0.01$) in DOR KO (60 ± 6.2%, $n = 7$, $p < 0.01$) than in WT (34 ± 7.6%, $n = 7$, $p < 0.01$) (Fig. 3, A and C) mice. Ethanol did not significantly alter the mean mIPSC amplitudes in either WT (−5 ± 3.4%, $n = 7$, $p = 0.24$) or KO mice (8 ± 7.8%, $n = 7$, $p = 0.35$) (Fig. 3, B and D), suggesting a lack of significant postsynaptic action. However, we did observe a slight increase in mIPSC amplitude with ethanol in 7 of 12 CeA neurons. This is similar to the findings of Roberto et al. (2003), where mean mIPSC amplitude across all CeA neurons was not significantly changed by ethanol, although some individual neurons did show an ethanol-induced increase.

ICI 174864 Augments Ethanol-Induced Increase of mIPSCs in CeA of WT Mice. We hypothesized that presynaptic inhibitory effects mediated by DOR were absent in KO mice, in a manner similar to pharmacological blockade of DOR in WT mice. We therefore studied the ethanol effect in CeA slices from WT mice after blocking DOR using a DOR inverse agonist, ICI 174864. Superfusion of ICI 174864 (1 μM) alone did not significantly affect the baseline mIPSC frequency (9.5 ± 3.8%, $p = 0.27$) or amplitude (−6.3 ± 5.5%, $p = 0.24$; Fig. 4). However, in the presence of ICI 174864, ethanol increased the frequency of mIPSCs to a significantly greater extent ($p = 0.04$) than in control conditions in CeA of WT mice. The mean ethanol-induced mIPSC frequency increase in CeA from WT mice in the presence of ICI 174864 was 77 ± 15.3% ($n = 5$, $p < 0.01$; Fig. 4C); that without ICI 174864 was 34 ± 7.6% ($n = 7$, $p < 0.01$; Fig. 3C).

DPDPE Reduces mIPSC Frequency in CeA of WT Mice. Because we hypothesized that presynaptic DOR regulate GABA release in CeA, we sought to verify actions of a DOR agonist directly on mIPSCs in CeA of WT mice. Superfusion of the selective DOR agonist DPDPE (1 μM) decreased the mean frequency of mIPSCs in 6 of 10 cells (Fig. 5, A and Ca); this effect was reversed by superfusion of the DOR antagonist naltrindole (1 μM). Averaged data from all 10 recorded cells showed a significant decrease in the mean frequency of mIPSCs from 1.4 ± 0.22 to 1.1 ± 0.16 Hz ($p < 0.01$, $n = 10$). There were no significant effects of DPDPE on mean mIPSC amplitude ($p = 0.46$, $n = 10$; Fig. 5, B and Cb).

Discussion

We observed here that ethanol enhanced evoked $GABA_A$ receptor-mediated IPSCs in CeA neurons to a greater extent in DOR KO mice than in WT mice. In addition, ethanol-induced increases in the frequency of mIPSCs were greater in DOR KO than WT mice. In WT mice, block of δ receptors by the DOR antagonist ICI 174864 enhanced the ethanol-induced increase in mIPSC frequency comparably with that seen in DOR KO mice. These results suggest that endogenous opioids negatively regulate ongoing and ethanol-elicited GABA release by actions on DOR in WT mice, and this effect is absent in DOR KO mice. Thus, the increased ethanol effect on $GABA_A$ IPSCs in DOR KO mice could be due, at least in part, to the absence of DOR-mediated inhibition of GABA release.

DOR-mediated negative modulation of GABA release has been reported previously in several brain regions including hippocampus, striatum, accumbens, globus pallidus, and locus coeruleus (Jiang and North, 1992; Yuan et al., 1992; Stanford and Cooper, 1999; Pan et al., 2002; Shen and Johnson, 2002). The colocalization of endogenous opioids and GABA in CeA neurons (Veinante et al., 1997; Cassell et al., 1998) and the localization of DOR on the axon terminals in CeA (Wilson et al., 2002) support a presynaptic function for DOR in regulating GABA release in this brain region.

Behavioral studies indicate that DOR KO mice show increased anxiety-like behaviors and greater self-administration of ethanol than do WT mice (Roberts et al., 2001). In addition, DOR KO mice show more sensitivity to the anxiolytic effect of ethanol than WT mice. Therefore, the functional significance of enhanced ethanol effects on GABAergic transmission in CeA may be related to the greater anxiolytic effects of ethanol observed in DOR KO mice. This stronger anxiolytic ethanol effect may lead to the increased ethanol self-administration in DOR KO mice, especially given the greater baseline anxiety.

In contrast, MOR KO mice show less anxiety-like behavior and less ethanol self-administration compared with WT mice (Filliol et al., 2000; Roberts et al., 2001; Sasaki et al., 2002). MOR KO mice also lack the potentiation of anxiolytic activity induced by the $GABA_A$ receptor agonist, muscimol (Sasaki et al., 2002). Therefore, MOR and DOR differentially modulate

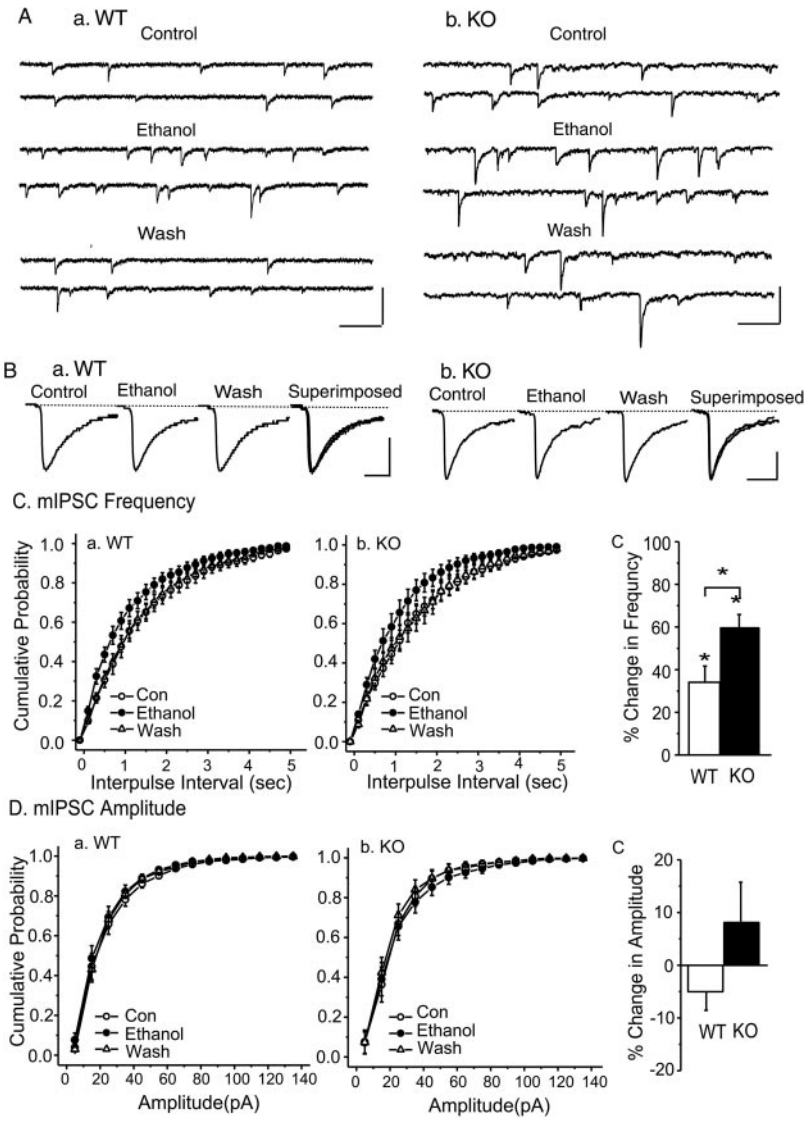

Fig. 3. Ethanol (40 mM) increases the frequency of mIPSCs to a greater extent in KO than in WT mice (all results in the presence of TTX). A, current traces from representative neurons from WT (a) and DOR KO (b) mice, respectively, showing mIPSCs. Scales, 50 pA and 500 ms. B, averaged traces of mIPSCs in control, after superfusion with 40 mM ethanol and after washout from the slices from WT (a) and DOR KO (b) mice. The traces were superimposed in the last panels to show the lack of change in the peak and decay of mIPSCs. Scales, 10 pA and 50 ms. C, averaged cumulative probability plots of the frequency of mIPSCs before (open circles), during superfusion with ethanol (closed circles), and after washout (open triangle) in slices from WT (a) and DOR KO (b) mice. The cumulative frequency histograms show a shift to the left during ethanol superfusion in CeA neurons from both WT and KO mice, indicating that ethanol increases the frequency of mIPSCs for both groups. Ethanol significantly increased the frequency of mIPSCs in WT mice and in KO mice. However, the magnitude of the leftward shift in the cumulative frequency histogram is greater in KO than WT mice. c, averaged increase in mean mIPSC frequency in neurons from WT mice (open column, $n = 7$) and from KO mice (closed column, $n = 7$). Ethanol induces a significant increase in the rate of mIPSCs for both WT ($34 \pm 7.6\%$, $n = 7$, $p < 0.01$) and KO mice ($60 \pm 6.2\%$, $n = 7$, $p < 0.01$), and this effect is significantly larger in KO than in WT mice ($p = 0.02$). D, averaged cumulative probability plots of the amplitude of mIPSCs before (open circles), during superfusion with ethanol (closed circles), and after washout (open triangle) in slice from WT (a) and DOR KO (b) mice. The mean cumulative amplitude histograms show no shift during ethanol superfusion in CeA neurons from either WT and KO mice, indicating that although ethanol did slightly increase mIPSC amplitude in a minority of WT cells (see text), it did not affect the mean amplitudes of mIPSCs for either group. c, effects of ethanol on mean mIPSC amplitude from WT mice (open column, $n = 7$) and from KO mice (closed column, $n = 7$). There was no significant difference in ethanol effects on the mean amplitude of mIPSCs: WT mice, $-5 \pm 3.4\%$, $n = 7$, $p = 0.24$; and KO mice, $8 \pm 7.8\%$, $n = 7$, $p = 0.35$.

$GABA_A$ receptor-mediated synaptic responses and ethanol reinforcement. Several studies have reported different modulation of synaptic functions by MOR and DOR. For example, MOR activation may elicit presynaptic inhibition of both excitatory and inhibitory neurotransmission, whereas DOR activation depresses only inhibitory neurotransmission (Pan et al., 2002; Shen and Johnson, 2002). In addition, in some brain areas, MOR and DOR modulate inhibitory neurotransmission differentially depending on the origin of neuronal projections; for example, although presynaptic MOR is located on striatopallidal terminals and pallidopallidal terminals, presynaptic DOR is preferentially located in quiescent globus pallidal cells (Stanford and Cooper, 1999). Within the CeA, DOR immunoreactive staining is only on the axon terminals, whereas MOR shows diffuse immunoreactivity in the neuropil (Wilson et al., 2002). In addition, morphine activates potassium channels in acutely dissociated amygdala neurons, and this effect is blocked by a MOR but not a DOR antagonist (Chen et al., 2000). Another recent study indicates that selective MOR agonists mediate direct postsynap-

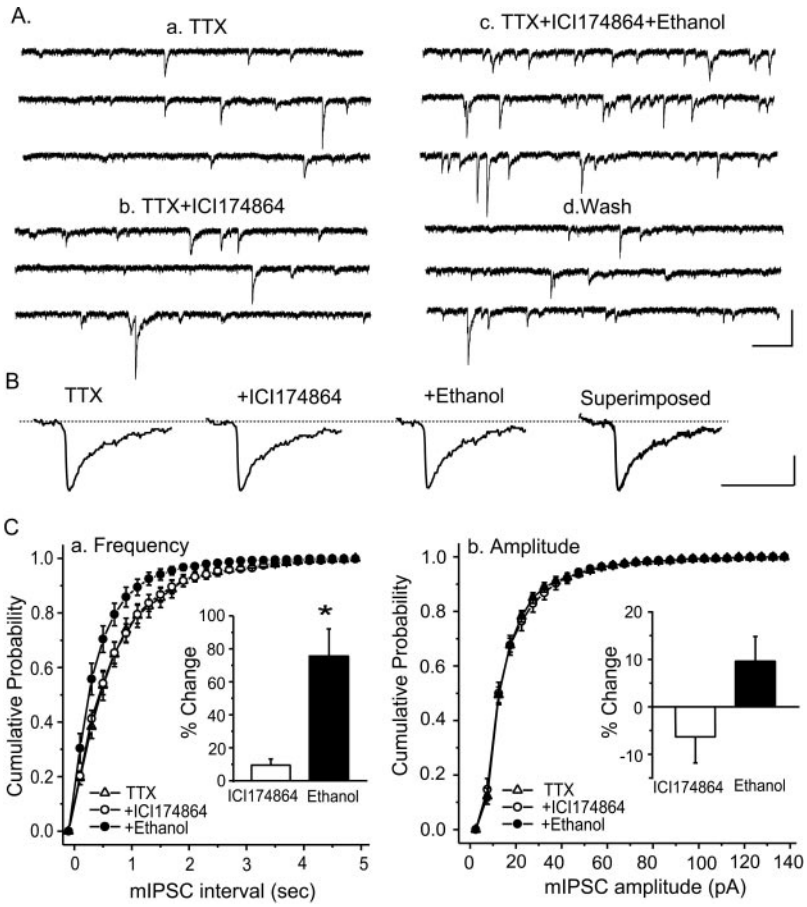

Fig. 4. ICI 174864 (1 μM) enhances the ethanol (40 mM) effect on the frequency of mIPSCs in CeA slices from WT mice (all results in the presence of TTX). A, representative traces showing the occurrence of mIPSCs in control (Aa), during superfusion with ICI 174864 (Ab), after addition of 40 mM ethanol (Ac), and after washout (Ad). Scales, 50 pA and 500 ms. B, averaged traces of mIPSCs in control, during superfusion with ICI 174864, and after addition of 40 mM ethanol. The traces were superimposed in the last panel to show the lack of changes in the peak and decay of mIPSCs. Scales, 10 pA and 50 ms. C, cumulative probability plot of the frequency (a) and the amplitude (b) of mIPSCs. C, cumulative probability plots of the amplitude of mIPSCs during TTX alone (open triangle), during the addition of ICI 174864 (open circles), and during the addition of ethanol (closed circles). a and b, inset, averaged ($n = 5$) increase in mIPSC frequency and amplitude, respectively, after superfusion with 1 μM ICI 174864 alone (open columns) and ICI 174864 plus 40 mM ethanol (closed columns). ICI 174864 itself did not affect mIPSC frequencies or amplitudes. However, ICI 174864 significantly enhanced the ethanol effect on the frequency of mIPSCs (by 77 $\pm$ 15.4%, $n = 5$, $p < 0.01$) compared with the ethanol effect without ICI 174864 (34 $\pm$ 7.6%; see Fig. 3B) in CeA slices from WT mice ($p = 0.04$). There was no significant difference in ethanol effects on the mean mIPSC amplitude: 10 $\pm$ 5.2% ($n = 5$, $p = 0.12$).

tic effects in CeA, whereas DOR agonists have no such effect (Zhu and Pan, 2004).

Regulation of inhibitory mechanisms in CeA is not fully understood, and it is likely that other peptidergic systems are involved in ethanol effects in CeA. Recently, it has been shown that the effect of ethanol and corticotropin-releasing factor (CRF) in enhancing $GABA_A$ receptor-mediated function is blocked by CRF antagonists and abolished in CRF receptor KO mice, an effect likely involving presynaptic GABAergic terminals (Nie et al., 2004).

Roberto and Siggins (2006) recently reported that the opioid-like peptide nociceptin also decreases both GABAergic neurotransmission and ethanol-induced enhancement of GABA release in CeA by actions at the nociceptin/orphanin FQ peptide receptor. Thus, actions of nociceptin and DOR agonists at the cellular level appear to overlap in CeA. However, the overall behavioral effects of centrally administered nociceptin differ significantly from DOR activation (Jenck et al., 2000), suggesting distinct roles for these peptide systems in regulating ethanol effects. The possibility of such a distinction is supported by the recent demonstration that oxytocin and vasopressin have similar cellular actions in CeA, yet opposing behavioral effects (Huber et al., 2005). In contrast, both peptide systems appear to counter actions of CRF in CeA (Nie et al., 2004). The observation that CRF and opioid peptides do not colocalize in CeA (Veinante et al., 1997) supports a model of distinct peptidergic functions modulating ethanol actions in CeA.

The interaction between acute ethanol and DOR may occur at any level of endogenous opioid action. First, ethanol could induce the release of endogenous opioids, which in turn activates DOR, leading to a decrease in GABAergic neurotransmission. Ethanol has been shown to induce synthesis and release of endogenous opioids in several brain regions (de Gortari et al., 2000; Marinelli et al., 2006). However, the cellular mechanisms underlying endogenous opioid release at neuronal terminals are not yet clearly understood. In the present study and elsewhere (Roberto et al., 2003), ethanol was shown to induce GABA release at the nerve terminal in the absence of Na^+ channel activation. Considering that in some inhibitory neurons endogenous opioids are colocalized with GABA, we speculate that a similar mechanism may

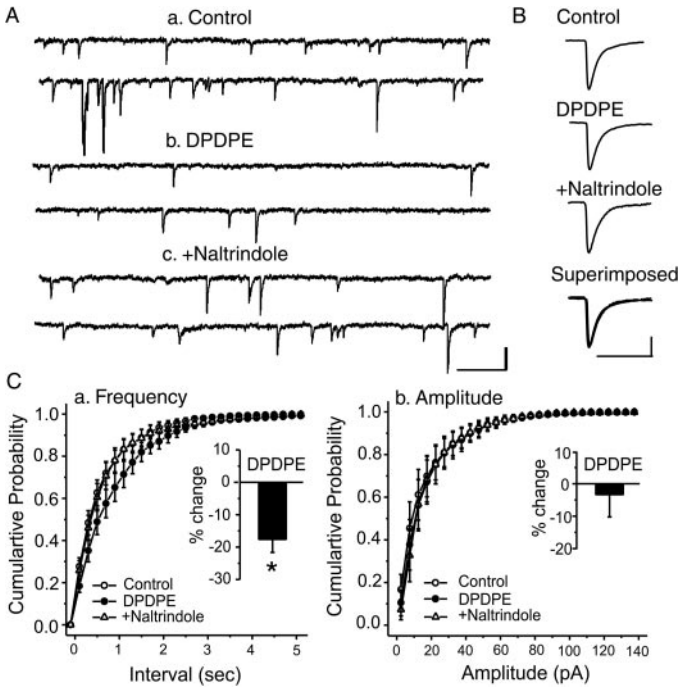

Fig. 5. DPDPE decreases the frequency of mIPSCs in CeA neurons from WT mice. A, representative current traces in control condition (a), after DPDPE (1 μM) (b), and after coapplication of naltrindole (1 μM), a DOR antagonist (c). Scales, 10 pA and 500 ms. B, averaged traces of mIPSCs in control, during superfusion with DPDPE, and during coapplication of naltrindole. The traces were superimposed in the last panel to show the lack of change in the peak and decay of mIPSCs. Scales, 10 pA and 50 ms. C, cumulative probability plots of frequency (a) and amplitude (b) of mIPSCs from DPDPE-sensitive cells ($n = 6$). Cumulative probability plots of the amplitude of mIPSCs during control (open circles), during the addition of DPDPE (closed circles), and during the addition of naltrindole (open triangles). Cumulative frequency histograms show a shift to the right during DPDPE superfusion, indicating a decrease in mIPSC frequency, whereas superfusion of naltrindole reverses the DPDPE effects. Mean cumulative amplitude probability plot shows no changes during DPDPE superfusion. a and b, insets, averaged frequency and amplitude from all 10 recorded cells, respectively. Superfusion of DPDPE decreases the mean frequency of mIPSCs in CeA from 1.4 to 1.1 Hz by 17.6 ± 4.0% ($p < 0.01$, $n = 10$) without significant change in mean amplitude.

corelease endogenous opioids as well as GABA. Alternatively, ethanol could activate DOR receptors directly. However, to our knowledge, there are no reports on the direct action of ethanol on DOR or any other G-protein-coupled receptors. Ethanol could interact with the DOR system reciprocally through common intracellular signal transduction mechanisms such as adenylyl cyclase, protein kinases, Ca^{2+}, or phosphatidyl inositol turnover. For example, ethanol has been shown to modulate metabotropic glutamate receptor function through protein kinase C (Olive et al., 2005). For this possibility, it is assumed that DOR is tonically activated by endogenous opioids. The presence of tonic DOR activity (either constitutive or due to tonic opioid release) was observed in the eIPSCs studies, even though we could not detect it in mIPSC studies.

DOR-mediated modulation of GABAergic neurotransmission could also play self-limiting a role for of ethanol effects, as reported for the self-limiting action of ethanol in hippocampus that is mediated through $GABA_B$ receptors (Ariwodola and Weiner, 2004). Increased GABAergic neurotransmission is suggested to play a critical role in ethanol consumption, reinforcement, and dependence. Therefore, we expect the effect of DOR in limiting the increase of GABAergic neurotransmission will modulate the reinforcing and dependence effects of ethanol.

It is remains unclear just how ethanol augments GABA release from the presynaptic terminal. DOR activation has been shown to decrease mIPSCs in other brain regions (Stanford and Cooper, 1999; Shen and Johnson, 2002). The opioid receptors can couple to K^+ and Ca^+ channels, adenylyl cyclase, protein kinases, and phosphatidyl inositol turnover (for review, see Williams et al., 2001). Any of these actions may be important in the regulation of neurotransmitter release. For example, activation of the inwardly rectifying K^+ channel plays a critical role in the modulation of action potential-independent neurotransmission (Piros et al., 2000). Considering that GABA and enkephalin are colocalized in about half of CeA neurons (Veinante et al., 1997), it seems possible that peptide release primarily occurs under conditions of GABA release. This would allow modulation of subsequent GABA release through negative feedback involving presynaptic DORs.

In conclusion, the present results suggest that the increased ethanol-induced enhancement of presynaptic GABAergic function in CeA neurons may be one of the cellular mechanisms underlying the enhanced anxiolytic effect of ethanol and increased ethanol self-administration observed in DOR KO mice. Our findings are consistent with the hypothesis that the anxiolytic effect of ethanol contributes to ethanol reinforcement and that presynaptic DORs in CeA are involved in modulating ethanol-induced anxiolysis. Given that the nonselective opiate antagonist naltrexone (likely acting preferentially at μ opiate receptors; O'Brien, 2005) reduces relapse in alcohol-dependent humans (O'Malley et al., 1992; Volpicelli et al., 1992), these new data support the idea of δ opioid receptors as attractive targets for pharmaceutical intervention of alcoholism.

Acknowledgments

We thank George Koob and Marisa Roberto for valuable comments.

References

Ariwodola OJ and Weiner JL (2004) Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: role of presynaptic GABA(B) receptors. *J Neurosci* **24:**10679–10686.

Brown SA, Vik PW, Patterson TL, Grant I, and Schuckit MA (1995) Stress, vulnerability and adult alcohol relapse. *J Stud Alcohol* **56:**538–545.

Cassell MD, Freedman LJ, and Shi C (1998) The intrinsic organization of the central extended amygdala. *Ann NY Acad Sci* **877:**217–241.

Chen X, Marrero HG, Murphy R, Lin Y-J, and Freedman JE (2000) Altered gating of opiate receptor-modulated K^+ channels on amygdala neurons of morphine-dependent rats. *Proc Nat Acad Sci USA* **97:**14692–14696.

Ciccocioppo R, Martin-Fardon R, and Weiss F (2002) Effect of selective blockade of $\mu(1)$ or δ opioid receptors on reinstatement of alcohol-seeking behavior by drug-associated stimuli in rats. *Neuropsychopharmacology* **27:**391–399.

Criado JR and Morales M (2000) Acute ethanol induction of c-Fos immunoreactivity in pre-pro-enkephalin expressing neurons of the central nucleus of the amygdala. *Brain Res* **861:**174–177.

de Gortari P, Mendez M, Rodriguez-Keller I, Perez-Martinez L, and Joseph-Bravob P (2000) Acute ethanol administration induces changes in TRH and proenkephalin expression in hypothalamic and limbic regions of rat brain. *Neurochem Int* **37:** 483–496.

Filliol D, Ghozland S, Chluba J, Martin M, Matthes HWD, Simonin F, Befort K, Gaveriaux-Ruff C, Dierich A, LeMeur M, et al. (2000) Mice deficient for δ- and μ-opioid receptors exhibit opposing alterations of emotional responses. *Nat Genet* **25:**195–200.

Foster KL, McKay PF, Seyoum R, Milbourne D, Yin W, Sarma PVVS, Cook JM, and June HL (2004) $GABA_A$ and Opioid receptors of the central nucleus of the amygdala selectively regulate ethanol-maintained behaviors. *Neuropsychopharmacology* **29:**269–284.

Froehlich JC (1996) The neurobiology of ethanol-opioid interactions in ethanol reinforcement. *Alcohol Clin Exp Res* **20:**181A–186A.

Herz A (1997) Endogenous opioid systems and alcohol addiction. *Psychopharmacology* **129:**99–111.
Heyser CJ, Roberts AJ, Schulteis G, and Koob GF (1999) Central administration of an opiate antagonist decreases oral ethanol self-administration in rats. *Alcohol Clin Exp Res* **23:**1468–1476.
Huber D, Veinante P, and Stoop R (2005) Vasopressin and oxytocin excite distinct neuronal populations in central amygdala. *Science (Wash DC)* **308:**245–248.
Hyytiä P and Koob GF (1995) GABA-A receptor antagonism in the extended amygdala decreases ethanol self-administration. *Eur J Pharmacol* **283:**151–159.
Ingman K, Salvadori S, Lazarus L, Korpi ER, and Honkanen A (2003) Selective δ-opioid receptor antagonist N,N(CH3)2-Dmt-Tic-OH does not reduce ethanol intake in alcohol-preferring AA rats. *Addiction Biol* **8:**173–179.
Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roever S, et al. (2000) A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: anxiolytic profile in the rat. *Proc Nat Acad Sci USA* **97:**4938–4943.
Jiang ZG and North RA (1992) Pre- and postsynaptic inhibition by opioids in rat striatum. *J Neurosci* **12:**356–361.
Kang-Park M-H, Roberts AJ, Siggins GR, and Moore SD (2004) Enhanced ethanol effects on GABAA receptor-mediated synaptic currents in neurons of the central amygdala nucleus from δ-opioid receptor knockout mice. *Alcohol Clin Exp Res* **28 (Suppl):**313.
Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytiä P, Merlo-Pich E, and Weiss F (1998) Neurocircuitry targets in ethanol reward and dependence. *Alcohol Clin Exp Res* **22:**3–9.
LeDoux J (2003) The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol* **23:**727–738.
Lupica CR (1995) δ and μ Enkephalins inhibit spontaneous GABA-mediated IPSCs via a cyclic AMP-independent mechanism in the rat hippocampus. *J Neurosci* **15:**737–749.
Marinelli PW, Lam M, Bai L, Quirion R, and Gianoulakis C (2006) A microdialysis profile of dynorphin A(1–8) release in the rat nucleus accumbens following alcohol administration. *Alcohol Clin Exp Res* **30:**982–990.
Matsuzawa S, Suzuki T, Misawa M, and Nagase H (1999) Different roles of μ-, δ- and κ-opioid receptors in ethanol-associated place preference in rats exposed to conditioned fear stress. *Eur J Pharmacol* **368:**9–16.
Mhatre MC, Carl K, Garrett KM, and Holloway FA (2000) Opiate δ-2-receptor antagonist naltriben does not alter discriminative stimulus effects of ethanol. *Pharmacol Biochem Behav* **66:**701–706.
Morales M, Criado JR, Sanna PP, Henrikson SJ, and Bloom FE (1998) Acute ethanol induces c-fos immunoreactivity in GABAergic neurons of the central nucleus of the amygdala. *Brain Res* **798:**333–336.
Nie Z, Roberts AJ, Madamba SG, Moore SD, and Siggins GR (2004) CRF1 receptors are involved in the ethanol enhancement of GABAergic transmission in the central amygdala. *Science (Wash DC)* **303:**1512–1514.
O'Brien CP (2005) Anticraving medications for relapse prevention: a possible new class of psychoactive medications. *Am J Psychiatry* **162:**1423–1431.
Olive MF, McGreehan AJ, Kinder JR, McMahon T, Hodge CW, Janak PH, and Messing RO (2005) The mGluT5 antagonist 6-methyl-2-(phenylethynyl)pyridine decreases ethanol consumption via a protein kinase C epsilon-dependent mechanism. *Mol Pharmacol* **67:**349–355.
O'Malley SS, Jaffe AJ, Chang G, Schottenfeld RS, Meyer RE, and Rounsaville B (1992) Naltrexone and coping skills therapy for alcohol dependence: a controlled study. *Arch Gen Psych* **49:**881–887.
Pan Y-Z, Li D-P, Chen S-R, and Pan H-L (2002) Activation of δ-opioid receptors excites spinally projecting locus coeruleus through inhibition of GABAergic inputs. *J Neurophysiol* **88:**2675–2683.
Park M-H, Roberts AJ, Siggins GR, Wilson WA, Moore SD (2004) δ Opioid receptor blocker increases ethanol effects on miniature inhibitory postsynaptic currents in the mouse central nucleus of amygdala cells. *Alcohol Clin Exp Res* **28:**38A.
Paxinos G and Franklin KBJ (2004) *The Mouse Brain in Stereotaxic Coordinates*, Academic Press, London, UK.
Piros ET, Charles RC, Song L, Evans CJ, and Hales TG (2000) Cloned δ-opioid receptors in GH(3) cells inhibit spontaneous $Ca^{(2+)}$ oscillations and prolactin release through K(IR) channel activation. *J Neurophysiol* **83:**2691–2698.
Roberto M, Madamba SG, Moore SD, Tallent MK, and Siggins GR (2003) Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc Nat Acad Sci USA* **100:**2053–2058.
Roberto M and Siggins GR (2006) Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. *Proc Nat Acad Sci USA* **103:**9715–9720.
Roberts AJ, Gold LS, Polis I, McDonald JS, Filliol D, Keiffer BL, and Koob GF (2001) Increased ethanol self-administration in δ-opioid receptor knockout mice. *Alcohol Clin Exp Res* **25:**1249–1256.
Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HWD, Koob GF, and Gold LH (2000) μ-Opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther* **293:**1002–1008.
Sah P, Faber ES, Lopez De Armentia M, and Power J (2003) The amygdaloid complex: anatomy and physiology. *Physiol Rev* **83:**803–834.
Sasaki K, Fan L-W, Tien L-T, Ma T, Loh HH, and Ho IK (2002) The interaction of morphine and γ-aminobutyric acid (GABA)ergic systems in anxiolytic behavior: using μ-opioid receptor knockout mice. *Brain Res Bull* **57:**689–694.
Shen K-Z and Johnson SW (2002) Presynaptic modulation of synaptic transmission by opioid receptor in rat subthalamic nucleus in vitro. *J Physiol (Lond)* **541:**219–230.
Stanford IM and Cooper AJ (1999) Presynaptic μ and δ opioid receptor modulation of $GABA_A$ IPSCs in the rat globus pallidus in vitro. *J Neurosci* **19:**4796–4803.
Sun N and Cassell MD (1993) Intrinsic GABAergic neurons in the rat central extended amygdala. *J Comp Neurol* **330:**381–404.
Veinante P, Stoeckel ME, and Freund-Mercier M-J (1997) GABA- and peptide-immunoreactivities co-localize in the rat central extended amygdala. *Neuroreport* **8:**2985–2989.
Volpicelli JR, Alterman AI, Hayashida M, and O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. *Arch Gen Psych* **49:**876–880.
Williams JT, Christie MJ, and Manzoni O (2001) Cellular and synaptic adaptations mediating opioid dependence. *Physiol Rev* **81:**299–343.
Wilson MA, Mascagni F, and McDonald AJ (2002) Sex differences in δ opioid receptor immunoreactivity in rat medial amygdala. *Neurosci Lett* **328:**160–164.
Yuan XR, Madamba S, and Siggins GR (1992) Opioid peptides reduce synaptic transmission in the nucleus accumbens. *Neurosci Lett* **134:**223–228.
Zhu W and Pan ZZ (2004) Synaptic properties and postsynaptic opioid effects in rat central amygdala neurons. *Neuroscience* **127:**871–879.

Address correspondence to: Scott D. Moore, 116A, 508 Fulton Street, Durham Veterans Administration Medical Center; Building 16, Room 25, Durham, NC 27705. E-mail: sdmoore@duke.edu