Published in final edited form as:
Biol Psychiatry. 2012 April 15; 71(8): . doi:10.1016/j.biopsych.2011.10.032.

Nociceptin/Orphanin FQ Blockade of Corticotropin-Releasing Factor-Induced Gamma-Aminobutyric Acid Release in Central Amygdala Is Enhanced After Chronic Ethanol Exposure

Maureen T. Cruz, **Melissa A. Herman**, **Marsida Kallupi**, and **Marisa Roberto**
Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, California

Abstract

Background—The central nucleus of the amygdala (CeA) mediates stress- and addiction-related processes. Corticotropin-releasing factor (CRF) and nociceptin/orphanin FQ (nociceptin) regulate ethanol intake and anxiety-like behavior. In the rat, CRF and ethanol significantly augment CeA gamma-aminobutyric acid (GABA) release, whereas nociceptin diminishes it.

Methods—Using electrophysiologic techniques in an in vitro slice preparation, we investigated the interaction of nociceptin and CRF on evoked and spontaneous GABAergic transmission in CeA slices of naive and ethanol-dependent rats and the mechanistic role of protein kinase A.

Results—In neurons from naive animals, nociceptin dose-dependently diminished basal-evoked $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) by decreasing GABA release and prevented, as well as reversed, CRF-induced augmentation of IPSPs, actions that required PKA signaling. In neurons from ethanol-dependent animals, nociceptin decreased basal GABAergic transmission and blocked the CRF-induced increase in GABA release to a greater extent than in naive controls.

Conclusions—These data provide new evidence for an interaction between the nociceptin and CRF systems in the CeA. Nociceptin opposes CRF effects on CeA GABAergic transmission with sensitization of this effect in dependent animals. These properties of nociceptin may underlie its anti-alcohol and anxiolytic properties and identify the nociceptin receptor as a useful therapeutic target for alcoholism.

Keywords

Central nucleus of the amygdala; CRF; electrophysiology; N/OFQ; neuroadaptation; nociceptin

Nociceptin/orphanin FQ (N/OFQ; nociceptin) is a 17-aminoacid opioid-like peptide that binds with high affinity to the opioid N/OFQ receptor (NOP), but it does not bind to mu-(μ) (MOP) opioid, delta-(δ) (DOP) opioid, or kappa-(κ) opioid (KOP) receptors. Nociceptin and NOP can be found in the cortex, amygdala, bed nucleus of the stria terminalis, and various fronto-cortical areas (1). Interestingly, these areas are implicated in regulating the motivational properties of drugs of abuse (2–7).

Address correspondence to: Marisa Roberto, Ph.D., Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, SP30-1160, 10550 N. Torrey Pines Road, La Jolla, CA 92037; mroberto@scripps.edu.

The authors reported no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

Behavioral studies suggest a role for the nociceptin-NOP system in alcohol addiction. Nociceptin inhibits stress-induced ethanol seeking, attenuates ethanol self-administration and conditioned reinstatement of alcohol seeking in alcohol-preferring rats, and has anxiolytic-like and anti-stress actions (8 –12). Ethanol-dependent rats exhibit neuroadaptive changes in nociceptin function that are associated with increased anxiety-like behavior and alcohol intake and display enhanced sensitivity to nociceptin (13). Repeated systemic administration of ethanol alters basal nociceptin levels in several brain regions (14), and site-specific changes in nociceptin levels have been demonstrated in alcohol-preferring rodent lines (15).

Application of gamma-aminobutyric acid (GABA) antagonists into the central nucleus of the amygdala (CeA) blocks the acute reinforcing effect of alcohol (16,17). We demonstrated that acute ethanol increases presynaptic GABA release in both rat (18) and mouse (19) CeA neurons. Like ethanol, corticotropin-releasing factor (CRF) augments $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs), and ethanol-induced augmentation of IPSCs involves presynaptic CRF_1 receptors (CRF_1) (19,20). Ethanol-dependent rats show enhanced basal GABA transmission, and acute ethanol increased evoked IPSC amplitudes and the miniature IPSC (mIPSC) frequency to an extent equivalent to that in neurons from naive rats, suggesting a lack of tolerance to acute ethanol (21). Ethanol-dependent rats also exhibit heightened sensitivity to CRF and CRF_1 antagonists (20). Nociceptin decreases basal GABAergic transmission and prevents the ethanol-induced augmentation of GABA IPSCs in the CeA of naive rats (13).

The ability of nociceptin to reduce ethanol intake suggests that this peptide may act as an endogenous functional antagonist of CRF_1 (5,11,12). For example, nociceptin is known to oppose the anorectic effect of CRF in rats (6,9,22). Currently, the mechanisms underlying functional antagonism of CRF_1 by nociceptin are not understood. Here, using electrophysiologic techniques in an in vitro slice preparation, we compared the action of nociceptin on basal GABAergic transmission and on the CRF-induced effects at GABAergic synapses in CeA neurons from naive and ethanol-dependent rats.

We recently reported that type 7 adenylate cyclase (AC7) plays an important role in ethanol and CRF modulation of presynaptic GABA release in the CeA (23). Protein kinase A (PKA) is inhibited by NOP receptors (via G_i protein) (24) and is also activated by CRF_1 (via G_s and G_q proteins) (25,26). Here, we investigated the role of PKA in the nociceptin-induced decrease and CRF-induced increase in spontaneous CeA GABA release in naive rats.

We found that nociceptin blocks acute CRF-induced augmentation of IPSPs in both naive and ethanol-dependent rats by inhibiting GABA release, and PKA signaling is required in this mechanism. In ethanol-dependent rats, nociceptin-induced inhibition of IPSPs was stronger than that in naive rats, suggesting an upregulation of the nociceptin system associated with the development of ethanol dependence.

Methods and Materials

Slice Preparation

As previously described (18,21), we prepared CeA slices from male Sprague-Dawley rats (150 – 300 g; 7–9 weeks old) that were anesthetized with halothane (3%) and decapitated.

Chronic Ethanol Treatment

We used the standard ethanol inhalation method of The Scripps Research Institute Alcohol Research Center to induce ethanol dependence (Supplement 1) (20,27). On experiment days, the chronic ethanol-treated rats were maintained in the ethanol vapor chamber until

preparation of slices (under ethanol-free conditions). We recorded from cells 2 to 8 hours after cutting the slices (depending on the amount of time required to locate and record from a viable cell), as previously described (21).

Blood Alcohol Level

We determined blood alcohol levels of the ethanol-dependent animals from tail-blood samples. The target range for blood alcohol levels was 150 to 200 mg%. Control animals were routinely sampled to control for possible effects of handling.

Intracellular Recording

We recorded from neurons in the medial subdivision of the CeA with sharp micropipettes filled with 3M KCl using discontinuous current-clamp mode (20,21). We held most neurons near their resting membrane potentials. We evoked pharmacologically isolated $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) by stimulating locally within the CeA through a bipolar stimulating electrode (Supplement 1). We examined paired-pulse facilitation (PPF) using paired stimuli at 50- and 100-msec inter-stimulus intervals (21,28,29). The stimulus strength was adjusted such that the amplitude of the first IPSP was half-maximal.

All measures were taken before drug superfusion (control), during drug superfusion (10 – 15 min), and following washout (20 – 30 min) and expressed as mean ± SEM. We used *t* test analyses for individual means comparisons (e.g., Figure 1B) and within-subject one-way repeated-measures analysis of variance (RM-ANOVA) to compare IPSPs within a group (naive or dependent). When appropriate, the Student Newman-Keuls post hoc test was used to assess significance between treatments (e.g., Figures 1, 3, and 4) with $p \leq .05$ considered significant. To assess differences resulting from ethanol exposure (naive × dependent) and drug interaction between groups, we used two-way RM ANOVA (e.g., Figure S3 in Supplement 1).

Whole-Cell Patch-Clamp Recording of Miniature IPSCs

We used the whole-cell patch-clamp method in the presence of 10 μmol/mL CNQX, 30 μmol/mL APV, 1 μmol/mL CGP 55845A, and 1 μmol/mL tetrodotoxin to isolate mIPSCs. All cells were clamped at − 60 mV (Supplement 1). All data are expressed as mean ± SEM. We used cumulative probability analysis and determined statistical significance using the Kolmogorov-Smirnov and nonparametric, two-sample *t* test, with $p < .05$ considered significant.

Drugs

We purchased AP5, CNQX, CGP 55845, CRF, PKI, [$Nphe^1$]Nociceptin-(1–13)NH_2, and nociceptin from Tocris Bioscience (Ellisville, Missouri) and tetrodotoxin, bicuculline, and Rpadenosine 3′,5′-cyclic mono-phosphorothioate triethylammonium salt hydrate (Rp-cAMP) from Sigma (St. Louis, Missouri).

Results

Nociceptin Reverses CRF-Induced Enhancement of GABAergic Synaptic Transmission

We recorded intracellularly from 104 CeA neurons by stimulating locally within the CeA. The mean resting membrane potential of these CeA neurons was −76.7 ± 1.3 mV with input resistance of 131.5 ± 5.8 MΩ.

In CeA neurons of naive rats, nociceptin dose-dependently reduced the mean amplitude of evoked IPSPs (Figures 1A and 1B). The lowest concentration of nociceptin tested (100 nmol/mL) produced a slight decrease in evoked IPSPs that was not statistically significant ($p > .05$; $df = 6$; $t = 2.22$), whereas 500 nmol/mL nociceptin significantly ($p < .05$; $df = 11$; $t = 13.78$) reduced the IPSP amplitude. This effect was reversible upon washout. The highest concentration of nociceptin tested (1 μmol/mL) significantly ($p < .05$; $df = 6$; $t = 2.91$) diminished evoked IPSPs with only partial recovery during washout (Figures 1A and 1B). Therefore, we used the maximally effective and reversible concentration of nociceptin, 500 nmol/mL throughout this study unless specified otherwise (e.g., Figure 3). A maximally effective concentration (20) of 200 nmol/mL CRF significantly ($p < .001$; $df = 7$; $t = 10.033$) increased evoked IPSPs in CeA neurons from naive rats (Figures 1A and 1B).

On the basis of behavioral studies of nociceptin and stress (8,11,12,30) and our cellular data on nociceptin (13) and CRF (20), we hypothesized that the anxiolytic and antistress properties of nociceptin may involve an interaction between nociceptin and CRF systems at GABAergic CeA synapses. Thus, to test for nociceptin-CRF interaction, we applied the two peptides concomitantly. We first applied 200 nmol/mL CRF and then coapplied 500 nmol/mL nociceptin onto CeA slices (Figure 1C). The statistics presented here refer to data collected at half-maximal stimulation intensities as determined from I/O relationships generated for each neuron. Overall ANOVA revealed a significant effect [$F(2,7) = 25.88$, $p < .01$] and post hoc analysis revealed that CRF significantly ($p < .01$) increased the mean amplitude of evoked IPSPs (137.3 ± 1.3% of control; Figure 1C). Subsequent application of nociceptin significantly ($p < .01$) reduced the mean evoked IPSP amplitude to 85.9 ± 1.1% of control with recovery upon washout (Figure 1C).

To determine the site of action of CRF, we examined PPF of IPSPs using 50- and 100-msec interstimulus intervals. CRF significantly [$F(2,8) = 7.64$, $p < .01$ for 50 msec and $F(2,8) = 15.63$, $p < .01$ for 100 msec] decreased the PPF ratio of IPSPs (Figure 1D). The ability of nociceptin to reverse the CRF-induced increase in IPSP amplitude correlated with a significant ($p < .01$) increase in the 50 and 100 msec PPF ratio; these effects returned to baseline values after washout.

We performed whole-cell patch clamp recordings of mIPSCs to confirm that CRF increased presynaptic GABA release in the CeA. Generally, an increase in mIPSC frequency suggests increased probability of transmitter release, and an increase in mIPSC amplitude reflects increased sensitivity of postsynaptic $GABA_A$ receptors (31,32). CRF significantly [$F(2,4) = 34.36$; $p < .01$] increased the mean frequency of mIPSCs to 146.8 ± 7.9% of control (control: .60 ±.08 Hz; CRF: .87 ±.12 Hz; $n = 5$; Figures 2A and 2B) and shifted the cumulative frequency distribution toward shorter interevent intervals, but had no effect on mean mIPSC amplitudes (control: 44.2 ± 5.8 pA; CRF: 46.1 ± 7.2 pA; Figures 2C and 2D), suggesting that the increase in mean evoked IPSP amplitude was due to increased presynaptic GABA release. CRF did not significantly ($p > .05$; $n = 5$) alter the decay or rise time of mIPSCs (Figure 2B). Subsequent cosuperfusion of nociceptin reversed the effects of CRF on mIPSC frequency ($p < .01$) and did not alter amplitude and kinetics (Figures 2A–2D).

Nociceptin Prevents the CRF-Induced Enhancement of GABAergic Synaptic Transmission

Because nociceptin counteracted the effects of CRF, we tested whether nociceptin could occlude the effects of CRF on GABA transmission. We inverted the order of the peptides and first applied 500 nmol/mL nociceptin and then coapplied 200 nmol/mL CRF (Figure 1E). Overall ANOVA demonstrated a significant effect of drug application [$F(2,15) = 43.13$, $p < .01$]. Post hoc analysis demonstrated that nociceptin significantly ($p < .01$) reduced IPSP amplitude. Interestingly, coapplication of CRF did not alter ($p > .05$) evoked

IPSP amplitudes (Figure 1E). Nociceptin occluded CRF-induced enhancement of IPSPs, and GABA transmission returned to baseline levels after 25 min of washout. To verify that CRF could increase IPSP amplitude in these neurons, we washed out both peptides for at least 30 min then applied CRF alone for 15 min. CRF responsivity was restored and this CRF-induced increase was reversible after 20 min washout ($n = 5$, Figure 3B).

Nociceptin significantly [$F(2,8) = 4.025$; $p < .05$ for 50 msec and $F(2,8) = 5.71$; $p < .05$ for 100 msec] increased the PPF ratio of IPSPs (Figure 1F), suggesting a decrease in presynaptic GABA release. In addition, CRF applied during ongoing nociceptin superfusion did not alter ($p > .05$) either the 50- or 100-msec PPF ratio. Rather, the increase in PPF ratio persisted and returned to baseline values during washout (Figure 1F). Nociceptin significantly [$F(2,4) = 13.90$; $p < .05$] decreased the mIPSC frequency to 77.1 ±2.4% of control in all five CeA neurons from naive rats (baseline: .58 ± .1 Hz; nociceptin: .44 ± .07 Hz; Figures 2E and 2F) and shifted the cumulative frequency distribution to longer interevent intervals (Figure 2G), suggesting that nociceptin reduces vesicular release of GABA. Nociceptin did not significantly ($p > .05$) alter the amplitude (control: 51.2 ± 5.07 pA; nociceptin: 49.3 ± 6.07 pA; Figures 2F and 2H) or decay or rise time (Figure 2F) of mIPSCs. Importantly, subsequent coapplication of CRF did not increase mIPSC frequency (.49 ±.1 Hz; Figure 2F) suggesting that nociceptin prevents CRF from increasing GABA release. CRF also did not alter amplitude, decay, and rise time under these conditions (Figure 2F). Consistent with previous findings (20), CRF applied alone significantly increased mIPSC frequency (Figures 2A).

Nociceptin-CRF Interaction Is Greater After Chronic Ethanol Exposure

We then examined whether chronic ethanol exposure would alter the nociceptin-CRF interaction in the CeA. In neurons of dependent rats, superfusion of 500 nmol/mL nociceptin reduced evoked IPSP amplitudes. Overall ANOVA indicated a significant effect [$F(2,10) = 45.6$, $p < .01$]. Post hoc analysis revealed that nociceptin significantly ($p < .01$) reduced IPSP amplitude (Figure 4A). Subsequent coapplication of CRF with nociceptin did not alter IPSP amplitude ($p > .05$). This effect was reversible upon washout. Two-way (ethanol exposure × drug) RM ANOVA indicated that nociceptin induced a significantly [$F(1,25) = 47.55$; $p < .05$] larger decrease in IPSPs in ethanol-dependent rats than in naive rats (Figure 4A vs. Figure 1E). Thus, in accord with our previous studies (13), nociceptin had an enhanced effect on IPSPs in CeA neurons of ethanol-dependent animals.

Notably, the baseline 50 and 100 msec PPF ratios were lower in ethanol-dependent rats (.91 ± .05 and .96 ± .07, respectively; Figure 4B) than in naive rats (1.07 ± .07 and 1.17 ± .08, respectively; Figures 1D and 1F), suggesting increased basal GABA release in dependent rats. Nociceptin alone significantly [$F(1,17) = 18.88$; $p < .01$) increased the 50- and 100-msec PPF ratios (Figure 4B) and significantly [$F(2,8) = 16.79$; $p < .01$] decreased mIPSC frequency (Figure 4D) without altering decay or rise time of mIPSCs (data not shown). In ethanol-dependent rats, the baseline frequency (.98 ±.2 Hz; $n = 5$) of mIPSCs was higher than that observed in naive controls (.58 ± .1 Hz; $n = 5$). Co-application of CRF with nociceptin did not alter the nociceptin-induced increase in PPF ($n = 8$; Figure 4B), mIPSP frequency, amplitude (Figure 4D) or kinetics (data not shown). Thus, in all measurements, nociceptin functionally antagonized the characteristic effects of CRF on GABA transmission.

The strong interaction of CRF and nociceptin prompted us to test whether a low concentration of nociceptin (100 nmol/mL) could antagonize an efficacious dose of CRF in both naive and ethanol-dependent rats. RM ANOVA (within-subject analysis for drug effect and between-subject analysis for ethanol exposure) indicated a significant main effect of drug treatment on IPSPs [$F(2,14) = 3.59$, $p < .05$]. Although 100 nmol/mL nociceptin had

no significant effect ($p > .05$) on evoked IPSP amplitudes in naive rats (Figures 3A and 3C), it still prevented the CRF-induced increase of IPSPs. This suggests that the anti-CRF actions of nociceptin were due to functionally independent actions and not simply to the summation of opposing effects on GABA release. Interestingly, in CeA neurons from ethanol-dependent rats, 100 nmol/mL nociceptin significantly ($p < .01$) decreased basal evoked IPSPs to 71.5 ± 7% of control (Figure 3D). Coapplication of CRF (200 nmol/mL) with nociceptin did not significantly ($p < .05$) increase the evoked IPSP amplitude compared with application of nociceptin alone.

Interestingly, 100 nmol/mL nociceptin significantly [$F(2,6) = 6.07$; $p < .05$] increased the 50 msec PPF ratio of IPSPs (Figure S1A in Supplement 1), but it did not have a significant ($p > .05$) effect on the 100 msec PPF, suggesting a weak effect on presynaptic GABA release. In addition, CRF applied after 100 nmol/mL nociceptin did not significantly alter the PPF ratio of IPSPs ($p > .05$).

Next, we tested a selective NOP antagonist ([Nphe^1]Nociceptin(1–13)NH_2) (33,34) (Figure S2 in Supplement 1). In naive rats, the NOP antagonist (1 μmol/mL) had no effect ($df = 6$; $t = 1.72$; $p > .05$) on basal evoked IPSPs, but significantly increased ($df = 8$; $t = 12.7$; $p < .01$) evoked IPSP amplitude by 20 ± 5% in neurons of ethanol-dependent rats, suggesting constitutive activation of NOPs associated with ethanol-dependence.

We examined the ability of nociceptin to antagonize the pronounced effect of CRF pretreatment in cells from ethanol-dependent rats. First, we superfused 200 nmol/mL CRF followed by coapplication of 500 nmol/mL nociceptin. In neurons from dependent rats, CRF significantly [$F(2,19) = 65.8$; $p < .01$] increased the mean IPSP amplitude to 153.8 ± 1.1% of control (Figure 4E). The maximum IPSP amplitude occurred after 10 to 15 min of CRF superfusion (Figure 4F). Subsequent coapplication of nociceptin significantly ($p < .01$) reversed the CRF effect on IPSP amplitudes (Figures 4E and 4F). The mean CRF-induced increase in IPSP amplitude in ethanol dependent rats was significantly ($p < .05$) larger than that observed in naive rats (Figure S3 in Supplement 1). Two-way (ethanol exposure × drug) RM ANOVA indicated a significant main effect of ethanol vapor treatment on IPSP amplitudes in CeA neurons [$F(2,1) = 3.798$, $p < .05$]. Also, application of nociceptin during ongoing CRF superfusion significantly [$F(2,10) = 24.087$] altered the 50-msec PPF ratio (Figure S1B in Supplement 1). CRF significantly ($p < .01$) reduced the 50-msec PPF ratio and coapplication of nociceptin significantly increased it ($p < .01$), suggesting that nociceptin counteracts CRF effects by diminishing presynaptic release of GABA. Similar effects were observed for the 100-msec PPF ($n = 11$; data not shown).

Inhibition of PKA Blocks Nociceptin and CRF Effects on GABA Release in Naive Rats

The PKA signaling pathway is downstream of $G\alpha_{i/s}$ activation (24,25). Therefore, we determined whether inhibiting the PKA pathway would occlude nociceptin from decreasing GABA release using Rp-cAMP, a PKA antagonist that occupies cAMP binding sites (35). Application of Rp-cAMP (10 μmol/mL) decreased mean baseline mIPSC frequency to 83.9 ± 7.5% of control ($p < .05$; $df = 17$; $t = 3.510$; data not shown). Rp-cAMP also prevented nociceptin from decreasing mIPSC frequency (98.6 ± 16.9% of control/Rp-cAMP; $p > .05$, $n = 5$; Figures 5A, 5B, and 5E). These results suggest that PKA plays a role in nociceptin-induced attenuation of spontaneous GABA release and are also consistent with the established role of PKA in neurotransmitter release (36 –38). To determine the site of action of the PKA antagonist, we included the membrane impermeable PKA antagonist, PKI (5 μmol/mL), in the pipette internal solution, thereby limiting the antagonist to the postsynaptic neuron (39). With PKI in the pipette, nociceptin still significantly decreased mean mIPSC frequency (48 ±3.9% of control, n ±6; Figures 5C and 5E). This effect was comparable to the nociceptin-induced decrease (58 ± 6.3%, $n = 5$) elicited with the control internal

solution. A cumulative probability curve shows that in the presence of PKI, nociceptin still shifts the interevent interval curve to the right (Figure 5C, right panel). These results suggest that PKA, acting in the presynaptic neuron, is a critical intermediary in the nociceptin-induced decrease in GABA release.

We then asked if the PKA pathway was involved in CRF effects on GABA release. Rp-cAMP (10 μmol/mL) prevented CRF from enhancing mIPSC frequency (107.1 μ 6.2% of control; $p > .05$, $n = 9$; Figures 6A, 6B, and 6E). With 5 μmol/mL PKI in the internal solution, CRF still significantly ($p < .05$) increased mIPSC frequency (177.4 ± 28.6% of control, $n = 4$; Figures 6C and 6E). This effect was comparable to the increase (163.1 ±22.07%, $n = 8$) observed with application of CRF and the control internal solution. A cumulative probability curve shows that CRF still shifts the interevent interval curve to the left with PKI in the internal solution (Figure 6C, right panel). These results suggest that PKA may represent a common intermediary in the presynaptic mechanism for the opposing actions of nociceptin and CRF on GABA release in CeA.

Discussion

To our knowledge, there are no previous electrophysiologic studies on the interaction between the nociceptin and CRF systems on GABAergic synapses in the CeA and the influence of alcohol dependence on this interaction. Here, we find that nociceptin pre-treatment blocks CRF-stimulated GABA release. Additionally, when applied subsequent to CRF, nociceptin reverses the action of CRF. The ability of nociceptin to oppose the CRF effect was stronger in ethanol-dependent rats. Our data also indicate that the PKA pathway plays an essential role in the effects of nociceptin and CRF on GABA release.

Within the CeA, CRF is expressed in GABAergic neurons, where it is coreleased with GABA (40,41). Both acute ethanol (18,21) and CRF (20) enhance GABAergic transmission via increased GABA release mediated by CRF_1 activation (19,23). Nociceptin decreases basal GABA transmission and opposes the effects of acute ethanol in rat CeA neurons (13).

Behavioral and neurochemical evidence has implicated the nociceptin-NOP system in the reinforcing effects of ethanol (6 –7,42) and suggests that nociceptin impedes the rewarding effects of ethanol, thereby reducing intake (9). Nociceptin reduces ethanol self-administration and conditioned reinstatement of alcohol-seeking behavior in ethanol-preferring rats (9 –12), inhibits stress-induced ethanol seeking (8) and attenuates withdrawal-related anxiety (12). Nociceptin putatively exerts general compensatory antistress-like effects by acting as a functional antagonist of extra-hypothalamic CRF_1 activation (6) and also blocks the anxiogenic effects of CRF (43).

In this study, nociceptin and CRF had opposite effects on CeA GABAergic transmission at the presynaptic level. Nociceptin diminished GABA transmission and blocked CRF-induced IPSP facilitation, indicating that nociceptin interferes with CRF effects and preempts the action of CRF in CeA neurons. NOP receptor activation is known to inhibit adenylate cyclase and decrease PKA activity (44) and the nociceptin-induced decrease of spontaneous GABA release in CeA involves PKA inhibition (45). PKA is also one of the second messengers engaged by CRF receptor activation (23,25). Here we demonstrated that a PKA antagonist blocked both nociceptin and CRF from regulating spontaneous GABA release, whereas a PKA antagonist limited to the postsynaptic neuron did not alter either nociceptin or CRF action on GABA release. These results suggest that the presynaptic PKA pathway represents a common signaling pathway targeted in opposing directions by nociceptin and CRF in regulating GABA release, thus playing an essential role in their interactions (24,46).

Importantly, the interaction between nociceptin and CRF is more than a mere summation of opposing effects because the inhibition of IPSP amplitudes by nociceptin constitutes complete blockade of the large amplitude increase elicited by CRF alone. Adding nociceptin to the superfusate containing CRF completely reversed the CRF-induced augmentation of evoked IPSPs and further decreased IPSP amplitudes to 85% of control. Furthermore, a low concentration of nociceptin that does not significantly decrease IPSP amplitudes in CeA neurons from naive rats still blocks the action of CRF. Thus, the inhibitory modulation of GABA release by nociceptin may either occur at a different (e.g., more proximal) intracellular site than that of CRF, or the two peptides may oppose each other's action at the same site (e.g., cAMP/PKA). Further studies are needed to elucidate the exact molecular mechanisms and sites involved.

During ethanol withdrawal, extrahypothalamic CRF systems become hyperactive, with increased extracellular CRF within the CeA (47 – 48). Notably, at the cellular level, we found that both basal GABA release and nociceptin-induced inhibition of IPSPs is augmented in CeA neurons of ethanol-dependent rats. The latter indicates enhanced sensitivity to nociceptin. The dependent state is also associated with an upregulation of the CRF system (20). Here we find that the interactive effects of nociceptin and CRF on GABAergic transmission in the CeA undergo significant neuroadaptation (enhancement) during ethanol dependence. On the basis of our experimental conditions, we cannot rule out the possibility that these effects are induced by ethanol withdrawal. However, preliminary data (Cruz et al., unpublished data) show that the increased sensitivity of GABAergic synapses to CRF persists 1 week after the cessation of ethanol vapor exposure, suggesting that these changes are not due to acute withdrawal. Similarly, we have shown that chronic ethanol treatment induces long-term changes in glutamatergic transmission (49,50). Future studies will characterize the persistence of the nociceptin/CRF interaction.

The effects of CRF on GABA transmission in the CeA may contribute to development of enhanced anxiety-like behaviors and dependence-related excessive alcohol consumption. Upregulation of the nociceptin system may reflect an adaptive response to a hyperactive extrahypothalamic CRF system. The nociceptin receptor antagonist ([Nphe^1]Nociceptin(1–13)NH_2) did not alter basal evoked IPSP amplitudes in naive rats but significantly increased IPSP amplitudes in ethanol-dependent rats (Figure S2 in Supplement 1) (13). These findings suggest a neuroadaptation of the nociceptin system during ethanol dependence (i.e., increased nociceptin tone, increased sensitivity of NOPs, or both) that may account for the increased sensitivity of the CeA GABA system. This enhancement of nociceptin-NOP receptor activity may represent an attempt to restore functional equilibrium in the CeA. Moreover, recent data (12) indicate that nociceptin prevents anxiety-like responses associated with ethanol withdrawal, suggesting that the anxiolytic-like effects of nociceptin reflect antagonism of CRF_1 and inhibition of ethanol- and CRF-induced GABA release. Blockade of CRF_1 with R121919 does not prevent nociceptin-induced decreases in GABAergic response (Figure S4 in Supplement 1), ruling out noncompetitive allosteric pharmacologic antagonism of CRF_1.

Although nociceptin has anxiolytic properties, it depresses GABA transmission in a brain region known to be important in mediating stress responses. We recorded from the medial subdivision of the CeA (51,52), and the total neuronal population responded equally to nociceptin by decreasing GABAergic responses. These CeA neurons are most likely GABAergic inhibitory neurons with recurrent or feedback connections to GABAergic interneurons or projection neurons (53,54). When nociceptin depresses inhibitory function in these neurons, it may inhibit downstream regions (e.g., bed nucleus of the stria terminalis) by increasing GABA and CRF release from projection neurons. Further investigation is needed to shed light on the amygdalar circuitry involved and the neuron types affected by

nociceptin and CRF. However, nociceptin has a similar inhibitory effect on CeA GABA transmission, as do other recognized anxiolytics such as gabapentin (55), cannabinoids (56), CRF_1 antagonists (20), and neuropeptide Y (57).

There is evidence that opiate receptor antagonists decrease the primary reinforcing effects of ethanol in animal models (16,42,58) and reduce relapse rates in animals (16) and in abstinent human alcoholics (59 – 61). The anti-anxiety and anti-stress properties of nociceptin (6,8,11,12) and its specificity for ethanol-seeking behavior (8) suggest that small molecule mimetics of nociceptin may be an effective therapy for alcoholism. Nociceptin prevents the anxiogenic effects of CRF and yet does not block hypothalamic-pituitary-adrenal axis responses to stressors (62), further supporting investigation of nociceptin or other agonists of the NOP receptor as possible treatment candidates for alcoholism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This is manuscript number 20852 from the Scripps Research Institute. We thank Drs. Floyd E. Bloom, George R. Siggins, George F. Koob, and Nicholas W. Gilpin for their valuable comments. We thank Christopher S. Oleata and Samuel Madamba for technical support. This work was supported by the Pearson Center for Alcoholism and Addiction Research and National Institute on Alcohol Abuse and Alcoholism Grant Nos. AA015566, AA06420, AA016985, and AA017447.

References

1. Neal CR Jr, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Watson SJ Jr. Localization of orphanin FQ (nociceptin) peptide and messenger RNA in the central nervous system of the rat. J Comp Neurol. 1999; 406:503–547. [PubMed: 10205026]
2. Everitt BJ, Wolf ME. Psychomotor stimulant addiction: A neural systems perspective. J Neurosci. 2002; 22:3312–3320. [PubMed: 11978805]
3. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, et al. Effects of moderate alcohol consumption on the central nervous system. Alcohol Clin Exp Res. 1998; 22:998–1040. [PubMed: 9726269]
4. Wise RA. Drug-activation of brain reward pathways. Drug Alcohol Depend. 1998; 51:13–22. [PubMed: 9716927]
5. Martin-Fardon R, Zorrilla EP, Ciccocioppo R, Weiss F. Role of innate and drug-induced dysregulation of brain stress and arousal systems in addiction: Focus on corticotropin-releasing factor, nociceptin/ orphanin FQ, and orexin/hypocretin. Brain Res. 2010; 1314:145–161. [PubMed: 20026088]
6. Ciccocioppo R, Economidou D, Fedeli A, Massi M. The nociceptin/ orphanin FQ/NOP receptor system as a target for treatment of alcohol abuse: A review of recent work in alcohol-preferring rats. Physiol Behav. 2003; 79:121–128. [PubMed: 12818717]
7. Ciccocioppo R, Angeletti S, Panocka I, Massi M. Nociceptin/orphanin FQ and drugs of abuse. Peptides. 2000; 21:1071–1080. [PubMed: 10998542]
8. Martin-Fardon R, Ciccocioppo R, Massi M, Weiss F. Nociceptin prevents stress-induced ethanol- but not cocaine-seeking behavior in rats. Neuroreport. 2000; 11:1939–1943. [PubMed: 10884047]
9. Ciccocioppo R, Economidou D, Fedeli A, Angeletti S, Weiss F, Heilig M, et al. Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/orphanin FQ in alcohol-preferring rats. Psychopharmacology (Berl). 2004; 172:170–178. [PubMed: 14624331]
10. Ciccocioppo R, Panocka I, Polidori C, Regoli D, Massi M. Effect of nociceptin on alcohol intake in alcohol-preferring rats. Psychopharmacology (Berl). 1999; 141:220–224. [PubMed: 9952048]

11. Economidou D, Hansson AC, Weiss F, Terasmaa A, Sommer WH, Cippitelli A, et al. Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. Biol Psychiatry. 2008; 64:211–218. [PubMed: 18367152]
12. Economidou D, Cippitelli A, Stopponi S, Braconi S, Clementi S, Ubaldi M, et al. Activation of brain NOP receptors attenuates acute and protracted alcohol withdrawal symptoms in the rat. Alcohol Clin Exp Res. 2011; 35:747–755. [PubMed: 21223310]
13. Roberto M, Siggins GR. Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. Proc Natl Acad Sci U S A. 2006; 103:9715–9720. [PubMed: 16788074]
14. Lindholm S, Ploj K, Franck J, Nylander I. Nociceptin/orphanin FQ tissue concentration in the rat brain. Effects of repeated ethanol administration at various post-treatment intervals. Prog Neuropsychopharmacol Biol Psychiatry. 2002; 26:303–306. [PubMed: 11817508]
15. Ploj K, Roman E, Gustavsson L, Nylander I. Basal levels and alcohol-induced changes in nociceptin/orphanin FQ, dynorphin, and enkephalin levels in C57BL/6J mice. Brain Res Bull. 2000; 53:219–226. [PubMed: 11044599]
16. Heyser CJ, Roberts AJ, Schulteis G, Koob GF. Central administration of an opiate antagonist decreases oral ethanol self-administration in rats. Alcohol Clin Exp Res. 1999; 23:1468–1476. [PubMed: 10512312]
17. Hyytia P, Koob GF. GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. Eur J Pharmacol. 1995; 283:151–159. [PubMed: 7498304]
18. Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci U S A. 2003; 100:2053–2058. [PubMed: 12566570]
19. Nie Z, Schweitzer P, Roberts AJ, Madamba SG, Moore SD, Siggins GR. Ethanol augments GABAergic transmission in the central amygdala via CRF1 receptors. Science. 2004; 303:1512–1514. [PubMed: 15001778]
20. Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, et al. Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. Biol Psychiatry. 2010; 67:831–839. [PubMed: 20060104]
21. Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Increased GABA release in the central amygdala of ethanol-dependent rats. J Neurosci. 2004; 24:10159–10166. [PubMed: 15537886]
22. Ciccocioppo R, Martin-Fardon R, Weiss F, Massi M. Nociceptin/ orphanin FQ inhibits stress- and CRF-induced anorexia in rats. Neuroreport. 2001; 12:1145–1149. [PubMed: 11338181]
23. Cruz MT, Bajo M, Magnoli EM, Tabakoff B, Siggins GR, Roberto M. Type 7 adenylyl cyclase is involved in the ethanol and CRF sensitivity of GABAergic synapses in mouse central amygdala. Front Neurosci. 2011; 4:207. [PubMed: 21258618]
24. Meunier JC. Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. Eur J Pharmacol. 1997; 340:1–15. [PubMed: 9527501]
25. Papadopoulou N, Chen J, Randeva HS, Levine MA, Hillhouse EW, Grammatopoulos DK. Protein kinase A-induced negative regulation of the corticotropin-releasing hormone R1 alpha receptor-extracellularly regulated kinase signal transduction pathway: The critical role of Ser(301) for signaling switch and selectivity. Mol Endocrinol. 2004; 18:624–639. [PubMed: 14657255]
26. Hanoune J, Defer N. Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol. 2001; 41:145–174. [PubMed: 11264454]
27. Rogers J, Wiener SG, Bloom FE. Long-term ethanol administration methods for rats: Advantages of inhalation over intubation or liquid diets. Behav Neural Biol. 1979; 27:466–486. [PubMed: 575037]
28. Zucker RS. Short-term synaptic plasticity. Annu Rev Neurosci. 1989; 12:13–31. [PubMed: 2648947]
29. Bonci A, Williams JT. Increased probability of GABA release during withdrawal from morphine. J Neurosci. 1997; 17:796–803. [PubMed: 8987801]

30. Ciccocioppo R, Biondini M, Antonelli L, Wichmann J, Jenck F, Massi M. Reversal of stress- and CRF-induced anorexia in rats by the synthetic nociceptin/orphanin FQ receptor agonist, Ro 64-6198. Psychopharmacology (Berl). 2002; 161:113–119. [PubMed: 11981590]
31. De Koninck Y, Mody I. Noise analysis of miniature IPSCs in adult rat brain slices: properties and modulation of synaptic GABAA receptor channels. J Neurophysiol. 1994; 71:1318–1335. [PubMed: 8035217]
32. Otis TS, De Koninck Y, Mody I. Lasting potentiation of inhibition is associated with an increased number of gamma-aminobutyric acid type A receptors activated during miniature inhibitory postsynaptic currents. Proc Natl Acad Sci U S A. 1994; 91:7698–7702. [PubMed: 8052645]
33. Calo G, Guerrini R, Bigoni R, Rizzi A, Marzola G, Okawa H, et al. Characterization of [Nphe(1)]nociceptin(1-13)NH(2), a new selective nociceptin receptor antagonist. Br J Pharmacol. 2000; 129:1183–1193. [PubMed: 10725267]
34. Florin S, Leroux-Nicollet I, Meunier JC, Costentin J. Autoradiographic localization of [3H]nociceptin binding sites from telencephalic to mesencephalic regions of the mouse brain. Neurosci Lett. 1997; 230:33–36. [PubMed: 9259457]
35. Lochner A, Moolman JA. The many faces of H89: A review. Cardiovasc Drug Rev. 2006; 24:261–274. [PubMed: 17214602]
36. Chheda MG, Ashery U, Thakur P, Rettig J, Sheng ZH. Phosphorylation of Snapin by PKA modulates its interaction with the SNARE complex. Nat Cell Biol. 2001; 3:331–338. [PubMed: 11283605]
37. Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev. 2005; 85:1303–1342. [PubMed: 16183914]
38. Trudeau LE, Emery DG, Haydon PG. Direct modulation of the secretory machinery underlies PKA-dependent synaptic facilitation in hippocampal neurons. Neuron. 1996; 17:789–797. [PubMed: 8893035]
39. Kelm MK, Criswell HE, Breese GR. The role of protein kinase A in the ethanol-induced increase in spontaneous GABA release onto cerebellar Purkinje neurons. J Neurophysiol. 2008; 100:3417–3428. [PubMed: 18945815]
40. Veinante P, Stoeckel ME, Freund-Mercier MJ. GABA- and peptide-immunoreactivities co-localize in the rat central extended amygdala. Neuroreport. 1997; 8:2985–2989. [PubMed: 9376543]
41. Day HE, Curran EJ, Watson SJ Jr, Akil H. Distinct neurochemical populations in the rat central nucleus of the amygdala and bed nucleus of the stria terminalis: Evidence for their selective activation by interleukin-1beta. J Comp Neurol. 1999; 413:113–128. [PubMed: 10464374]
42. Gianoulakis C. Endogenous opioids and addiction to alcohol and other drugs of abuse. Curr Top Med Chem. 2004; 4:39–50. [PubMed: 14754375]
43. Rodi D, Polidori C, Bregola G, Zucchini S, Simonato M, Massi M. Pro-nociceptin/orphanin FQ and NOP receptor mRNA levels in the fore-brain of food deprived rats. Brain Res. 2002; 957:354–361. [PubMed: 12445978]
44. Hawes BE, Graziano MP, Lambert DG. Cellular actions of nociceptin: Transduction mechanisms. Peptides. 2000; 21:961–967. [PubMed: 10998529]
45. Buzas B, Rosenberger J, Cox BM. Activity and cyclic AMP-dependent regulation of nociceptin/orphanin FQ gene expression in primary neuronal and astrocyte cultures. J Neurochem. 1998; 71:556–563. [PubMed: 9681445]
46. Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, et al. Isolation and structure of the endogenous agonist of opioid receptor- like ORL1 receptor. Nature. 1995; 377:532–535. [PubMed: 7566152]
47. Merlo Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, et al. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci. 1995; 15:5439–5447. [PubMed: 7643193]
48. Olive MF, Koenig HN, Nannini MA, Hodge CW. Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. Pharmacol Biochem Behav. 2002; 72:213–220. [PubMed: 11900791]

49. Roberto M, Bajo M, Crawford E, Madamba SG, Siggins GR. Chronic ethanol exposure and protracted abstinence alter NMDA receptors in central amygdala. Neuropsychopharmacology. 2006; 31:988–996. [PubMed: 16052244]
50. Roberto M, Schweitzer P, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: An in vitro and in vivo analysis. J Neurosci. 2004; 24:1594–1603. [PubMed: 14973247]
51. Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, et al. Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature. 2010; 468:270–276. [PubMed: 21068836]
52. Lopez de Armentia M, Sah P. Firing properties and connectivity of neurons in the rat lateral central nucleus of the amygdala. J Neurophysiol. 2004; 92:1285–1294. [PubMed: 15128752]
53. Lang EJ, Pare D. Synaptic responsiveness of interneurons of the cat lateral amygdaloid nucleus. Neuroscience. 1998; 83:877–889. [PubMed: 9483571]
54. Cassell MD, Freedman LJ, Shi C. The intrinsic organization of the central extended amygdala. Ann N Y Acad Sci. 1999; 877:217–241. [PubMed: 10415652]
55. Roberto M, Gilpin NW, O'Dell LE, Cruz MT, Morse AC, Siggins GR, et al. Cellular and behavioral interactions of gabapentin with alcohol dependence. J Neurosci. 2008; 28:5762–5771. [PubMed: 18509038]
56. Roberto M, Cruz M, Bajo M, Siggins GR, Parsons LH, Schweitzer P. The endocannabinoid system tonically regulates inhibitory transmission and depresses the effect of ethanol in central amygdala. Neuropsychopharmacology. 2010; 35:1962–1972. [PubMed: 20463657]
57. Gilpin NW, Misra K, Herman MA, Cruz MT, Koob GF, Roberto M. Neuropeptide Y opposes alcohol effects on gamma-aminobutyric acid release in amygdala and blocks the transition to alcohol dependence. Biol Psychiatry. 2011; 69:1091–1099. [PubMed: 21459365]
58. Krystal JH, Cramer JA, Krol WF, Kirk GF, Rosenheck RA. Naltrexone in the treatment of alcohol dependence. N Engl J Med. 2001; 345:1734–1739. [PubMed: 11742047]
59. O'Malley SS, Krishnan-Sarin S, Farren C, Sinha R, Kreek MJ. Naltrexone decreases craving and alcohol self-administration in alcohol-dependent subjects and activates the hypothalamopituitary-adreno-cortical axis. Psychopharmacology (Berl). 2002; 160:19–29. [PubMed: 11862370]
60. O'Brien CP. The mosaic of addiction. Am J Psychiatry. 2004; 161:1741–1742. [PubMed: 15465965]
61. Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP. Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry. 1992; 49:876–880. [PubMed: 1345133]
62. Devine DP, Watson SJ, Akil H. Nociceptin/orphanin FQ regulates neuroendocrine function of the limbic-hypothalamic-pituitary-adrenal axis. Neuroscience. 2001; 102:541–553. [PubMed: 11226692]

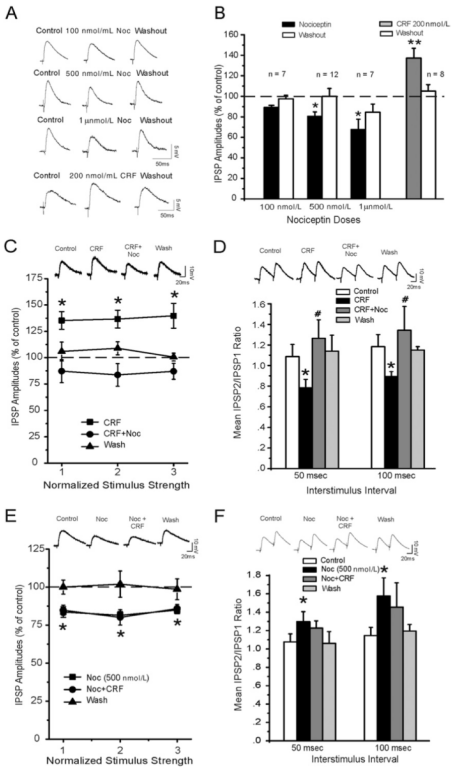

Figure 1.

(A) Nociceptin (Noc) dose-dependently decreases gamma-aminobutyric acid (GABA)ergic transmission in central nucleus of the amygdala (CeA) neurons. Representative recordings of evoked inhibitory postsynaptic potentials (IPSPs) in a CeA neuron from a naive rat recorded before, during Noc or corticotropin-releasing factor (CRF; 20 min), and washout (30 min). **(B)** Histograms representing percent decrease in mean ± SEM evoked IPSP amplitudes. *$p < .05$ and **$p < .01$. **(C)** Top panel: representative recordings of evoked IPSPs. Bottom panel: CRF increases mean IPSP amplitudes and subsequent application of Noc diminishes the CRF-induced effect. **(D)** Top panel: representative recordings of evoked 50-msec paired-pulse IPSPs in a CeA neuron. Bottom panel: CRF significantly decreases the 50- and 100-msec paired-pulse facilitation ratio of IPSPs. #$p < .05$ indicates significance between CRF alone and concurrent application of CRF and Noc. **(E)** Noc prevents CRF-induced enhancement of GABAergic transmission in CeA neurons. Top panel: Representative recordings of evoked IPSPs. Bottom panel: superfusion of 500 nmol/mL Noc significantly decreases mean IPSPs and prevents the CRF-induced enhancement. **(F)** Top panel: representative recordings of evoked 50-msec paired-pulse IPSPs. Bottom panel: Noc significantly increases the 50- and 100-msec paired-pulse facilitation ratio of IPSPs and blocks the CRF-induced decrease in paired-pulse facilitation ratio ($p < .05$).

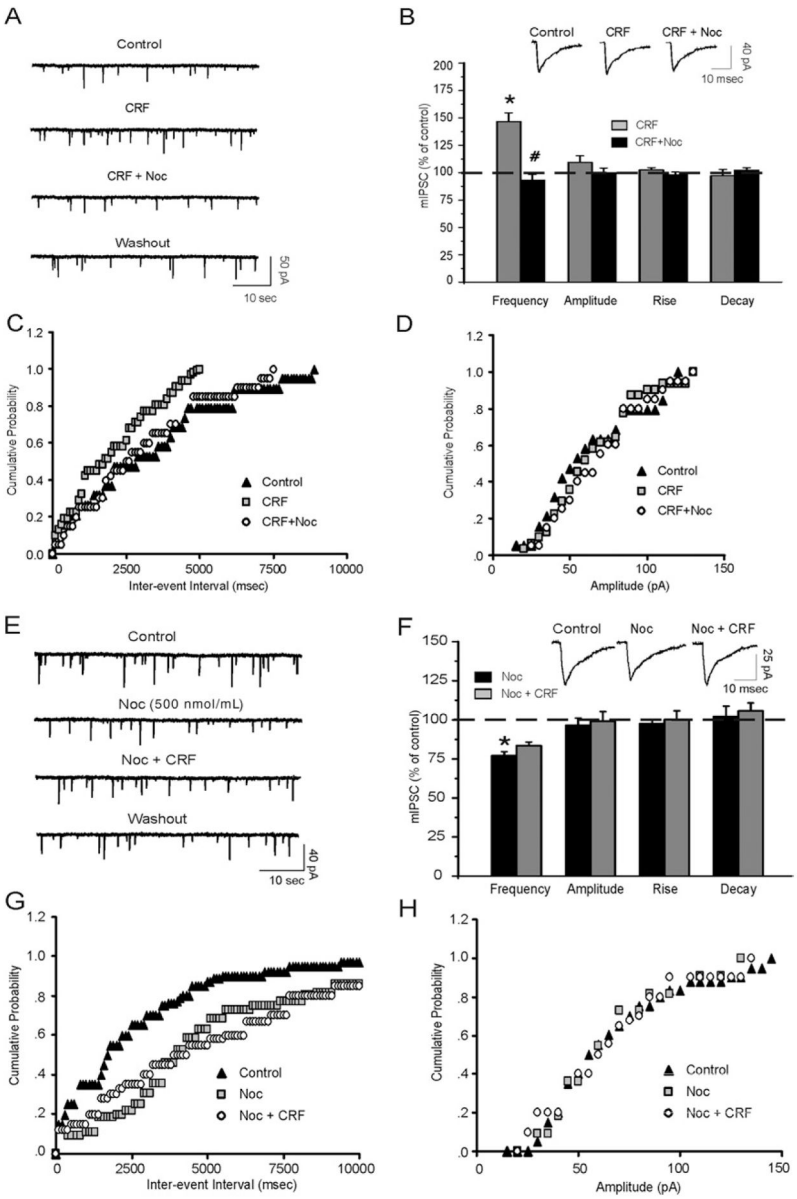

Figure 2.

(A) Representative miniature inhibitory post-synaptic currents (mIPSC) recordings in central nucleus of the amygdala (CeA) neurons from naive rats. **(B)** Top panel: representative recordings of scaled average mIPSCs from the traces depicted in the CeA neuron shown in Figure 2A. Bottom panel: mean ± SEM frequency, amplitude, rise, and decay time of mIPSCs for CeA neurons. Corticotropin-releasing factor (CRF) significantly (*$p < .01$) increased the mean mIPSC frequency but does not change mean mIPSC amplitude, rise, and decay time. #$p < .05$ indicates significance between CRF alone and concurrent application of CRF and nociceptin (Noc). **(C)** Cumulative frequency distribution for the naive neuron in Panel A showing a leftward shift, indicating a shorter interevent interval during CRF application and a rightward shift produced by adding Noc. **(D)** Cumulative mIPSC amplitude distribution from the neuron in Figure 2A showing no significant change during either CRF or concurrent CRF and Noc application. **(E)** Representative mIPSC recordings in CeA neurons from naive rats. **(F)** Top panel: Representative recordings of scaled average

mIPSCs from the traces depicted in the CeA neuron in Panel E. Bottom panel: Mean ± SEM frequency, amplitude, rise and decay time of mIPSCs for CeA neurons from naive rats. Noc significantly (*$p < .05$) decreased the mean mIPSC frequency. **(G)** Cumulative frequency distribution for the naive neuron in Panel E showing a rightward shift, indicating a longer interevent interval during Noc application. The addition of CRF did not change the cumulative frequency curve. **(H)** Cumulative mIPSC amplitude distribution from the neuron in Panel E showing no significant change during either Noc or concurrent Noc and CRF application.

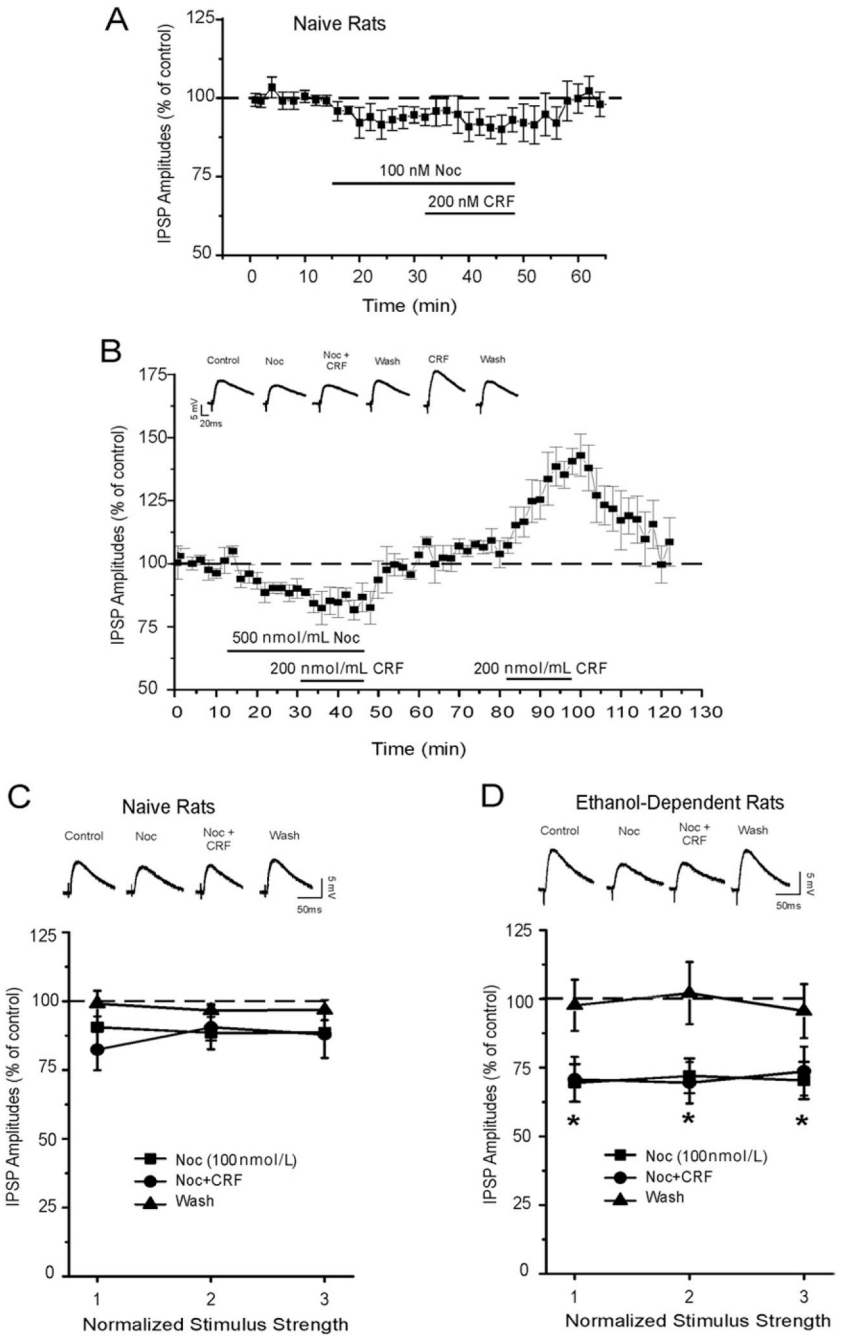

Figure 3.

A low concentration of nociceptin (Noc) prevents corticotropin-releasing factor (CRF)-induced enhancement of gamma-aminobutyric acid (GABA)ergic transmission in central nucleus of the amygdala (CeA) neurons. **(A)** Time course of changes in inhibitory post-synaptic potential (IPSP) amplitude evoked by Noc, concurrent application of CRF (n =7), and washout of the two peptides. **(B)** Top insert: evoked IPSPs in a CeA neuron from a naive rat. Noc decreased evoked IPSP amplitude and blocked the CRF-induced increase in IPSPs. In the same cells, Noc and CRF were washed out and application of CRF alone increased evoked IPSP amplitude. **(C)** Top panel: representative recordings of evoked IPSPs

in a neuron from a naive rat. Bottom panel: superfusion of 100 nmol/mL Noc slightly decreases the mean IPSP amplitude and prevents the enhancement induced by CRF (200 nmol/mL). **(D)** Noc (100 nmol/mL) significantly (*$p < .05$) decreased evoked IPSPs in CeA neurons from ethanol-dependent rats. Coapplication of CRF did not increase evoked IPSP amplitude.

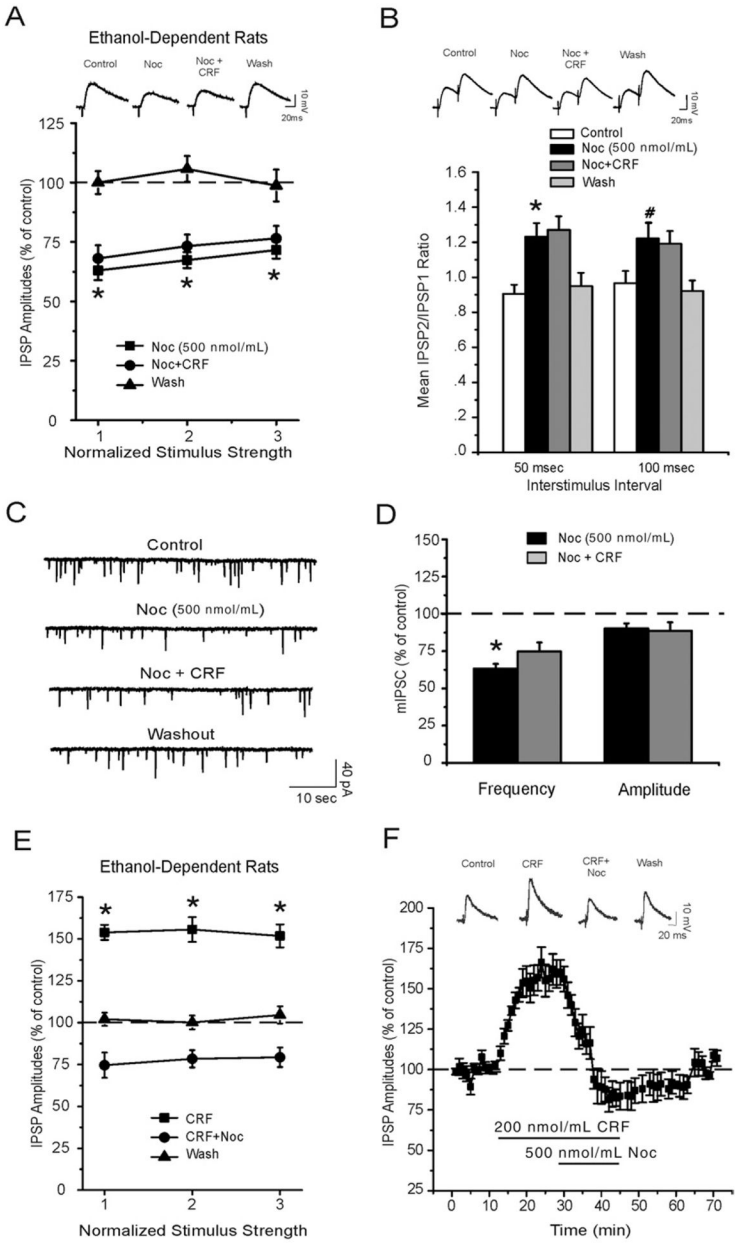

Figure 4.

The effects of the nociceptin (Noc)–corticotropin-releasing factor (CRF) interaction are enhanced in ethanol-dependent rats. **(A)** Top panel: evoked inhibitory postsynaptic potentials (IPSPs) from an ethanol-dependent rat. Bottom panel: Noc (500 nmol/mL) significantly (*$p < .01$) decreases mean IPSP amplitudes and prevents enhancement of IPSPs induced by subsequent application of CRF (200 nmol/mL). **(B)** Top panel: Representative recordings of evoked paired-pulse IPSPs in a central nucleus of the amygdala (CeA) neuron. Bottom panel: Noc significantly increases the mean 50- and 100-msec PPF ratio of IPSPs (*$p < .01$; #$p < .05$). Application of CRF after Noc does not alter paired-pulse facilitation ratio. **(C)** Representative traces of miniature inhibitory postsynaptic currents (mIPSCs) from neurons of ethanol-dependent rats. **(D)** Mean ± SEM frequency and amplitude of mIPSCs for CeA neurons from ethanol-dependent rats. Noc significantly (*$p < .01$) decreased the

mean mIPSC frequency. Subsequent coapplication of CRF did not alter mIPSC frequency. **(E)** Superfusion of CRF alone significantly (*$p < .01$) increases mean IPSP amplitudes. Subsequent application of Noc reverses CRF-induced facilitation of evoked IPSP amplitude. **(F)** Top panel: representative recordings of evoked CeA IPSPs from an ethanol-dependent rat. Bottom panel: time course depicting changes in IPSP amplitude evoked by CRF, concurrent application of CRF and Noc ($n = 6$), and washout of the two peptides.

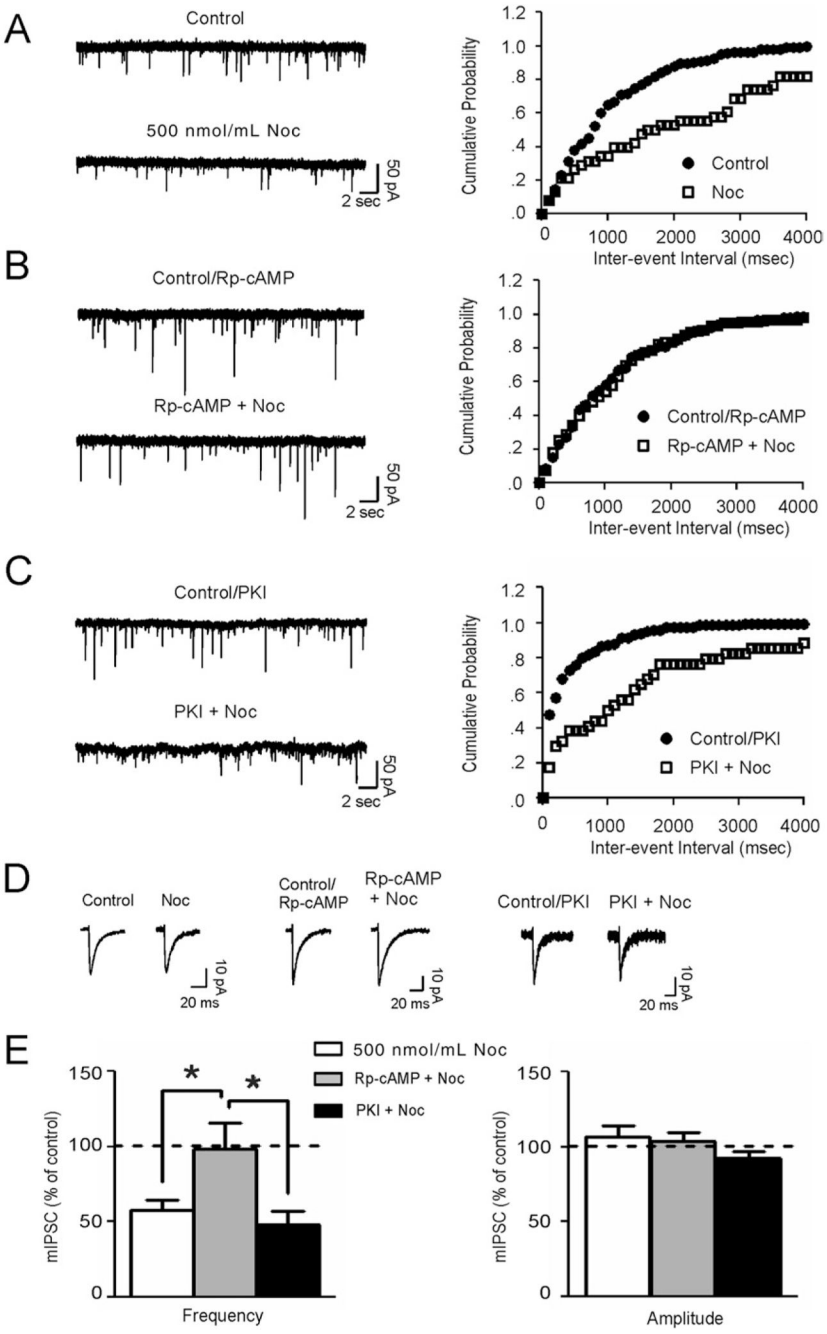

Figure 5.

Nociceptin (Noc) decreases gamma-aminobutyric acid release via a protein kinase A (PKA)-dependent pathway. **(A)** Left panel: representative miniature inhibitory postsynaptic currents (mIPSCs) from a central nucleus of the amygdala (CeA) neuron before and during 500 nmol/mL Noc application. Right panel: a rightward shift in the cumulative frequency plot for the neuron in Figure 5A indicating a longer interevent interval during the application of Noc. **(B)** Left panel: representative mIP-SCs from a CeA neuron before and during application of nociceptin in the presence of Rp-cAMP. Pretreatment with Rp-cAMP prevented Noc from decreasing mIPSC frequency. Right panel: cumulative frequency plot for the CeA neuron in Figure 5C. **(C)** Left panel: representative mIPSCs before and during

application of nociceptin with 5 μmol/mL PKI. Right panel: a rightward shift in the cumulative frequency plot for the CeA neuron in Figure 5E indicates a longer interevent interval during the application of nociceptin with PKI in the internal solution. **(D)** Scaled average mIPSCs from the traces depicted in Figures 5A–C. **(E)** Histograms depicting the average change in mIPSC frequency (left panel) and amplitude (right panel) with nociceptin alone, nociceptin in the presence of Rp-cAMP, and nociceptin with PKI in the internal solution (between subjects one-way analysis of variance: [$F(2,13) = 5.55$; $*p < .05$]).

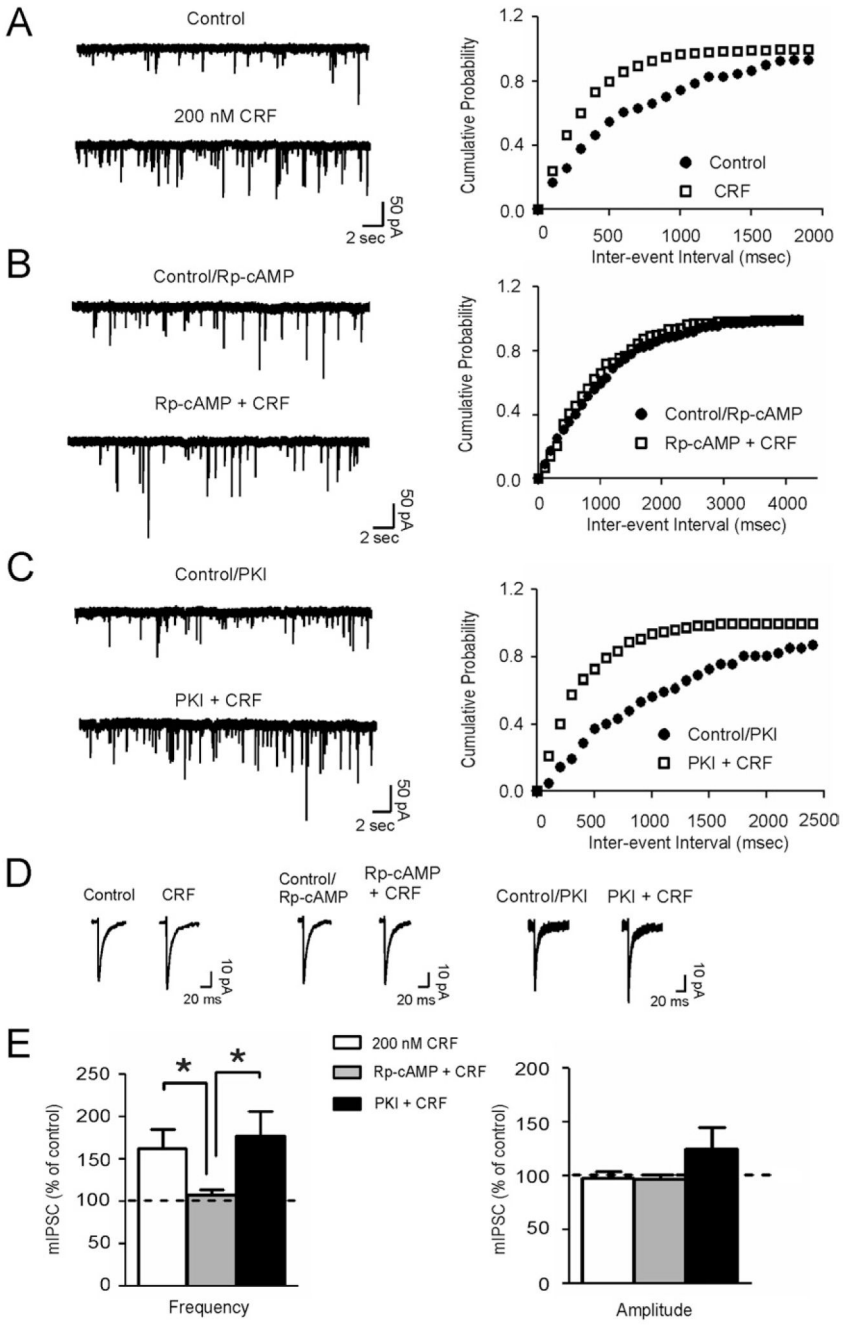

Figure 6.

Corticotropin-releasing factor (CRF) increases gamma-aminobutyric acid release via a protein kinase A (PKA)-dependent pathway. **(A)** Left panel: representative miniature inhibitory postsynaptic currents (mIPSCs) from a central nucleus of the amygdala (CeA) neuron before and during 200 nmol/mL CRF application. Right panel: a leftward shift in the cumulative frequency plot for the CeA neuron in Figure 6A indicates a shorter interevent interval during CRF application. **(B)** Left panel: representative mIPSCs before and during CRF application in the presence of Rp-cAMP. Right panel: cumulative frequency plot for the neuron in Figure 6C. **(C)** Left panel: representative mIPSCs before and during CRF application with 5 μmol/mL PKI in the internal solution. Left panel: cumulative frequency

plot for the CeA neuron in Figure 6E. The leftward shift indicates a shorter interevent interval during the application of CRF with PKI. **(D)** Scaled average mIPSCs from the traces in Panels A, C, and E. **(E)** Histograms depicting the average change in mIPSC frequency (left panel) and amplitude (right panel) with CRF alone, CRF in the presence of Rp-cAMP, and CRF with PKI in the internal solution (between-subjects one-way analysis of variance: [$F(2,18) = 4.409$; $*p < .05$]).