Interaction of CRF and Kappa Opioid Systems on GABAergic Neurotransmission in the Mouse Central Amygdala

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ABSTRACT

The corticotropin-releasing factor (CRF) and kappa-opioid receptor (KOR) systems are both implicated in stress-related behaviors and drug dependence. Although previous studies suggest that antagonism of each system blocks aspects of experimental models of drug dependence, the possible interaction between these systems at the neuronal level has not been completely examined. We used an in vitro brain slice preparation to investigate the interaction of these two peptide systems on inhibitory neurotransmission in the central nucleus of the amygdala (CeA). Application of exogenous CRF increased the mean frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSC) by 20.2%, suggesting an increase in presynaptic GABA release. Although the pharmacological blockade of KORs by norBNI alone did not significantly affect mIPSC frequency, it significantly enhanced the effect of CRF (by 43.9%, \( P = 0.02 \)). Similarly, the CRF effects in slices from KOR knockout (KO) mice (84.0% increase) were significantly greater than in wild-type (WT) mice (24.6%, \( P = 0.01 \)), although there was no significant difference in baseline mIPSC frequency between slices from KOR KO and WT mice. The increase in CRF action in the presence of norBNI was abolished by a CRF-1 receptor antagonist but was unaffected by a CRF-2 receptor antagonist. We hypothesize that CRF facilitates the release of an endogenous ligand for KORs and that subsequent activation of KOR receptors modulates presynaptic effects of CRF in CeA. These results suggest that potential pharmacotherapies aimed at neurobehavioral and addictive disorders may need to involve both the KOR/dynorphin and the CRF systems in CeA.

Introduction

The brain stress system is activated during withdrawal states from various drugs of abuse, and avoiding drug withdrawal stress is known to provide strong motivation for continuing use of the drugs, leading to a drug-dependent state (Koob et al., 2014). Therefore, intervening within brain stress systems has been proposed as a strategic approach to decrease or block reinstatement of drug-seeking and/or drug-consuming behaviors. Such brain stress systems include corticotropin-releasing factor (CRF) and the dynorphin/kappa opioid receptor (KOR) neuropeptide systems. In support of this view, CRF receptor antagonists block ethanol self-administration in ethanol-dependent rats (Funk et al., 2007) and cocaine self-administration (Boyson et al., 2011), whereas the block of KOR receptors decreases ethanol drinking in ethanol-dependent rats (Walker and Koob, 2008; Walker et al., 2011) and also decreases heroin intake (Scholsburg et al., 2013).

Considering this apparent overlap in actions between CRF and dynorphin/KOR systems in regulating stress-related behavior, an interaction between these two systems has been proposed. It has been reported that CRF induces dynorphin release in the hypothalamus (Almeida et al., 1986), striatum (Srinathingshji et al., 1989), and spinal cord (Song and Takemori, 1992). Furthermore, the KOR antagonist norbinaltorphimine (norBNI) was shown to block CRF-induced anxiety-like behavior and CRF-induced conditioned place aversion, a measure of dysphoric effects (Land et al., 2008; Bruchas et al., 2009; Bruchas et al., 2010). However, KOR agonist-induced conditioned place aversion is still intact in animals lacking CRF1 receptors (Contrarino and Papaleo, 2005). In contrast, Valdez et al. (2007) reported that selective CRF1-receptor antagonists blocked KOR agonist–induced reinstatement of cocaine seeking in primates. However, interactions between KOR and CRF systems may be reciprocal, as Wittmann et al. (2009) reported downregulation of CRF mRNA in select brain regions from dynorphin KO mice.

The central nucleus of the amygdala (CeA) is a brain locus mediating stress and anxiety-like behavior and is also

ABBRIVIATIONS: ACSF, artificial cerebrospinal fluid; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; CRF R1, CRF type 1 receptor; CRF R2, CRF type 2 receptor; KO, knockout; KOR, kappa-opioid receptor; mIPSCs, miniature inhibitory postsynaptic currents; NBI, NBI-27914 ([cyclopropylmethyl]-2-methyl-N-propyl-N’-[2,4,6-trichlorophenyl]-4,6-pyrimidinediamine HCl); norBNI, norbinaltorphimine; WT, wildtype.
regarded as a critical region mediating drug dependence (Gilpin et al., 2014). The CeA contains both CRF and dynorphin, with colocalization reported in many neurons (Marchant et al., 2007), suggesting that it is a possible site for interaction of CRF and KOR systems. We previously observed that KOR activation decreases inhibitory neurotransmission in the CeA (Kang-Park et al., 2013), whereas CRF activation increases the inhibitory neurotransmission (Nie et al., 2004). Therefore, in the present study we have examined interactive effects of the CRF and KOR systems on CeA inhibitory GABAergic synaptic neurotransmission, and particularly on miniature inhibitory postsynaptic currents (mIPSCs), to characterize a possible interaction at the cellular and synaptic levels.

Methods

Animals. Female C57BL/6 mice (60–90 days old; Charles River Laboratories, Raleigh, NC), and homozygous KOR knockout (KO) mice and wild-type (WT) littermate mice (120–180 days old, shipped from the Scripps Research Institute, La Jolla, CA) were used for this study. We exclusively used female mice, as it has been reported that female animals showed a greater or selective sensitivity to KOR-mediated behaviors such as anxiolysis, motivation, mood-related disorders, and social interaction (Chang et al., 2000; Chakrabarti et al., 2009; Lawson et al., 2010; Liu et al., 2011; Robles et al., 2014; Russell et al., 2014). The genetic background of KOR KO and WT mice was a hybrid C57BL/6Orl Russell et al., 2014). The genetic background of KOR KO and WT mice (120–180 days old, shipped from Charles River Laboratories, Raleigh, NC), and homozygous KOR knockout (KO) mice (7.7 ± 1.0 Hz; 129/Sv background). To examine the interaction between CRF and KOR in the CeA, we measured the modulation of GABAergic mIPSCs by CRF after pharmacological blockade of KORs and compared it with the effect of CRF alone (control) in brain slices from drug-naive C57 mice from Charles River Laboratories. CRF (100 nM) alone significantly increased the mean frequencies of mIPSCs by 20.2% (from 7.9 ± 0.8 Hz to 9.4 ± 0.9 Hz, P = 0.02; n = 5 neurons from four mice; Fig. 1). Pharmacological blockade of KORs with the KOR antagonist norBNI (1 µM) alone had no significant effect on mean mIPSC frequency (baseline 7.81 ± 0.78 Hz versus norBNI 7.13 ± 1.25 Hz; n = 5). However, after the addition of norBNI, CRF further increased the frequency of mIPSCs (from 7.1 ± 1.2 Hz to 9.8 ± 1.4 Hz; P = 0.02; n = 5; Fig. 1) in the same cells; the magnitude of increase (43.9%) in the presence of the KOR blocker was significantly greater than in the control (CRF alone) condition (P = 0.02; Fig. 1, B and C) in the same cells of this set. By contrast, CRF did not significantly change the amplitudes of mIPSCs either in the control condition (from 67.0 ± 17.0 pA to 60.2 ± 12.1 pA, P = 0.8; n = 5) or in the presence of KOR blockade (from 66.1 ± 12.3 pA to 65.3 ± 11.4 pA; n = 5; data not shown). These results suggest that an active KOR system dampens CRF effects on GABAergic mIPSCs by a pre-synaptic mechanism.

Next, we examined the effect of deletion of KORs on CRF effects using KOR KO littermate control mice. There was no significant difference in baseline mean mIPSC frequency between the WT (8.01 ± 1.0 Hz; n = 10 neurons from 7 mice) and KOR KO mice (7.7 ± 1.6 Hz; n = 8 neurons from 4 mice). However, CRF (100 nM) enhanced the mean frequencies of mIPSCs significantly more (P = 0.01) in CeA neurons from KOR KO than in WT mice (Fig. 2, B and C). Thus CRF
increased the mean frequency of mIPSCs by 24.6 ± 6.5% (from 8.0 ± 1.0 Hz to 9.7 ± 1.2 Hz) in WT mice (n = 10), but increased the mean frequency of mIPSCs by 84.0 ± 17.8% (from 7.6 ± 1.6 Hz to 12.9 ± 2.3 Hz) in KOR KO mice (n = 8) (Fig. 2). By contrast, CRF did not significantly change the mean amplitudes of mIPSCs either in WT (from 64.9 ± 8.4 pA to 59.3 ± 6.1 pA) or in KOR KO mice (from 40.0 ± 5.2 pA to 42.0 ± 5.6 pA, data not shown). These results further support the suggestion that CRF activates the KOR system, which then reduces the CRF effect on IPSCs in CeA.

Of the two major CRF receptors that have been identified, blockade of the CRF type 1 receptor (CRFR1) is associated with decreased stress responsiveness and decreased ethanol intake (Funk et al., 2007; Lowery et al., 2010), whereas activation of the CRF type 2 receptor (CRFR2) is associated with decreased ethanol intake (Funk and Koob, 2007; Lowery et al., 2010). In the present experiments we examined the subtype of CRF receptors involved in the CRF-KOR interaction using female C57 mice from Charles River Laboratories. In the presence of the CRFR1 antagonist NBI-27914 (100 nM), CRF no longer significantly (n = 5 neurons from three mice, P = 0.27) affected the mean frequencies of mIPSCs in the control condition: Mean frequency increase was only 2.3% of control (from 9.0 ± 0.8 Hz to 9.21 ± 0.9 Hz; Fig. 3). Although mean mIPSC frequency with NBI-27914 pretreatment was somewhat higher than without pretreatment (Figs. 1 and 2), this difference was not statistically significant. In addition, CRF in the presence of NBI-27914 did not significantly affect the mean frequency of mIPSCs even in the norBNI-pretreated condition (n = 5, P = 0.01), by 51.3% (from 7.8 ± 2.1 Hz to 11.2 ± 2.4 Hz). The effect of CRF on mean mIPSC frequency in the presence of astressin 2B was not statistically different from that in the absence of astressin 2B. As in the control condition, in the presence of astressin 2B the magnitude of the CRF effect was significantly greater with norBNI in the bath than with CRF alone (P = 0.03) (Fig. 4, B and C). By contrast, the mean amplitudes of mIPSCs were not significantly different with CRF treatment either in astressin 2B alone (from 61.0 ± 4.5 pA to 67.9 ± 11.1 pA) or in astressin 2B plus norBNI (from 56.2 ± 8.1 pA to 64.2 ± 9.5 pA. These results suggest that the enhanced release of GABA (and dynorphin, the endogenous KOR agonist; see below) by CRF is mediated by CRFR1 but not by CRFR2 receptors.

Discussion

In this study, we observed that pharmacological blockade of KORs by norBNI or genetic deletion of KORs enhanced the response to CRF in the CeA. Specifically, blockade or loss of KORs enhanced the effectiveness of CRF to increase
presynaptic GABA release. Our data suggest that the KOR system in the CeA is activated by exogenous CRF, probably owing to release of the putative endogenous ligand dynorphin, as has been previously reported in the amygdala and other brain regions (Chavkin et al., 1982; Sirinathsinghji et al., 1989; Song and Takemori, 1992; Bruchas et al., 2009; Lam and

Fig. 2. The genetic deletion of KORs increases the effect of CRF to enhance mIPSC frequency in CeA neurons. Representative mIPSC traces are shown in (A); traces from WT mice and KOR KO mice are shown in (A), a and b, respectively, and each panel shows traces before, during, and after bath application of CRF. In (B), the cumulative probability plot of interpulse intervals of mIPSCs was compared before and during CRF application in slices from WT mice (A) and KOR KO mice (B). The averaged percentage changes in mIPSC frequencies are presented in (C), showing that percentage change in mIPSC frequency in neurons from KOR KO mice is significantly greater than that in WT mice.

Fig. 3. CRF R1 antagonism abolishes the CRF-KOR interaction. Pretreatment with the CRFR1 antagonist NBI-27914 not only blocked the CRF effect on mIPSC frequency in the control condition but also blocked the enhancement of the CRF effect in the presence of the KOR antagonist. The representative mIPSC traces are shown in (A); traces from the NBI-27914-alone–pretreated condition and both NBI-27914 and norBNI-pretreated condition are shown in (A), a and b, respectively, and each panel shows traces before, during, and after bath application of CRF. In (B), the cumulative probability plot of interpulse intervals of mIPSCs was compared before and during CRF application in the NBI-27914-pretreated condition (A) and NBI-27914 plus norBNI–pretreated condition (B). The averaged percentage changes in mIPSC frequencies in NBI-27914-pretreated condition (closed columns) are presented in (C) compared with control result (open columns).
Gianoulakis, 2011). However, a microdialysis study of CRF effects in CeA suggested that dynorphin release was mediated by CRF2 rather than CRF1 receptors (Lam and Gianoulakis, 2011). The latter observation raises the possibility that CRF2 receptors mediate dynorphin release, whereas CRF1 receptors enhance GABA release. Our laboratory and others have previously shown that the CRF and KOR systems modulate GABAergic inhibitory neurotransmission in an opposite manner in the CeA: KOR activation decreases GABA neurotransmission (Kang-Park et al., 2013), whereas CRF activation increases this neurotransmission (Nie et al., 2004). If the KOR system is activated downstream from CRF receptors, KOR-mediated mechanisms may compensate for the CRF-mediated increase of the inhibitory synaptic neurotransmission in the control condition, whereas blockade of KORs would exaggerate the CRF-mediated increase of this inhibitory neurotransmission (Figs. 1 and 2). By contrast, if the CRF system is independent of the KOR system or acts downstream from KORs, exogenous CRF-evoked responses should not be different between control and KOR blockade conditions. In addition, we observed that the KOR-mediated CRF response is abolished by the CRFR1 antagonist NBI-27914 but not by the CRFR2 antagonist astressin 2B, suggesting that CRFR1-mediated mechanisms underlie this particular CRF-KOR interaction. However, the observation that activation of either CRF or kappa receptors produces anxiogenic effects (Land et al., 2008; Keen-Rhinehart et al., 2009) suggests that the CeA contains discrete circuitries mediating both anxiolytic and anxiogenic effects of neuropeptides and that these circuits are differentially sensitive to CRF and dynorphin. We propose this possibility to account for the behavioral similarities of CRF-R1 and kappa receptor activation in the context of apparent opposite actions on terminal GABA release in the CeA.

In a previous study, we observed that ethanol activates the KOR system in the CeA in a manner similar to the exogenous CRF effect found in the present study, and ethanol enhancement of inhibitory synaptic transmission was greater in KOR KO mice than WT mice (Kang-Park et al., 2013). Because ethanol is known to release CRF in the CeA (Nie et al., 2004), and ethanol’s effect in modulating inhibitory neurotransmission is mediated through the CRF system (Nie et al., 2004), we propose that ethanol activation of the KOR system is mediated by the sequential activation through the CRF system, although we are still testing this possibility. Further, the CRF system may be critical for KOR actions, as a CRF1-R antagonist can block KOR-mediated reinstatement of alcohol seeking (Funk et al., 2014) or cocaine seeking (Valdez et al., 2007). However, the suggestion that the KOR system is dependent on CRF may be overly simplistic, as mice lacking CRF1 receptors still exhibit KOR-dependent place aversion (Contarino and Papaleo, 2005). As noted above, it may be possible that the release of dynorphin, the putative endogenous KOR agonist, is mediated by CRF2 receptors (Lam and Gianoulakis, 2011), whereas release of GABA is mediated by CRF1 receptor activation (Nie et al., 2004; Roberto et al., 2010).

The functional significance of the CRF-KOR interaction remains to be determined. The characteristics of reciprocal CRF-KOR interactions could be compensatory or regulatory. Zhou et al. (2013) reported differential ethanol regulation of CRF and dynorphin mRNA levels: chronic ethanol decreased CRF mRNA but increased dynorphin mRNA. Therefore, the functional significance of these CRF-KOR interactions could result from different permutations of responses to their combination.
Currently, there are several studies on CRF-KOR interactions that support the idea that CRF and KOR systems in the amygdala (Bruchas et al., 2009), and our study expands this a connection into the functional level of the CeA. This information could inform the development of more efficient therapeutic strategies for anxiety disorders and for drug and alcohol abuse. Thus, future pharmacotherapies may be improved by combining agents acting simultaneously on CRF and kappa opioid systems.

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Authorship Contributions

Participated in research design: Kang-Park, Moore, Siggins.

Conducted experiments: Kang-Park.

Conducted new reagents or analytic tools: Kieffer, Roberts.

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References


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