Kappa Opioid Receptor Signaling in the Basolateral Amygdala Regulates Conditioned Fear and Anxiety in Rats

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Background: The kappa opioid receptor (KOR) system contributes to the prodepressive and aversive consequences of stress and is implicated in the facilitation of conditioned fear and anxiety in rodents. Here, we sought to identify neural circuits that mediate KOR system effects on fear and anxiety in rats.

Methods: We assessed whether fear conditioning induces plasticity in KOR or dynorphin (the endogenous KOR ligand) messenger RNA (mRNA) expression in the basolateral (BLA) and central (CeA) nuclei of the amygdala, hippocampus, or striatum. We then assessed whether microinfusions of the KOR antagonist JDTic (0–10 μg/side) into the BLA or CeA affect the expression of conditioned fear or anxiety. Finally, we examined whether fear extinction induces plasticity in KOR mRNA expression that relates to the quality of fear extinction.

Results: Fear conditioning upregulated KOR mRNA in the BLA by 65% and downregulated it in the striatum by 22%, without affecting KOR levels in the CeA or hippocampus, or dynorphin levels in any region. KOR antagonism in either the BLA or CeA decreased conditioned fear in the fear-potentiated startle paradigm, whereas KOR antagonism in the BLA, but not the CeA, produced anxiolytic-like effects in the elevated plus maze. Effective fear extinction was associated with a 67% reduction in KOR mRNA in the BLA.

Conclusions: These findings suggest that fear conditioning and extinction dynamically regulate KOR expression in the BLA and provide evidence that the BLA and CeA are important neural substrates mediating the anxiolytic-like effects of KOR antagonists in models of fear and anxiety.

Key Words: Anxiety, conditioned fear, elevated plus maze, fear-potentiated startle, JDTic, quantitative PCR

The kappa opioid receptor (KOR) system is widely implicated in mediating the prodepressive emotional and behavioral consequences of stress. KOR activation produces dysphoria in humans (1,2) and prodepressive-like behavioral signs in rodents, including dysphoria (3,4), anhedonia (5–7), and passive coping strategies (8–13). In contrast, KOR antagonism or ablation of the genes encoding KORs or their endogenous ligand dynorphin has antidepressant-like effects (9–14). We reported that systemic administration of KOR antagonists also produces anxiolytic-like effects in models of conditioned fear and anxiety in rats (15). Recent work has confirmed these findings (16,17) and demonstrated an anxiolytic-like phenotype in independent lines of prodynorphin (PDyn; the dynorphin precursor) knockout mice that is reproduced in wild-type mice treated with a KOR antagonist (18,19). Thus, the KOR system appears to be an important mediator of both prodepressive and anxious mood states. Given the high comorbidity of clinical depression and anxiety disorders (20,21) and their association with stress (20,22,23), these findings suggest that dysregulation of KOR signaling within neural circuits involved in mood and motivation contributes to the etiology of depression and anxiety disorders.

Studies characterizing the role of KOR systems in depressive behavior have focused mainly on the mesocorticolimbic dopamine system and hippocampus (HIP) (5,7,11,12,24–26). However, little is known about the neural circuits that mediate KOR system effects on fear and anxiety. KORs and dynorphin are expressed throughout brain areas involved in fear and anxiety, including in the basolateral (BLA) and central (CeA) nuclei of the amygdala and the HIP, in humans and rodents (27–30). The amygdala is critically involved in conditioned fear and anxiety-related behaviors, and many stress hormones and neuropeptides modulate fear learning and memory via effects in this region (31–39). Although it is not known if KOR signaling in the amygdala modulates fear learning and memory, evidence suggests that plasticity in KOR system gene expression in limbic brain regions contributes to the enduring prodepressive consequences of stress (11–13,25,26,40). Because fear memory is associated with altered gene expression in a broad neural circuitry that includes the amygdala and HIP (41–46), we hypothesized that fear conditioning might induce plasticity in KOR system gene expression in brain regions involved in fear and anxiety and that KOR signaling in these regions might facilitate conditioned fear and anxiety.

Here, we used quantitative real-time reverse transcriptase polymerase chain reaction (qPCR) to examine whether fear learning induces plasticity in KOR or PDyn messenger RNA (mRNA) expression in the BLA, CeA, HIP, and striatum (STR). We then examined the behavioral consequences of disrupting KOR signaling in the amygdala using local microinfusions of the KOR antagonist JDTic into the BLA or CeA before testing conditioned fear and anxiety. Finally, we examined whether fear extinction training alters KOR mRNA expression in the BLA in a manner that relates to the quality of extinction.

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Received Jan 26, 2011; revised Mar 7, 2011; accepted Mar 10, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.03.017

Methods and Materials

Rats

A total of 198 male Sprague-Dawley rats (325–375 g) (Charles River Laboratories, Raleigh, North Carolina) were used. Rats were singly housed following surgery and maintained on a 12-hour light-dark cycle with unrestricted access to food and water. Protocols were approved by McLean Hospital's Institutional Animal Care Committee and consistent with National Institutes of Health policies.

Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction

Thirty-eight rats were used to determine if fear conditioning affects KOR or PDyn mRNA expression in the BLA, CeA, and HIP; we also assessed gene expression in a portion of the STR dorsal to the amygdala in anticipation of using this region as a dorsal control site in drug microinfusion studies. An additional 20 rats were used to determine if fear extinction affects KOR mRNA expression in the BLA. We assessed levels of glutamic acid decarboxylase 65 kDa (GAD65) and glutamic acid decarboxylase 67 kDa (GAD67) and PDyn in the BLA and CeA as an index of dissection accuracy. GAD65 levels are decreased in the BLA 24 hours after fear conditioning (47,48), and thus GAD65 expression was used to confirm that our training parameters induced molecular correlates of conditioned fear. On 4 consecutive days, rats were briefly handled and then placed for 30 minutes into unilluminated fear-potentiated startle (FPS) chambers (60 dB background noise) for acclimation. The next day, rats received a training session consisting of 10 light-shock presentations (Light+Shock) (3.7 sec light conditioned stimulus [CS] co-terminating with a .5 sec, .6 mA foot-shock unconditioned stimulus; ~3 min intertrial interval). A separate group received 10 light presentations (Light Alone). Eighteen hours after training, 20 rats (10 Light+Shock, 10 Light Alone) were killed by decapitation for qPCR analyses, and 24 hours after training, 18 rats were tested for FPS to confirm that our training parameters induced conditioned fear. The remaining 20 rats were used to assess the molecular effects of fear extinction. Methods for tissue collection and qPCR are provided in Supplement 1.

Drugs

JDTic was synthesized at Research Triangle Institute (Research Triangle Park, North Carolina) and dissolved in artificial cerebrospinal fluid (Harvard Apparatus, Holliston, Massachusetts); drug doses are based on the salt form of JDTic.

Surgery and Microinfusions

To determine the effect of KOR antagonism in the amygdala on fear and anxiety, rats received surgery to implant bilateral cannulas in the BLA ($n = 56$) or CeA ($n = 54$), as previously described (49). Cannulas (23-gauge; Plastics One, Roanoke, Virginia) were directed 1.5 mm above the BLA (relative to bregma in mm: anteroposterior [AP] −2.8, mediolateral [ML] ± 5.1, ventral to dura from the stylet tip [DV] −7.7) or CeA (AP −2.6, ML ± 4.5, DV −7.3). Stainless steel obturators and infusion stylets (30-gauge) extended 1.5 mm beyond the cannula. A dorsal control group had bilateral cannulas implanted in the STR, 2.5 mm above the BLA (AP − 2.8, ML ± 5.1, DV −5.2) or 1.5 mm above the CeA (AP − 2.6, ML ± 4.5, DV −5.8) infusion sites. Behavioral data for sites dorsal to the BLA or CeA did not differ and thus were combined into a single group.

Microinfusions were administered as previously described (49). Rats received infusions of JDTic (3–10 μg/side; 5.4–18.0 nmol) in .5 μL/side over 5 minutes, 24 hours before behavioral testing. JDTic is a highly selective KOR antagonist (50) that has a slow onset of antagonism (effects peak ~24 hours after systemic administration) that is maintained for at least 7 days (51,52).

Fear-Potentiated Startle

Seventy-five rats were used to test the effect of KOR antagonism in the amygdala on FPS (BLA, $n = 38$; CeA, $n = 24$; STR, $n = 13$). A description of the FPS apparatus (Med Associates, St. Albans, Vermont) is provided in Supplement 1. Before surgery, rats received a habituation session consisting of 100 startle stimuli (50-msec, 100-dB, 30-sec interstimulus interval to determine baseline startle magnitudes. Following recovery from surgery, rats received two habituation sessions separated by 48 hours to acclimate them to the holding chambers and startle stimuli. Two and 5 days later, rats received conditioning sessions consisting of 10 light-shock pairings (3.7-sec light co-terminating with a .5-sec, .6-mA foot-shock, ~3-min intertrial interval). Three days after conditioning, rats received a pretest consisting of 15 habituating startle stimuli and 20 startle stimuli in which half were preceded by the light CS, to form experimental groups with similar levels of fear. Rats received microinfusions of JDTic or vehicle 20 minutes after the pretest and received a full-length FPS test 24 hours later consisting of 15 habituating startle stimuli and 60 startle stimuli in which half were preceded by the CS. The operational measure of fear was the difference in startle in the presence and absence of the CS (percentage of FPS = [(startle in the presence of the light − startle in the dark)/startle in the dark] × 100).

Fear Extinction

To determine if fear extinction alters KOR mRNA expression in the BLA, 20 rats were subjected to fear conditioning as described above. One day later, rats were tested for acquisition of FPS and the following day were given extinction training consisting of 60 presentations of the CS (3.7-sec light, 30-sec interstimulus interval) alone. The next day, rats were retested for FPS to assess the magnitude of fear extinction. Rats with the lowest (good extinction, $n = 4$) or highest (poor extinction, $n = 4$) levels of FPS were inferred to have effectively or ineffectively extinguished conditioned fear, respectively, and were killed immediately after testing by decapitation; gene expression was analyzed using qPCR (Supplement 1).

Data for each brain region were analyzed separately using one-way (treatment) analysis of variance, and significant effects were analyzed using Dunnett's post hoc tests. Two-way (treatment × time, group × time) analyses of variance were used to analyze the time course of FPS, followed by Bonferroni post hoc tests. Studies comparing two treatment groups were analyzed using Student *t* tests.

Elevated Plus Maze

Sixty-five rats were used to test the effect of KOR antagonism in the amygdala on anxiety in the elevated plus maze (EPM) (BLA, $n = 18$; CeA, $n = 30$; STR, $n = 17$). Methods for EPM and histology are presented in Supplement 1.

Results

Tissue punches were obtained from the BLA, CeA, HIP, and STR, which have varying degrees of dynorphin expression (27,29) (Figure 1A,B). Rats that received Light+Shock training displayed marked conditioned fear, as indicated by a 72% increase in startle in the presence of the light (Figure 1C). Rats exposed to Light Alone did not show altered startle in the presence of the light and had significantly lower FPS (4%) than rats receiving Light+Shock training [$t(16) = 2.87$, $p < .05$] (Figure 1C), demonstrating conditioned fear only in rats that received paired training. We confirmed the