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Review

The role of the dynorphin/κ opioid receptor system in anxiety

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Anxiety disorders are the most common and prevalent forms of psychiatric disease, although the biological basis of anxiety is not well understood. The dynorphin/κ opioid receptor system is widely distributed in the central nervous system and has been shown to play a critical role in modulating mood and emotional behaviors. In the present review, we summarize current literature relating to the role played by the dynorphin/κ opioid receptor system in anxiety and κ opioid receptor antagonists as potential therapeutic agents for the treatment of anxiety disorders.

Keywords: dynorphin; κ opioid receptor; stress; anxiety

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Introduction

Anxiety is a recognized symptom of various anxiety disorders, with more than 3.6 million individuals in European countries suffering an anxiety disorder at some point in their lifetime[1]. The clinical anxiety disorders recognized in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) include the following: generalized anxiety disorder, obsessive compulsive disorder, panic disorder, acute and chronic posttraumatic stress disorder, and various phobias, including agoraphobia, social phobia, and specific phobia (*eg*, fear of flying)[2]. Anxiety disorders, commonly occurring with depression and drug abuse, can be triggered or promoted by stress[3, 4]. The most widely used therapeutic agents for the treatment of anxiety disorders include selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, benzodiazepine anxiolytic and NMDA receptor modulators[5]. However, currently available anxiety disorder modulators are inadequate for patients because of the existence of "nonresponders" or unwanted side effects, such as ataxia, drowsiness, and impairment of cognition[6–8]. Increasing evidence indicates that the dynorphin/κ opioid receptor system plays an important role in the regulation of anxiety disorders. In this review, we describe existing data from preclinical studies using animal models to present an overview of the dynorphin/κ opioid receptor system in anxiety.

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The dynorphin/κ opioid receptor system

κ Opioid receptors belong to the rhodopsin sub-family of the G protein-coupled receptor (GPCR) family. In the brain, κ opioid receptors are present primarily in the claustrum, cortex, hypothalamus, endopiriform nucleus, nucleus accumbens, caudate putamen, and substantial nigra[9–11]. Stimulation of κ opioid receptors results in the dissociation of G proteins into Gα and Gβγ subunits, in turn affecting a variety of effectors including adenylyl cyclase, potassium/calcium channels, phospholipase C and the p42/44 mitogen-activated protein kinase pathway[12]. Activation of the κ opioid receptor *in vivo* produces various effects, including analgesia/antinociception, psychomimesis, dysphoria/aversion, diuresis, antipruritic and blockade of psychostimulant effects[12]. In contrast, the activation of μ opioid receptors is known to induce euphoria and mediates positive reinforcement. Previous studies have demonstrated that κ opioid receptor agonists functionally attenuate cocaine-induced behavioral sensitization[13, 14], place preference[14, 15], and self-administration[16, 17]. These inhibitory effects of κ opioid receptor agonists on cocaine-induced abuse-related behaviors are achieved potentially through the inhibition of dopamine release from dopaminergic neurons[18, 19].

Dynorphin peptides, potent endogenous κ opioid recep-

tor ligands[20], consist of dynorphin A (Dyn A), dynorphin A(1-8), dynorphin B (Dyn B), α-neoendorphin (α-Neo), β-neoendorphin (β-Neo), leumorphin, and big dynorphin (Big Dyn, which contains both Dyn A and Dyn B)[21] and have been found to modulate neuronal excitability and to regulate nociception, motivation, cognitive function and stress-induced mood disorders[22].

Rodent models of anxiety

The validity of anxiety models rests on three criteria: face validity, predictive validity and construct validity[2]. In the anxiolytic drug discovery field, the most commonly used rodent models include elevated plus-maze (EPM), light/dark box, social interaction, Vogel conflict, open field, ultrasonic distress vocalization, conditioned fear, Geller-Seifter conflict and stress-induced hyperthermia[2]. Among these, EPM, light/dark box and open field have been main stay tests for many years. The details of these models and their uses in anxiety have been previously described[2, 23]. Pharmacological data involving different anxiety models are often inconsistent across studies. For example, mice with ablation of κ opioid receptors from brain dopamine neurons displayed anxiolytic effects in the open field and light/dark box tests but not in the EPM test[24]. This discrepant result may be due to genetic and environmental influences[25]. Therefore, it will be important to use multiple tests to obtain a broad understanding of the molecular mechanisms of anxiety and to develop new medications for the treatment of anxiety disorders.

Role of the dynorphin/κ opioid receptor system in anxiety

Chronic stress may lead to anxiety and depression[4]. Moderate to high levels of dynorphin mRNA and κ opioid receptors are expressed in regions of the brain that are stress-related in rodents, including the hypothalamic paraventricular nucleus (PVN), amygdala (AMY), hippocampus (Hip) and bed nucleus of the stria terminalis (BNST)[11, 26, 27], and stress exposure has been shown to increase endogenous dynorphin levels[28]. A growing body of evidence reveals that the dynorphin/κ opioid receptor system plays an important role in stress[29–31].

κ Opioid receptor agonists and antagonists

Human studies show that selective κ opioid receptor agonists produce dysphoria, anxiety and abnormal behavior along with psychotomimesis at higher doses[29]. The benzomorphan κ opioid receptor agonist MR2033 elicited dose-dependent dysphoric and psychotomimetic effects, which were antagonized by naloxone[29]. This was consistent with work demonstrating that salvinorin A, a highly selective κ opioid receptor agonist, caused a certain degree of anxiety according to the state-trait anxiety inventory-S, a 20-item self-rating scale[32].

However, κ opioid receptor agonists exert biphasic effects on anxiety in rodents. Increasing evidence shows that selective κ opioid receptor agonists produce anxiety-like behaviors in the EPM test[33–39]. These findings were further supported by findings that anxiolytic effects are produced by deficiencies in the κ opioid receptor system in mice. Mice lacking prodynorphin displayed increased anxiolytic parameters of explorative behavior in the open field as well as EPM and light-dark tests[38]. Ablation of κ opioid receptors from brain dopamine neurons produced reduced anxiety-like behaviors in the open field and light-dark tests but not in the EPM test[40]. In addition, intra-amygdala microinjection of dynorphin A increased anxiety-like behavior in the light-dark test[41]. However, inconsistent with these observations is the finding that the κ opioid receptor agonist U50488 significantly increased time spent in open arms during the EPM test[42, 43]. This is consistent with work demonstrating that U69593 and salvinorin A both produced anxiolytic effects in rodents[44, 45]. Microinjection of U69593 into the infralimbic cortex reduced anxiety-like behavior in the EPM test[46]. Kuzmin *et al* (2006) showed that big dynorphin, a prodynorphin-derived precursor peptide, induced anxiolytic-like behavior in mice in the EPM test[47]. Whereas, deletion of the prodynorphin gene increased anxiety-like behaviors in the EPM and light-dark tests[48]. Similarly, ablation of prodynorphin showed increased anxiety-like behaviors in zero-maze and startle-response tests[49]. It must be noted that some lines of constitutive κ opioid receptor knockout (KO) mice did not display altered anxiety-like behaviors[50, 51]. Discrepancies among these studies may be due to, but are not limited to, the use of specific genetic constructs for generating mutant mice, experimental paradigms, size of the apparatus, intensity of illumination, test conditions, animal strains, and lab specific basal stress levels. Although with these limitations and variables, the findings clearly demonstrate that the dynorphin/κ opioid receptor system is involved in anxiety-related behavior[33–47, 49–51] (see Table 1 for a summary of current literature), but it is difficult to define the exact role of κ opioid receptor signaling because both anxiolytic- and anxiogenic-like effects are reported with κ opioid receptor agonists. Indeed, THC, a CB1 receptor agonist, microinjected at low doses in the prefrontal cortex and ventral hippocampus induced an anxiolytic-like response, while high doses caused an anxiogenic reaction[52]. Considering that κ opioid receptors are widely expressed in the central nervous system[11], it is not surprising that specific brain regions (*ie*, prefrontal cortex, amygdala and hypothalamus) may have opposite and complementary roles in the regulation of anxiety by κ opioid receptors. Further studies are clearly needed to understand the mechanism involved in biphasic effects induced by κ opioid receptor agonists.

Although κ opioid receptor agonists present conflicting profiles in mood disorders, the administration of κ opioid receptor antagonists have been shown to exert consistent anxiolytic effects in different animal models[34, 37–39, 53–60] (see Table 2 for a summary of the current literature). The κ opioid receptor antagonists nor-BNI and JDTic increased open arm exploration in EPM tests and decreased conditioned fear in the fear-potentiated startle paradigm[55, 60]. Similarly, DIPPA produced anxiolytic-like effects in both novelty-induced hypophagia and defensive burying tests[57]. In addition, it was demonstrated that animals treated with GNTi displayed increased open arm exploration in the EPM test and increased center area explora-

Table 1. Evidence that dynorphin/κ opioid receptors (KOPRs) play a diphasic role in anxiety disorders.

Behavior	Activate dynorphin/ KOPRs system	KOPRs agonists/dynorphin/ gene abation	Paradigm/model	Outcome	Reference
Anxiogenic	Yes	U50488 (ip, 10 mg/kg)	Elevated plus maze	Decreased open time	33
	Yes	U50488 (ip, 10 mg/kg)	Elevated plus maze	Decreased open time	34
	Yes	U50488 (ip, 5 mg/kg)	Elevated plus maze	Decreased open time	35
	Yes	U50488 (0.1, 1, and 10 mg/kg)	Open field	Decreased center time	36
	Yes	U50488 (ip, 10 mg/kg)	Elevated plus maze	Decreased open time	37
	Yes	U50488 (ip, 2.5 mg/kg)	Elevated plus maze	Decreased open time	38
	Yes	U50488 (sc, 5 mg/kg)	Elevated plus maze	Decreased open time	39
	Yes	Dynorphin A	Light-dark box	Decreased lit compartment time	41
	No	Prodynorphin$^{-/-}$	Elevated plus maze; Open field; Light-dark box	Increased anxiolytic parameters of explorative behavior	38
	No	DAT-KOR$_{lox/lox}$	Open field; Light-dark box	Decreased anxiety-like behavior	40
Anxiolytic	Yes	U50488 (sc, 2.5 mg/kg)	Elevated plus maze	Increased open time	43
	Yes	U50488 (sc, 2.5 mg/kg, in the losers)	Elevated plus maze	Increased open time	42
	Yes	U69593 (Microinjections in the IL cortex)	Elevated plus maze	Increased open time	46
	Yes	U50488 (ip, 10–1000 μg/kg) U69593 (ip, 100 μg/kg)	Elevated plus maze	Increased open time	44
	Yes	Salvinorin A (sc, 0.001–1000 μg/kg)	Elevated plus maze	Increased open time	45
	Yes	Big dynorphin	Elevated plus maze	Increased open time	47
	Yes	Prodynorphin$^{-/-}$	Zero-maze test; Startle-response test	1. Increased latency of first area change and decreased activity in the open part 2. Increased amplitude of the startle reaction	49
No effect	No	KOR$^{-/-}$	Elevated plus maze; Light-dark box		50
	No	KOR$^{-/-}$	Elevated zero-maze; Elevated plus maze; Open field		51

tion in the open field test[38]. Together, these studies suggest that κ opioid receptor antagonists may be particularly effective for the treatment of anxiety disorders[61, 62].

Link between the dynorphin/κ opioid receptor system and corticotrophin-released factor

The neuropeptide corticotrophin-release factor (CRF) plays a critical role in the stress response by its regulation of the hypothalamic-pituitary axis (HPA) and subsequent adrenocorticosteroid release[63]. CRF and dynorphin are co-expressed in the hypothalamus[64, 65] and central amygdala[66, 67]. CRF causes dynorphin release[68, 69] and dynorphin-dependent κ opioid receptor activation in several anxiety-related brain regions[30]. Land *et al* (2008) reported that CRF-induced anxiety-like behaviors were blocked by a κ opioid receptor antagonist[30]. Recent work further showed that the anxiogenic-like effects of CRF were triggered by CRF1-R activation of the dynorphin/κ opioid receptor system[59]. These results reveal a connection between CRF and the dynorphin/κ opioid receptor system[70] and support that the CRF-induced dynorphin/κ opioid receptor-dependent pathway is involved in the modulation of anxiety-like behaviors[62].

Brain regions involved in κ opioid receptor-mediated anxiety

The mesocorticolimbic dopamine (DA) system originates in the ventral tegmental area (VTA) and projects to the amygdala, BNST, nucleus accumbens, prefrontal cortex, and hippocampus[40, 71]. κ Opioid receptors are located on both the cell bodies and terminals of mosocorticolimbic DA neurons[72, 73]. Activation of κ opioid receptors leads to the inhibition of DA neurons in the VTA[74] and decreases DA release in regions that receive VTA input[75, 76]. κ Opioid receptor agonists produce dysphoria, anhedonia and depressive-like effects, which are partially mediated by decreased function of the mesocorticolimbic DA system[76, 77]. Recently, two lines of mice with mutations in the κ opioid receptor system were generated[24]. One

Table 2. Evidence that κ opioid receptor (KOPR) antagonists can prevent anxiety-like behaviors from preclinical studies.

Behavior	Inactivate KOPR/system	KOPR antagonists	Paradigm/model	Outcome	Reference
Anxiolytic	Yes	norBNI (sc, 30 mg/kg)	The defensive withdrawal test	Decreased the latency to leave the withdrawal box	53
	Yes	JDTic (ip, 10 mg/kg)	Elevated plus maze	Increased open time	54
	Yes	norNI (ip, 10, 20 mg/kg)	Elevated plus maze	Increased open time	34
	Yes	JDTic (BLA, 0–10 μg/side)	Elevated plus maze	Increased open time	55
	Yes	AZ-MTAB (sc, 30 μmol/kg) LY-DMPF (sc, 24 μmol/kg)	Elevated plus maze	Increased open time	56
	Yes	DIPPA (sc, 2.5, 5 mg/kg)	Novelty-induced hypophagia	Decreased the latency to feed	57
	Yes	DIPPA (sc, 1, 5 mg/kg)	Defensive burying test	Decreased burying time	57
	Yes	JDTic (sc, 1, 4, 8, 16 mg/kg)	Elevated plus maze	Increased open time	58
	Yes	norBNI (ip, 20 mg/kg)	Elevated plus maze	Increased open time	37
	Yes	norBNI (ip, 10 mg/kg)	Elevated plus maze	Increased open time	59
	Yes	norBNI (ip, 10 mg/kg)	Open Field	Increased center time	38
	Yes	GNTI (ic, 3 nmol)	Open Field	Increased center time	38
	Yes	norBNI (sc, 20 mg/kg)	Elevated plus maze	Increased open time	39
	Yes	norBNI (ip, 3–30 mg/kg); JDTic (ip, 1-10 mg/kg)	Elevated plus maze	Increased open time	60
	Yes	norBNI (ip, 3–30 mg/kg); JDTic (ip, 1–10 mg/kg)	Fear-potentiated startle	Decreased conditioned fear	60

is a constitutive κ opioid receptor knockout ($KOR^{-/-}$), the other is a conditional knockout (DAT-$KOR_{lox/lox}$) in which κ opioid receptors are lacking in DA-containing neurons. Behavioral characterization demonstrated that DAT-$KOR_{lox/lox}$ mice displayed reduced anxiety-like behaviors in the open field and light/dark box tests. These findings suggest that the activation of κ opioid receptors in the mesocorticolimbic DA system plays a key role in anxiety.

The amygdala, a target of VTA dopamine neurons, is critical for anxiety-related responses. Knoll *et al* (2011) found that the microinjection of κ opioid receptor antagonist into the basolateral amygdala (BLA) produced anxiolytic-like responses in the EPM test[55]. The importance of the amygdala in anxiety has also been confirmed by other researchers who report that stress- or CRF-induced anxiety is mediated by dynorphin release in the BLA, which can be blocked by a local injection of the κ opioid receptor antagonist norBNI[59].

The dorsal raphe nucleus (DRN), the primary source of serotonin that sends projections to multiple forebrain limbic regions, is critical for regulating affective states and stress[78]. Land *et al* (2009) demonstrated that the aversive properties of κ opioid receptor activation was encoded by DRN to NAc serotonergic projections because κ opioid receptor KO mice failed to develop κ opioid receptor agonist U50488-induced CPA; however, lentivirus expression of κ opioid receptors in the DRN restored the aversive response[79], whereas lentivirus expression of mutated κ opioid receptors that were unable to activate p38 MAPK in the DRN did not restore the aversive response. In addition to mediating the dysphoric responses of stress, p38 MAPK activation within the DRN has also been found to contribute to depressive-like and drug-seeking behaviors[80].

The locus coeruleus (LC) is one of the primary sources of norepinephrine (NE) in the forebrain[81]. Dynorphin and κ opioid receptors are coexpressed within the LC on noradrenergic (NA) neurons[67, 82–85]. Previous reports have shown that both stress and CRF engage in LC NA cell firing[86, 87]. Ai-Hasani *et al* (2013) first reported that κ opioid receptors within the LC NA nuclei modulate the reinstatement of cocaine place preference through a noradrenergic mechanism[88]. Because the LC-NE system is a critical stress response system[81], κ opioid receptor pathway interactions with the NA system may influence κ opioid receptor-mediated aversion and anxiety-like behaviors. Evidence supports a model in which the dynorphin/κ opioid receptor system and CRF coordinate in stress-induced anxiety behaviors. κ Opioid receptors and CRF are co-expressed in the hypothalamus[64] and central amygdala[65]. Both stress and CRF cause dynorphin-dependent κ opioid receptor activation in the BLA, nucleus accumbens, dorsal raphe and hippocampus[30]. Recent evidence indicates that κ opioid receptors are expressed on the terminal of amygdala inputs to BNST[89], a brain region strongly involved in fear and anxiety[90]. Thus, there is a considerable possibility that the dynorphin/κ opioid receptor system within these regions may play a role in anxiety.

Various stress paradigms differentially influence κ opioid receptor-induced responses

Previous studies implicate stress activation of the κ opioid receptor in increased anxiety-like behaviors, dysphoric responses, and potentiation of drug seeking behaviors[29–31, 38, 76, 91]. It has been reported that acute stress

activates the hypothalamic-pituitary-adrenal (HPA) axis and influences amygdala CRF gene expression, which is a key mediator of the stress response[61]. Acute stress has also been found to affect κ opioid receptor activation in the BLA and κ opioid receptor transcription in the PVN of the hypothalamus[55, 59]. Using an acute swim stress method, Bruchas *et al* (2009) demonstrated that CRF1-R activation of the dynorphin/κ opioid receptor system in the BLA mediates anxiety-like behaviors[59]. Moreover, recent work demonstrates that single acute swim stress-induced cocaine seeking reinstatement occurred via κ opioid receptor activation[88]. Similar to acute stress, repeated exposure to stress also results in dynorphin release and subsequent κ opioid receptor activation[92]. Following repeated swim stress, κ opioid receptor mRNA expression was regulated in a region-specific manner in the brain[93]. In recent reports, exposure to repeated stress resulted in the dysregulation of κ receptor signaling in the DRN through a p38 MAPK-dependent mechanism[92]. In this study, repeated swim stress significantly reduced κ opioid receptor-mediated G-protein gated inwardly rectifying potassium channel currents in serotonergic neurons post-synaptically, without affecting pre-synaptic excitatory transmission[92]. The functional consequences of repeated stress exposure on κ opioid receptor-dependent behaviors have not been well investigated; however, several studies reveal that repeated swim stress-induced activation of the dynorphin/κ opioid receptor system potentiates nicotine conditioned place preference[35] and cocaine rewarding effects[94]. Chronic mild stress is a widely used animal model for inducing anxiety-like behavior; however, the significance of κ opioid receptors in chronic mild stress is unclear. Recently, Ai-Hasani *et al* (2013) compared different types of exposure to stress (acute, sub-chronic, and chronic) to study the impact on κ opioid receptor-induced reinstatement[95]. They found that following an acute swim stress, the activation of κ opioid receptors potentiated cocaine reinstatement; however, repeated swim stress and chronic mild stress blocked κ opioid receptor-induced cocaine or nicotine reinstatement. These findings indicate that various types of stress paradigms affect dynorphin/κ opioid receptor system-mediated reinstatement. Although these studies do not definitively prove that stress types differentially influence κ opioid receptor-mediated affective states, they do provide the basis for further investigation.

Ligand-directed signaling at the κ opioid receptor

Numerous studies support that GPCRs exist in multiple conformation states. Agonists can initiate distinct receptor conformations that produce distinct signaling cascades to mediate various behavioral effects. This concept is referred to as ligand-directed signaling or biased agonism[96]. It has been recognized that biased μ opioid receptor agonists may be promising analgesics with less abuse potential, whereas biased κ opioid receptor agonists can be used for the treatment of pain and other disorders with less risk of convulsions[97]. Ligand-directed signaling at the κ opioid receptor also has important implications because the activation of the κ opioid receptor produces analgesia with a low risk of addiction or dysphoria, unlike the action of the μ opioid receptor, which induces euphoria[62]. κ Opioid receptor agonists activate a variety of kinase cascades including ERK1/2, JNKs, PKC, and p38 MAPKs[62]. The link between κ opioid receptor ligand-selective signaling cascades and *in vivo* responses has not been fully characterized, but recent behavioral studies demonstrate that arrestin-dependent p38 activation is selectively involved in dysphoria induced by κ opioid receptor activation, whereas Gβγ-dependent signaling underlies analgesic responses. Bruchas *et al* (2006) found that κ opioid receptors activate the p38 MAPK pathway through a GRK3- and arrestin-dependent mechanism[98]. κ Opioid receptor activation of the p38 MAPK pathway in the DRN, a serotonergic nucleus, is important for κ opioid receptor agonist U50488-induced conditioned place aversion and stress-induced reinstatement of drug seeking[79]. Further selective inactivation of p38 signaling in serotonergic neurons of the DRN blocked defeat-induced social aversion[79]. Therefore, arrestin-dependent p38 activation is required for κ opioid receptor-mediated dysphoric and proaddictive effects. Together, these findings demonstrate that κ opioid receptor agonist-biased signaling exerts behavioral consequences. From a therapeutic perspective, signaling pathway-selective κ opioid receptor agonists may have clinical applications for the treatment of mood disorders. In contrast to κ opioid receptor agonists, κ opioid receptor antagonists such as norBNI, GNTI and JDTic have long durations of action and cause κ opioid receptor inactivation through a c-Jun N-terminal kinase (JNK)-dependent signaling cascade; the underlying mechanisms need to be elucidated[99].

Summary

The dynorphin/κ opioid receptor system has a wide range of biological effects, including affecting mood disorders, cognition and reward. Activation of the κ opioid receptor by agonists may have biphasic effects in anxiety-like behaviors. These inconsistent findings require further study to examine the underlying mechanism of the dynorphin/κ opioid receptor system in the neurobiology of anxiety. Another view based on preclinical studies is that κ opioid receptor antagonists produce profoundly consistent anxiolytic effects in animal behavioral models, although solid evidence from clinical studies is lacking. It is not fully understood how the κ opioid receptor blockade will eventually affect behavior. Answering this question may provide insights into the mechanism of anxiety. So far, the available κ opioid receptor antagonists have an extremely long duration of action. If shorter acting agents become available and are effective, κ opioid receptor antagonists may have therapeutic potential for the treatment of anxiety disorders.

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