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Fundamentals of the Dynorphins / Kappa Opioid Receptor System: From Distribution to Signaling and Function

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Abstract

This chapter provides a general introduction to the dynorphins (DYNs) / kappa opioid receptor (KOR) system, including DYN peptides, neuroanatomy of the DYNs / KOR system, cellular signaling, and *in vivo* behavioral effects of KOR activation and inhibition. It is intended to serve as a primer for the book and to provide a basic background for the chapters in the book.

1. Historical perspectives

Opium, the dried latex or gum obtained from lancing the outer surface of the seed pods of the opium poppy *Papaver somniferum*, has been used for centuries for medicinal and recreational practices to relieve pain, cough and diarrhea, and to cause euphoria (Presley and Lindsley 2018). The euphoria produced by opium and iatrogenic-induced positive mood effects have been the basis for its abuse. It was in the 20th century when there were major advances made in understanding how the active constituents of opium, such as morphine, act to produce their beneficial and harmful effects.

Opioid receptors were first hypothesized in 1954 by Beckett and Casy (1954) and stereospecific saturable binding of levorphanol was proposed to be the opiate receptor (Goldstein et al. 1971). In 1973, the existence of specific receptors for opioid drugs was demonstrated in brain preparations by radioligand binding assays (Pert et al. 1973; Simon et al. 1973; Terenius 1973). Multiplicity of opioid receptors was reported, with different classes of opioid drugs having distinct pharmacological activities (Lord et al. 1977; Martin

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et al. 1976). Following the identification of opioid receptors, an intense search for the endogenous ligands ensued and cumulated in the discovery of enkephalins, β-endorphin and dynorphins (DYNs) as ligands for the delta, mu and kappa opioid receptors (DOR, MOR and KOR) (Chavkin et al. 1982; Goldstein et al. 1979; Hughes et al. 1975; Loh et al. 1976). Subsequently, the precursors of these peptides, proenkephalin, proopiomelanocortin and prodynorphin (pDYN), were cloned and their amino acid sequences deduced [see (Höllt 1986) for a review]. These precursors are synthesized as large proteins in cell bodies by the protein synthesis machinery, transported via axonal transport to nerve terminals and cleaved by peptidases to produce the final active peptides during the transport process. In the 1980s, great efforts by chemists and pharmacologists resulted in synthesis and characterization of selective MOR, KOR and DOR agonists and antagonists, which facilitated *in vitro* and *in vivo* opioid pharmacological studies. The first prototypical selective KOR agonist U50,488H and antagonist nor-binaltorphimine (norBNI) were reported by von Voigtlander et al. (1983) and Portoghese et al. (1987), respectively. Roth et al. (2002) demonstrated that salvinorin A, a compound isolated from the mint family plant *Salvia divinorum,* was a selective KOR agonist and also the first nonnitrogenous KOR agonist.

In 1992, the laboratories of Evans and Kieffer independently cloned the DOR (Evans et al. 1992; Kieffer et al. 1992). Subsequently, the KOR was cloned from several species, along with the MOR and a highly homologous receptor named opioid receptor-like (ORL) receptor [which subsequently renamed as nociceptin/orphanin FQ receptor (NOP receptor)] were cloned [see (Knapp et al. 1995) for a review]. Following cloning of the receptors, many studies demonstrated the structural bases of ligand binding selectivity using chimeric receptor and site-directed mutagenesis approaches. In addition, genetic deletion of the KOR or pDYN in mice revealed the *in vivo* physiological and pharmacological roles of the DYNs/KOR system and in sustaining drug and alcohol abuse (Chavkin 2013; Simonin et al. 1998). In the meantime, deletion of the MOR, DOR and NOP receptor was used to demonstrate their functional roles [see (Gaveriaux-Ruff and Kieffer 2002) for review]. In 2012, X-ray crystal structures of an inactive state of the human KOR complexed with the selective antagonist JDTic was revealed (Wu et al. 2012), and in 2018, the structure of an active state of the KOR was reported (Che et al. 2018). During this period, structures of active and inactive states of the MOR, DOR and NOP receptor were also published [see (Marino et al. 2018) for a review].

2. Dynorphin peptides

Endogenous opioid peptides are processed from three precursors, proopiomelanocortin, proenkephalin, and pDYN. Endogenous activation of the KOR primarily occurs via the opioid peptides derived from pDYN (Chavkin et al. 1982). Dynorphins such as DYN A (1–7, 1–8, 1–13, and 1–17), DYN B, big DYN (DYN A + DYN B), α-neoendorphin, and β-neoendorphin are derived from the processing of pDYN (Figure 1) by various non-selective proteases, including cathepsin L, prohormone convertases 1, 2 and 3 and carboxypeptidase E [see (Chavkin 2013; Fricker et al. 2020; Schwarzer 2009) for reviews]. Dynorphins and their endogenous target, the KOR, constitute the DYNs/KOR system (Chavkin et al. 1982). In addition, several peptides derived from proenkephalin, such as metorphamide and BAM-18, have high affinity for the KOR (Hurlbut et al. 1986). Although most peptide

products elicit opioid-like effects, whether the different peptides have different physiological functions remains unclear. In addition, there is differential processing of pDYN throughout the brain as suggested by early work showing that the ratios of DYNs and other pDYN derivative levels varied across different regions of the mesocorticolimbic and nigrostriatal dopamine (DA) systems (Zamir et al. 1984). Further, pDYN and DYNs are colocalized mostly on presynaptic sites of hippocampal and striatal neurons, but more evenly distributed throughout the soma of amygdala and cortex neurons. Of the different DYN peptides, DYN A(1-17) is considered the primary KOR ligand having higher potency at the KOR than other DYN peptides [see (Chavkin 2013; Fricker et al. 2020; Schwarzer 2009) for reviews]. Notably, DYNs have affinity for other opioid and non-opioid receptors in addition to the KOR. For example, DYNs bind to the MOR and DOR in brain tissue, with equal or lower affinities than to the KOR [see (Chavkin 2013; Fricker et al. 2020; Schwarzer 2009) for reviews]. Des-Tyr^{1} DYNs have direct effects on N-methyl-D-aspartate (NMDA) receptors in the spinal cord and brain (Caudle and Dubner 1998; Shukla and Lemaire 1994). Dynorphins also show activity at bradykinin receptors, which have been implicated in pain (Lai et al. 2006). Thus, DYN peptides, typically considered a monolithic entity in the field, may be more diverse in their processing and actions than has been appreciated.

In contrast to classical neurotransmitter systems whereby an action potential results in pre-synaptic transmitter release from the synaptic active zone, neuropeptide signaling parameters may vary widely in terms of spatio-temporal release, target activation, and receptor fidelity (Gomes et al. 2020). Following sustained neuronal activity, DYNs are released from large dense core vesicles (Cho and Basbaum 1989; Drake et al. 1994) and decreases cellular activation following binding to KOR. Dynorphins can be released from pre-synaptic neurons onto post-synaptic neurons or act in an auto-inhibitory manner by activating presynaptic KORs in cells that release DYNs, or from be released from dendrites to produce retrograde inhibition of KOR-sensitive presynaptic inputs [see (Chavkin 2013; Schwarzer 2009) for reviews].

Dynorphin peptides are widely expressed throughout the brain and spinal cord, with only the cerebellum and dorsal thalamus having no DYNs (Fallon and Leslie 1986; Mansour et al. 1994; Mansour et al. 1988) (Figure 2). For example, expression of pDYN is high in the substantia nigra pars reticulata, various hypothalamic nuclei, nucleus of solitary tract, hippocampus, globus pallidus, spinal trigeminal nucleus and substantia gelatinosa of spinal cord. Medium to low pDYN levels are found in the amygdala, nucleus accumbens, olfactory tubercle, caudate putamen, bed nucleus stria terminalis, preoptic area, periaqueductal grey, parabrachial nucleus, raphe nucleus, cortex, periventricular nucleus of thalamus, substantia nigra pars compacta, ventral tegmental area and superior and inferior colliculus. Although it is important to consider that DYN peptides are released from the terminals of these neurons and rarely from the soma of the neurons. Thus, low pDYN levels do not mean that DYN projection neurons may not release peptide in these locations.

Insight into the role of pDyn-derived peptides in behaviors has been gained utilizing mutant mice lacking pDyn-derived peptides (Schunk et al. 2008; Sharifi et al. 2001). Mice with genetic deletion of pDyn have been demonstrated to (1) show impaired extinction learning of contextual fear (Bilkei-Gorzo et al. 2012); (2) have diminished impairment in cognition

associated with aging and stress (Carey et al. 2009; Ménard et al. 2013; Nguyen et al. 2005); (3) exhibit increased alcohol and drug self-administration and decreased stress-induced reinstatement of drug preference (Femenía and Manzanares 2012; Galeote et al. 2009; Redila and Chavkin 2008); (4) display anxiolytic phenotypes relative to controls (Ménard et al. 2013; Wittmann et al. 2009) or show increased anxiety-like behavior relative to controls (Femenía et al. 2011); (5) have decreased pain in models of chronic pain [see (Tseng and Hoon 2020) for a review]. Therefore, endogenous dynorphins likely play a pivotal role in regulating cognition, fear-related behaviors, reward, anxiety, and nociception.

Cloning of the KOR

The KOR has been cloned from several species including humans (Mansson et al. 1994; Simonin et al. 1995; Zhu et al. 1995). The KOR genes were mapped at position q11-12 in human chromosome 8 (Simonin et al. 1995) and mouse chromosome 1A2-3 (Nishi et al. 1994). Like all other G protein-coupled receptors (GPCRs), the KORs have seven transmembrane domains with a N terminal outside the cell and an intracellular C terminal domain. The sequences of mouse, rat, guinea pig and human KORs have high homology. The amino acid sequence of the human KOR is 94% identical to those of the rat and mouse and 91% identical to that of the guinea pig (Simonin et al. 1995). The N- and C-terminal domains have more sequence variations than the transmembrane domains among the KORs of different species. The amino acid sequences of the human KOR, MOR, DOR and NOP receptor share ~ 60% overall identity and ~80% identity among the transmembrane domains (Zhu et al. 1995). The N- and C-terminal domains have the highest sequence divergence among the four opioid receptors, hence antibodies are typically generated against the N- and C-terminal domain peptides.

Neuroanatomy of the KOR

The distribution of the KOR has been investigated with several different methods, including biochemical techniques, i.e. receptor autoradiography and immunohistochemistry (IHC), and recently with mutant mouse lines expressing KOR-Cre or KOR-tdTomato. The KOR IHC, discussed below, has been plagued by lack of highly specific antibodies. The KOR mRNA distribution has been examined using *in situ* hybridization.

Receptor autoradiography was performed on sections of fresh frozen tissues with radioactive labeled KOR-selective ligand such as [^{3}H]U69,593 (Mansour et al. 1994; Unterwald et al. 1991; Wang et al. 2011) or [^{3}H]CI-977 (Slowe et al. 1999), and the sections were exposed to ^{3}H-senstive films or screens. The most important advantage of receptor autoradiography is its high specificity because of the use of highly selective ligands and anatomical definition. However, it takes a relatively long exposure time to obtain the results due to the low energy of β-particles emitted from ^{3}H-labeled ligands. In addition, autoradiography has low resolutions, making it difficult to visualize small brain regions.

Immunohistochemistry of the KOR has been carried out with antibodies against synthetic peptide fragments of partial sequences of N- and C-terminal domains the KOR [for example, (Appleyard et al. 1997; Arvidsson et al. 1995; Drake et al. 1996; Mansour et al. 1996)].

Because these studies yielded significant different results among themselves and also from those of receptor autoradiography, these studies are not described here. Issues related to KOR antibodies are discussed elsewhere in this book.

Recently, reporter protein approaches were employed to map the distribution of the KOR in the mouse brain. The Ross lab generated a KOR-Cre mouse line, in which the exon 2 coding region of the *oprk1* gene was replaced with that of *Cre* recombinase (Cai et al. 2016). They then bred KOR-Cre mice with mice expressing the Cre-dependent allele $Rosa^{lsl\ tdTomato}$ (also known as *Ai14*), which resulted in tdTomato (tdT) being expressed in KOR-containing cells. The KOR distribution in the KOR-Cre x Ai14 mouse brain was similar to that of receptor autoradiography in most regions, however, there were significant differences, such as the presence of the KOR in the cerebellum, high expression in the striatum and low expression in the claustrum. These differences are due to constitutive and cumulative expression of Cre from all the developmental stages. This can be circumvented by viral injection of Cre-dependent reporter gene into brain regions of interests.

Liu-Chen's lab produced a knockin mouse line that expresses a fusion protein of KOR conjugated in frame with tdTomato 5' to the stop codon (KOR-tdT) (Chen et al. 2020). The KOR-tdT has similar distribution as KOR in receptor autoradiography of adult mouse brains. As the expression of KOR-tdT is under the control of KOR promotor like the wildtype KOR, KOR-tdT mice do not have developmental issues as KOR-Cre mice. One unique feature is that these mice display prominent KOR-tdT-containing neuronal fibers not seen with receptor autoradiography, for example, projections from the striatum to substantia innominata and substantia nigra reticulata. The KOR-Cre x Ai14 and KOR-tdT mouse lines provide resolutions at the cellular level and are valuable tools for KOR neuroanatomy studies. Liu-Chen's lab, employing the CLARITY technique (Chung and Deisseroth 2013) to clear mouse brains followed by 3-D imaging, produced the first 3-D images of the KOR in the brain, which is also the first for any GPCRs (Chen et al. 2020)(Video 1).

Results from receptor autoradiography and reporter protein approaches show that the KOR has a widespread distribution in the brain. The claustrum, a brain region implicated in consciousness, has the highest level, followed by the endopiriform nucleus (separate regions in rodents that are a single continuous structure in primates); however, the functions of KOR in these areas are not clear. The KOR is present in the areas involved in mood, reward, motivation and addiction, including the ventral tegmental area, nucleus accumbens, prefrontal cortex, anterior cingulate cortex, amygdala nuclei, bed nucleus of stria terminalis and raphe nucleus, with the nucleus accumbens shell exhibiting particularly intense signal. Moderate levels of KOR are observed in pain pathways, including the periaqueductal gray, parabrachial nucleus, some nuclei in thalamus, primary and secondary somatosensory cortices. The KOR is found in several nuclei in the hypothalamus, indicating roles of the KOR in neuroendocrine regulation. The KOR is also found in paraventricular nucleus and nucleus reunion of thalamus, but its functions in these brain regions is currently unknown (Figure 3, for KOR distribution, signaling and ligands see https://www.guidetoimmunopharmacology.org/GRAC/ObjectDisplayForward?objectId=318). Discrepancies in KOR binding versus KOR mRNA expression may in part be due to trafficking of KOR to terminals of long-range neuronal projections. The KOR mRNA

is localized in cell bodies, whereas KOR binding in any given region is comprised of KOR in local cells and KORs localized on afferent inputs to that region.

The KOR is also found in the dorsal horn of the spinal cord, and is involved in pain and itch regulation. Additionally, the KOR is distributed throughout the body, including in the lungs, heart, spleen, kidneys, liver and small intestines (Peckys and Landwehrmeyer 1999; Peng et al. 2012), though its precise function in each of these regions is yet to be fully described.

There are species differences in the KOR distribution in the brain, with the receptor distribution in the human brain being more similar to that in the guinea pig than in rat or mouse. For example, in the guinea pig and human, but not the rat or mouse, the KOR is abundant in the cerebellum and deep layers (layers V and VI) of the cortex, and is found in striosomes of the striatum (having patchy distribution) (Mansour et al. 1988; Quirion and Pilapil 1991; Quirion et al. 1987). Transcript for the KOR also shows some divergence between humans and rodents where unlike rodents, there was low level of transcript in the human substantia nigra and hippocampus (Simonin et al. 1995).

There are also differences in the KOR level in the brain among species. Kitchen et al. (1990) reported that B_{max} value of [^{3}H]U69,593 binding to the KOR in adult rat brain was 7.3 fmol/mg protein. The KOR levels in the human frontal cortex and forebrains of animals are in the order of pigeon (8x) > guinea pig (~3x) ~ human (~2.5x) > mouse (~1.5x) > rat brain (1x) (Mansour et al. 1988; Quirion and Pilapil 1991; Quirion et al. 1987). Moreover, the KOR level in the adult rat brain is about 1/20 of that of the MOR, which is second to the NOP receptor that has the highest expression in the brain of all opioid receptors. However, it was reported that the KOR mRNA was the most abundantly expressed over MOR and DOR in the human during development (Wang et al. 2006).

KOR signaling at the cellular level

In the brain, DYNs are released upon membrane depolarization and activates the KOR to modulate presynaptic and postsynaptic neural activity (Chavkin et al. 1983; Wagner et al. 1991). Opioid receptors, including the KOR, belong to the rhodopsin sub-family of GPCRs. At the cellular level, activation of the KOR stimulates Gi/o proteins and promotes receptor phosphorylation. Phosphorylated KOR, in turn, recruits β-arrestins, which lead to β-arrestin-mediated signaling (Bruchas et al. 2006; Law 2011; McLennan et al. 2008). It has also been reported that the KOR signals via Gz and G16 proteins (Lee et al. 1998; Tso and Wong 2000).

Activation of KOR results in dissociation of guanosine 5'-diphosphate (GDP) from $G_{\alpha i}$ and association of guanosine 5'-triphosphate (GTP) with $G_{\alpha i/o}$ as well as dissociation of the $G_{\beta\gamma}$ from G_{α} subunits. Activated $G_{\alpha i/o}$ proteins inhibit adenylate cyclase resulting in decreases in cyclic AMP production and a host of effects in downstream targets, and inhibit Ca^{2+}-channel activity and attenuate presynaptic neurotransmitter release [see (Law 2011) for a review]. $G_{\beta\gamma}$ proteins activate G protein-gated inwardly rectifying potassium channels (GIRKs) and other potassium channels, which causes a reduction in neuronal or axonal excitability [see (Law 2011) for a review]. $G_{\beta\gamma}$ proteins also enhance ERK1/2 phosphorylation (early phase)

via L-type Ca^{2+} channels, phospholipase C (PLC), intracellular Ca^{2+} release, protein kinase C (PKC) (Bohn et al. 2000; Law 2011). β-Arrestins-mediated signaling includes activation of ERK1/2 (late phase) and p38 MAPK (Al-Hasani and Bruchas 2011; Bruchas and Chavkin 2010). The KOR also activates c-Jun N-terminal kinase (JNK) and protein kinase B (Akt) pathways. Hence, activation of the KOR results in signal transduction via a variety of intracellular pathways with diverse effects on cells (Bruchas and Chavkin 2010; Pradhan et al. 2012). Biased agonism for the KOR has been demonstrated *in vitro*, in which agonists preferentially activate G protein- or β-arrestin-mediated pathways [for a review, (Bohn and Aubé 2017)]. Several KOR biased agonists have been reported, which may provide beneficial pharmacological effects with reduced unwanted side effects [see (Bohn and Aubé 2017; Faouzi et al. 2020; Mores et al. 2019) for reviews]. The predominance of one signaling cascade over another may vary by brain region, demonstrating the potential utility of biased KOR ligands that may target one downstream cascade over another (Crowley and Kash 2015). Activation of JNK signaling was reported to inactivate the KOR, which is one of the mechanisms for prolonged durations of action (up to weeks) of KOR antagonists, such as norBNI and JDTic (Bruchas et al. 2007).

Agonist-promoted KOR phosphorylation and regulation

Binding of an agonist, such as U50,488H to the KOR, caused KOR phosphorylation at S356, T357, T362 and S369 in the C-terminal domain (Chen et al. 2016) in cultured cells. The KOR phosphorylation at all the residues was mediated by $G_{i/o}$ alpha proteins and G protein-coupled receptor kinases (GRK2, GRK3, GRK5, GRK6), and PKC (Chiu et al. 2017). GRKs-mediated, but not PKC-mediated, KOR phosphorylation followed by β-arrestin recruitment desensitized U50,488H-induced ERK1/2 response and [^{35}S]GTPgS binding and was involved in agonist-induced KOR internalization (Liu-Chen 2004). PKC activation by phorbol ester induced agonist-independent KOR phosphorylation. Compared with U50,488H, PKC activation induced much higher S356/T357 phosphorylation, much lower T363 phosphorylation, and similar levels of S369 phosphorylation. PKC activation caused a lower level of agonist-independent KOPR internalization, compared with U50,488H.

U50,488H promoted KOR internalization in a process that depends on GRKs, β-arrestin, and dynamin proteins (Li et al. 1999). KOR internalization precedes downregulation which, in addition, involves Rab5- and Rab7-dependent process and requires ubiquitination of the KOR (Li et al. 2000). The KOR appears to be internalized into early endosomes then trafficked via late endosomes to lysosomes and proteosomes for degradation (Liu-Chen 2004).

X-ray Crystal Structures of the KOR

The understanding of the DYNs / KOR system function and signaling continues to be enhanced by the publication of the high-resolution crystal structures of the receptor. Wu et al. (2012) reported an X-ray crystal structure of an inactive state of the human KOR in complex with the selective KOR antagonist JDTic. Che et al. (2018) provided a crystal structure of an active state of the human KOR in complex with the epoxymorphinan opioid agonist MP1104 and an active-state-stabilizing nanobody. The active structure

provides significant information for the structural basis of biased agonism (Che et al. 2018) and allosteric modulation (Che et al. 2020). Comparisons between inactive- and active-state KOR structures reveal substantial conformational changes in the binding pocket and intracellular and extracellular regions. The characterization of the crystal structures of inactive and active states of the KOR has provided insight for ligand-receptor interactions allowing new concepts for novel drug design. This structural characterization together with identification of the signaling events that elicit antinociceptive versus dysphoric and psychotomimetic effects, has provided extensive advancement in novel chemical entities that hold promise as new pain treatments with minimal aversive effects including depressive or addictive properties.

In vivo pharmacology of the DYNs/KOR system

Activation of the KOR *in vivo* produces many physiological effects and behavioural responses, including analgesia, antipruritic effects, water diuresis, dysphoria / aversion, sedation, motor incoordination and hypothermia (Mucha and Herz 1985; Pfeiffer et al. 1986; Simonin et al. 1998; Togashi et al. 2002; von Voigtlander et al. 1983). KOR agonists are effective analgesics without causing respiratory depression and abuse liabilities associated with MOR-selective or preferring analgesics (Paton et al. 2020). In light of the recent opioid epidemic, KOR agonists may be re-examined as analgesics, either alone or as part of pharmacological regimen. Generation of KOR-deficient animals has provided significant knowledge on the *in vivo* physiological role of the DYNs / KOR system (Ansonoff et al. 2006; Simonin et al. 1998). Dynorphins are released in the central nervous system during pain states and activate KORs to produce analgesia, with KOR-knockout mice exhibiting enhanced pain sensitivity (Simonin et al. 1998).

The KOR is also involved in the pathophysiology of pruritus, with KOR agonists as promising antipruritic agents [for a review see Cowan et al. (2015)]. However, to date, nalfurafine is the only KOR agonist approved for clinical use (marketed in Japan). One of the limitations that has hampered the development of KOR agonists as safer analgesics and effective anti-pruritic agents is the negative effects that emerge following KOR agonist administration. KOR agonists produce dysphoria, depressive-like symptoms and psychotomimetic effects in humans (Pfeiffer et al. 1986; Wadenberg 2003; Walsh et al. 2001), and elicit place aversion and depressive-like behaviors (Mucha and Herz 1985; Shippenberg and Herz 1986) as well as stimulate drug-seeking (Bruchas et al. 2010; Chavkin and Koob 2016; Knoll and Carlezon 2010) in rodents. Activation of the DYNs/KOR system also elicits signs of anxiety and fear in animals and humans (Chartoff and Mavrikaki 2015; Chavkin and Koob 2016; Darcq and Kieffer 2018).

Chavkin and colleagues have proposed that antinociception produced by KOR agonists is mediated by G protein pathways, whereas aversion is mediated by β-arrestin2-dependent p38 MAP kinase phosphorylation [reviewed in (Bruchas and Chavkin 2010)]. However, White et al. (2015) showed that U69,593, salvinorin A and RB-64 (a salvinorin A analog) produced similar levels of aversion in the conditioned place preference (CPA) test in wildtype and β-arrestin2−/− mice, indicating that either β-arrestin2 is not involved in CPA or other pathways besides β-arrestin2 are involved. In addition, βarrestin2 deletion impaired

KOR-mediated motor incoordination, but did not affect antinociception or hypolocomotion (White et al. 2015). Morgenweck et al. (2015) demonstrated that β-arrestin2 deletion did not affect the anti-scratch effects of U50,488H or the G protein-biased KOR agonist isoquinone 2.1, indicating that the anti-scratch effect of KOR agonists is not mediated by β-arrestin2.

Several groups have been actively searching for G protein-biased KOR agonists [see (Bohn and Aubé 2017; Faouzi et al. 2020; Mores et al. 2019) for reviews] to avoid the dysphoric and psychotomimetic effects. Whether G protein-biased KOR agonists will fulfill the promise of retaining beneficial analgesic and antipruritic effects with reduced side effects in humans remains to be determined. Another approach is to develop peripherally acting KOR agonists to avoid the central nervous system-mediated side effects. An example is CR845/difelikefalin, which is under clinical trials for treatment of pruritus associated with chronic kidney or liver disease or atopic dermatitis and treatment of pain in post-operative settings (Fishbane et al. 2020; Therapeutics 2020) (https://www.caratherapeutics.com/pipeline-technology/our-pipeline/).

Kappa opioid receptor antagonists have been demonstrated to have antidepressant- and anxiolytic-like activities in rodents. In addition, KOR antagonists were found to reduce stress-induced instatement of drug seeking for several drugs of abuse in animals. Thus, KOR antagonists may be useful for treatment of depression, anxiety and drug addiction [see (Al-Hasani and Bruchas 2011; Bruchas et al. 2010; Carroll and Carlezon 2013; Van't Veer and Carlezon 2013) for reviews]. Buprenorphine, having MOR partial agonist and KOR antagonist activities, in combination with the MOR antagonist samidorphan was investigated in clinical trials for treatment of depression, but the combination did not meet the primary endpoint goal (Zajecka et al. 2019). JDTic underwent clinical trials for cocaine use disorder, but failed in phase I due to cardiac side effects (Buda et al. 2015). Aticaprant (formerly JNJ-67953964, CERC-501 and LY2456302) is currently in clinical trials for management of anhedonia (an important symptom of major depressive disorder) (Krystal et al. 2020; Pizzagalli et al. 2020).

Conclusion

The KOR is found throughout the peripheral and central nervous systems and participate in a range of physiological functions. The potential therapeutic use of KOR ligands as analgesics, anti-pruritic, mood and substance use disorders are of continued interest and being pursued by various pharmaceutical companies. The following chapters will provide more thorough insights of KOR functions in various physiological and pathological states, together with recent developments in chemistry and pharmacology of novel KOR ligands and their therapeutic potentials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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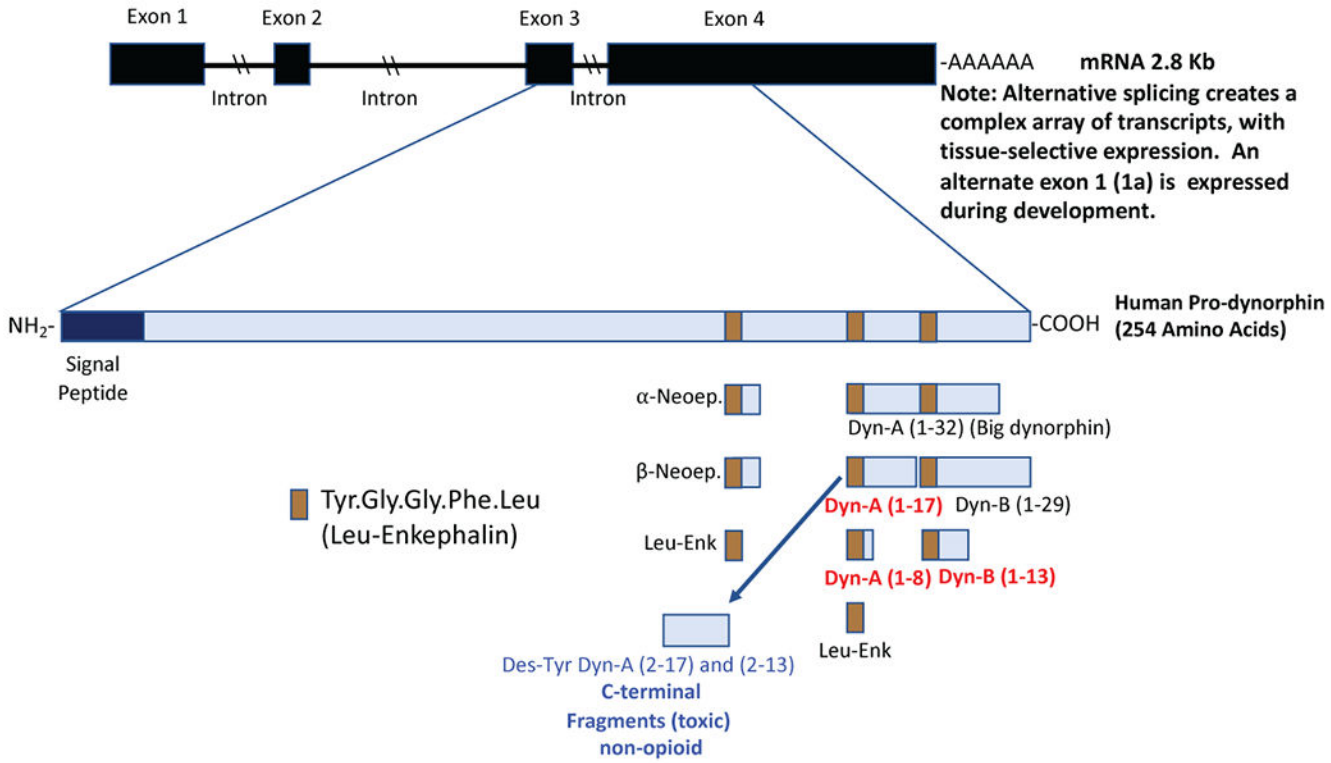

Figure 1. Summary of post-translational PDYN products, changes in chronic pain and effects on nociception, and expression patterns.

A. Dynorphin peptides are up-regulated in chronic neuropathic and inflammatory pain models (**marked in red**). Spinal (intrathecal) administration of DYN peptides that produce acute antinociception include Dyn A (1-8), Dyn A (1-17), Dyn B (1-13). Spinal administration of DYN peptides, Dyn A (1-17), Dyn B (1-13), Dyn A (2-17), Dyn A (2-13), **produces pain-like (allodynia and hyperalgesia)** responses via activation of NMDA or bradykinin receptors. [Reviewed in (Podvin et al. 2016)]. Dyn A (2-17 or 2-13) have no activity at KORs.

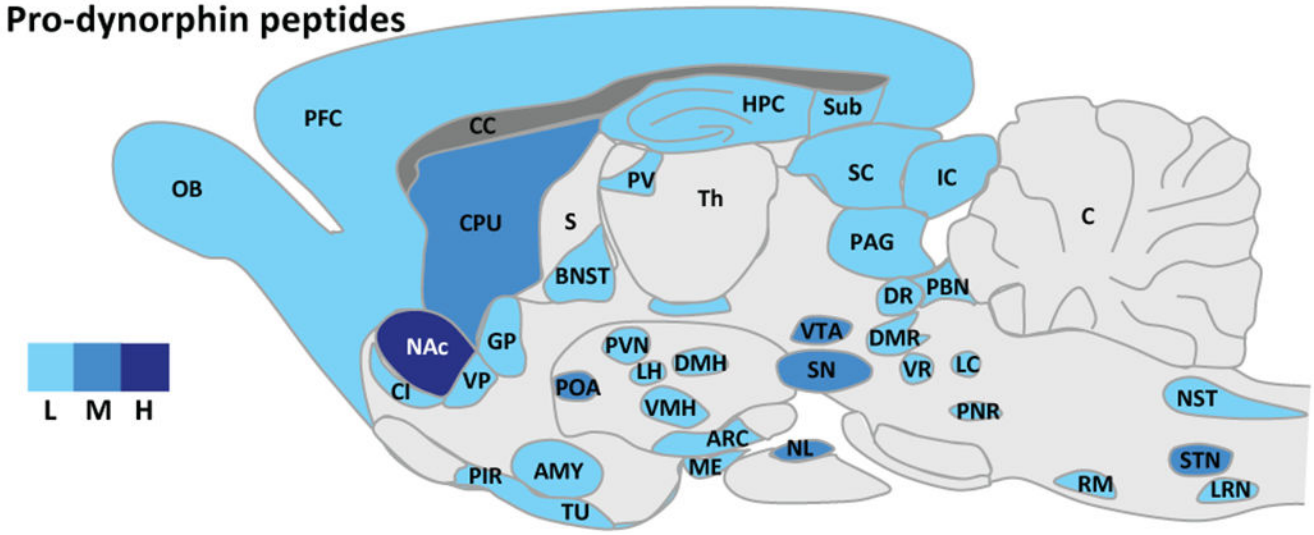

Figure 2.

Dynorphin peptide expression in the rodent brain. L: low, M: moderate, H: high expression. Abbreviations. ARC: arcuate nucleus, hypothalamus, AMY: amygdala, BLA: basolateral nucleus, amygdala, BNST: bed nucleus of the stria terminalis, C: cerebellum, CC: corpus collosum, CeA: central nucleus, amygdala, CI: claustrum, CL: centrolateral thalamus, CM: centromedial thalamus, COA: cortical nucleus, amygdala, CPU: caudate putamen, DMH: dorsomedial hypothalamus, DMR: dorsal and medial raphe, DR: dorsal raphe, DTN: dorsal tegmental nucleus, EN: endopiriform cortex, GP: globus pallidus, HPC: hippocampus, IC: inferior colliculus, IP: interpeduncular nucleus, LC: locus coeruleus, LD: laterodorsal thalamus, LH: lateral hypothalamus, LRN: lateral reticular nucleus, ME: median eminence, MeA: median nucleus, amygdala, NAc: nucleus accumbens, NST: nucleus tractus solitarius, NL: neuronal lobe, pituitary, NRGC: nucleus reticularis gigantocellularis, OB: olfactory bulb, OCX: occipital cortex, PAG: periaqueductal gray, PBN: parabrachial nucleus, PCX: parietal cortex, PFC: prefrontal cortex, PIR: piriform cortex, PNR: pontine reticular, POA: preoptic area, PV: paraventricular thalamus, PVN: paraventricular hypothalamus, RE: reuniens thalamus, RM: raphe magnus, S: septum, SC: superior colliculus, SN: substantia nigra, STN: spinal trigeminal nucleus , Sub: subiculum, TCX: temporal cortex, Th: thalamus, TU: olfactory tubercule, VMH: ventromedial hypothalamus, VP: ventral pallidum, VR: ventral raphe, VTA: ventral tegmental area, Zi: zona incerta. [Adapted from Le Merrer et al. (2009)].

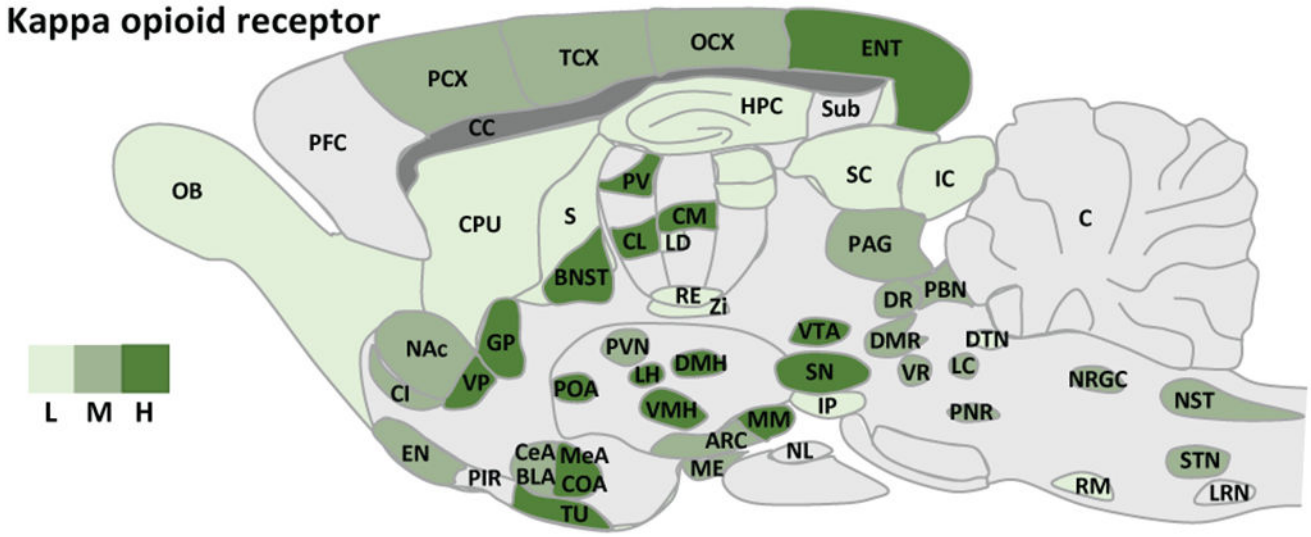

Figure 3.

Expression of the KOR in rat brain. L: low, M: moderate, H: high expression. Abbreviations. ARC: arcuate nucleus, hypothalamus, AMY: amygdala, BLA: basolateral nucleus, amygdala, BNST: bed nucleus of the stria terminalis, C: cerebellum, CC: corpus collosum, CeA: central nucleus, amygdala, CI: claustrum, CL: centrolateral thalamus, CM: centromedial thalamus, COA: cortical nucleus, amygdala, CPU: caudate putamen, DMH: dorsomedial hypothalamus, DMR: dorsal and medial raphe, DR: dorsal raphe, DTN: dorsal tegmental nucleus, EN: endopiriform cortex, GP: globus pallidus, HPC: hippocampus, IC: inferior colliculus, IP: interpeduncular nucleus, LC: locus coeruleus, LD: laterodorsal thalamus, LH: lateral hypothalamus, LRN: lateral reticular nucleus, ME: median eminence, MeA: median nucleus, amygdala, NAc: nucleus accumbens, NST: nucleus tractus solitarius, NL: neuronal lobe, pituitary, NRGC: nucleus reticularis gigantocellularis, OB: olfactory bulb, OCX: occipital cortex, PAG: periaqueductal gray, PBN: parabrachial nucleus, PCX: parietal cortex, PFC: prefrontal cortex, PIR: piriform cortex, PNR: pontine reticular, POA: preoptic area, PV: paraventricular thalamus, PVN: paraventricular hypothalamus, RE: reuniens thalamus, RM: raphe magnus, S: septum, SC: superior colliculus, SN: substantia nigra, STN: spinal trigeminal nucleus , Sub: subiculum, TCX: temporal cortex, Th: thalamus, TU: olfactory tubercule, VMH: ventromedial hypothalamus, VP: ventral pallidum, VR: ventral raphe, VTA: ventral tegmental area, Zi: zona incerta. [Adapted from Le Merrer et al. (2009)].