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Review Article

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Peptide Kappa Opioid Receptor Ligands: Potential for Drug Development

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Abstract. While narcotic analgesics such as morphine, which act preferentially through mu opioid receptors, remain the gold standard in the treatment of severe pain, their use is limited by detrimental liabilities such as respiratory depression and drug dependence. Thus, there has been considerable interest in developing ligands for kappa opioid receptors (KOR) as potential analgesics and for the treatment of a variety of other disorders. These include effects mediated both by central receptors, such as antidepressant activity and a reduction in cocaine-seeking behavior, and activity resulting from the activation of peripheral receptors, such as analgesic and anti-inflammatory effects. While the vast majority of opioid receptor ligands that have progressed in preclinical development have been small molecules, significant advances have been made in recent years in identifying opioid peptide analogs that exhibit promising *in vivo* activity. This review will focus on possible therapeutic applications of ligands for KOR and specifically on the potential development of peptide ligands for these receptors.

KEY WORDS: antidepressant; blood–brain barrier; cocaine abuse; dynorphin analogs; peptide metabolism.

INTRODUCTION

Opioid receptors are important targets for the treatment of pain. Clinically used narcotic analgesics such as morphine act primarily through mu opioid receptors (MOR) and remain the gold standard for the treatment of severe pain, but their use is limited by serious side effects including respiratory depression and dependence liability (1). In an attempt to avoid the detrimental effects of MOR agonists, there has been considerable interest in developing ligands for other opioid receptors as potential therapeutic agents (2). In addition to MOR, delta (DOR) and kappa opioid receptors (KOR) have been pharmacologically characterized and cloned (1). While DOR agonists can produce analgesia, they also have serious side effects, most notably convulsions (see (3) for a review), that have limited their therapeutic development. In contrast, new potential therapeutic applications have recently been recognized for KOR agonists and antagonists (reviewed below), sparking renewed interest in the development of ligands for this receptor.

The vast majority of opioid receptor ligands that have progressed in preclinical development have been small molecules (see (2,4,5) for detailed reviews of small-molecule KOR agonists and antagonists). Significant advances have also recently been made in the identification of opioid peptide analogs with promising *in vivo* activity at MOR and DOR (see (6,7)). This review will discuss the possible therapeutic applications of ligands for KOR, focusing on promising results of peptide ligand activity *in vivo*, and discussing the advantages and challenges in developing peptide ligands for these receptors as potential therapeutic agents.

ACTIONS OF THE ENDOGENOUS KAPPA OPIOID SYSTEM: MODULATION OF DOPAMINE AND RESPONSES TO STRESS

The three opioid receptors all possess the putative seven transmembrane domains characteristic of the superfamily of G-protein-coupled receptors (8). All three opioid receptors couple to and utilize G_i and G_o heterotrimeric G proteins for signal transduction (9). Agonist-induced activation of opioid receptors has been shown to inhibit adenylyl cyclase and calcium channel activity while stimulating potassium channel activity (9). Opioid receptors also regulate the activity of a number of kinases, notably mitogen-activated protein kinase cascades (10). The endogenous ligands for opioid receptors

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ABBREVIATIONS: BBB, blood–brain barrier; BBMEC, bovine brain microvessel endothelial cell; CPP, conditioned place preference; DOR, delta opioid receptor; Dyn, dynorphin; GNTI, 5'-guanidinonaltrindole; i.c.v., intracerebroventricular; i.t., intrathecal; KOR, kappa opioid receptor; MOR, mu opioid receptor; norBNI, nor-binaltorphimine; s.c., subcutaneous; SIA, stress-induced analgesia.

are peptides (see (11) for a recent review), with the dynorphins and related peptides derived from the precursor protein prodynorphin functioning as endogenous ligands for KOR.

The endogenous kappa opioid system is involved in a variety of physiological processes including analgesia, antipruritic activity (12), and diuresis (13,14) that offer opportunities for drug development. The involvement of the KOR system in the regulation of dopamine levels and responses to stress has important implications for several potential therapeutic applications of KOR ligands and will be reviewed below. This article is not intended as a comprehensive review of the endogenous kappa opioid system; for this, readers are referred to the annual review series in *Peptides* (see for example (15)) for detailed reviews of opioid pharmacology.

The endogenous opioid system modulates dopaminergic signaling in the brain. Activation of MOR and KOR results in opposing modulation of the mesolimbic dopamine system, with MOR agonists increasing dopamine levels while KOR agonists decrease levels of this neurotransmitter (16). Much of this effect likely arises from the location of KOR and the distribution of the dynorphin peptides in the brain. In the neostriatum, the endogenous dynorphins are localized within the GABAnergic medium spiny neurons that express dopamine D_1 receptors (17). Neurons expressing dynorphin project to the substantia nigra, hyperpolarizing the dopaminergic cell bodies located there (18). KOR are located on the presynaptic terminals of mesolimbic dopaminergic neurons synapsing in the nucleus accumbens shell, providing a cellular site for dynorphin to hyperpolarize and inhibit dopamine release (19). Accordingly, acute activation of KOR results in tonic inhibition of dopamine signaling (19,20) by modulating the release of this neurotransmitter (19,21) and its uptake through the dopamine transporter (20).

The endogenous kappa opioid system has also been implicated in multiple behavioral responses to stress. When exposed to an inescapable physical or psychological stressor, rodents demonstrate stress-induced analgesia (SIA) that is blocked by KOR-selective antagonists (22–24) or by the disruption of the prodynorphin gene (23), suggesting that KOR activation contributes to SIA. Moreover, administration of the 1-13 and 1-10 fragments of the endogenous KOR peptide dynorphin (Dyn) A at subanalgesic doses prolonged the SIA produced by mice exposed to forced-swim stress, suggesting that the analgesic effects of dynorphins are potentiated by stressors (25). Intracerebroventricular (i.c.v.) administration of Dyn A or a stable analog (E2078, see below) potentiates the immobility responses to a stressor, an effect blocked by administration of the nonselective opioid antagonist Mr2266 (26). Exposure to forced-swim stress increases the concentration of Dyn A in the hypothalamus in mouse (27) and in the hippocampus in rat (28), consistent with the behavioral findings. Interestingly, elevated cyclic adenosine monophosphate response element-binding protein (CREB) levels in rats and mice produce increased immobility behaviors in the forced swim and learned helplessness paradigms that were blocked by administration of the KOR-selective antagonist nor-binaltorphimine (norBNI), suggesting that CREB-mediated induction of dynorphin induced the immobility (29). Together, these reports suggest that the activation of the endogenous kappa opioid system may potentiate the immobility response to a stressor, an animal model of depression-like behavior (see for example ref. (30)), as well as stress-induced analgesia.

Animal and human studies have shown a clear correlation between exposure to an inescapable stressor and apparent increases in both the rewarding properties and the self-administration of abused drugs (31–33). The endogenous kappa opioid system has been proposed to play a role in mediating these effects (see (19) for a review). In support of this hypothesis, repeated forced-swim stress also dramatically increases the reinforcing effects of cocaine by a mechanism that involves KOR activation (23). The prevention of this increase by KOR antagonist pretreatment prior to conditioning or deletion of the genes for either dynorphin or KOR (23,24) suggested that activation of the endogenous kappa opioid system potentiates the response to the reinforcing effects of cocaine, although the underlying mechanisms are not yet understood.

POTENTIAL THERAPEUTIC APPLICATIONS OF KAPPA OPIOID RECEPTOR LIGANDS

While activation of KOR can produce analgesia, the effectiveness of KOR agonists in antinociceptive assays varies significantly with the type of noxious stimuli (see (34)). KOR agonists are generally less effective in thermal antinociceptive assays involving more intense stimuli but produce maximal responses against mechanical stimuli and chemical irritants. In addition to analgesia mediated by KOR in the central nervous system (CNS), KOR agonists produce antinociceptive effects through interaction with peripheral KOR in inflammatory pain models (see (14,35)) and in thermal hyperalgesia induced by capsaicin (36). KOR agonists not only have analgesic activity but also exhibit anti-inflammatory activity (see (35)). Since many painful conditions are associated with inflammation, this suggests that KOR agonists could be very useful in the treatment of these conditions. KOR agonists also have shown antiarthritic activity and decrease signs of joint damage as well as decreasing inflammation (35,37). Interestingly, there appear to be gender differences in the analgesic activity of KOR agonists, but this is not observed for their anti-inflammatory effects (35). Peripheral KOR also appears to mediate visceral pain, and KOR agonists are potent analgesics in a variety of visceral pain models (see (38) for a review), with chronic inflammation of the viscera enhancing KOR agonist activity (39).

Activation of KOR by selective agonists also produces other effects, including antipruritic activity (12), diuresis (13,14), neuroprotective effects (40), modulation of immune responses (41), and suppression of HIV-1 expression (42). Thus, KOR agonists could have applications as therapeutic agents for a variety of disease states.

KOR agonists generally lack reinforcing effects and can abolish the reinforcing effects of morphine (see (43)). KOR agonists have been reported to reduce tolerance to morphine in a variety of antinociceptive tests, and dynorphin can inhibit opiate withdrawal symptoms in opiate-dependent animals (for a review, see (43)) and in humans (see (44,45) and references cited therein). In addition, KOR agonists generally have opposite subjective effects from MOR agonists (dys-

phoria for KOR agonists *vs.* euphoria for MOR agonists). Some of the centrally mediated effects of KOR agonists are undesirable, particularly dysphoria and sedation (46,47), and have limited the therapeutic development of this class of ligands (13,14). However, the effects of KOR agonists on mood could be beneficial in the treatment of the manic phase of bipolar disorder. In a recent small trial, acute treatment with the nonselective KOR agonist pentazocine decreased symptoms of mania in bipolar disorder patients with no adverse effects (48).

Importantly, because KOR agonists can modulate mesolimbic dopamine levels, these ligands have been investigated as potential treatments of drug abuse, particularly of cocaine. Cocaine blocks dopamine reuptake, increasing extracellular dopamine. Considerable evidence indicates that cocaine's reinforcing effects are mediated by these increases in extracellular dopamine (see (19,47)). Since KOR agonists can decrease dopamine levels, they can act as functional antagonists of cocaine. A number of studies have shown that the acute administration of KOR agonists decreases cocaine self-administration (19,47) and suppresses the rewarding effects of cocaine in animal models, including drug discrimination (49,50) and cocaine conditioned place preference (CPP) assays (24,50).

However, a growing number of studies have found that repeated KOR agonist administration can paradoxically increase extracellular dopamine levels (51) and enhance dopamine signaling (52,53), thereby potentiating cocaine-seeking behavior (24,54). In behavioral studies, repeated infusion of the KOR-selective agonist U50,488 produced a dose-dependent leftward shift in the cocaine choice dose–effect curve in rhesus monkeys self-administering cocaine that was reversed by the KOR-selective antagonist norBNI (54), and in CPP studies KOR agonist administration first suppressed and then potentiated cocaine CPP in a time-dependent norBNI-sensitive manner (24). Both authors speculated that chronic activation of KOR, either from exogenous or endogenous sources, may enhance the relative reinforcing efficacy of cocaine. This paradoxical time-mediated phenomenon is poorly understood, and while it is consistent with the effects of stress on drug-seeking behavior, the mechanism of this KOR agonist-induced potentiation has yet to be established. Nevertheless, it would appear that KOR agonists are better suited therapeutically for acute intervention to suppress cocaine reward and craving than for long-term treatment.

Based on the evidence of endogenous kappa opioid mediation of the immobility in the forced-swim test, a rodent assay for depression-like behavior, KOR antagonists were found to have antidepressant-like activity (30,55). KOR antagonists have also recently been reported to have anxiolytic activity (56). Because of the proposed involvement of the endogenous KOR system in responses to stress and the antidepressant activity of KOR antagonists, Beardsley *et al.* (57) hypothesized that by blocking the KOR response to stress, a KOR antagonist could block reinstatement of cocaine-seeking behavior. Both the nonpeptide antagonist JDTic and the peptide KOR antagonist arodyn (see below) have been shown to prevent stress-induced reinstatement of cocaine-seeking behavior (57,58). In addition, based on a "kappa overdrive" hypothesis for opiate addiction, Rothman *et al.* (59) also tested a functional KOR antagonist (buprenorphine plus naltrexone to antagonize buprenorphine's MOR agonist activity) in human addicts and demonstrated that it could be used to treat opiate addiction. Overall, these results suggest that KOR antagonists could have therapeutic value as a novel class of antidepressants as well as aiding in the maintenance of drug abstinence and the prevention of stress-induced reinstatement of drug abuse.

SMALL-MOLECULE LIGANDS FOR KOR

While a variety of small-molecule ligands, both agonists and antagonists, selective for KOR have been developed (see (2,4,5) for reviews), only a limited number have advanced into clinical trials in humans (see (13,38) for reviews) and none have yet been approved for marketing. Some clinically used analgesics, the so-called mixed agonists–antagonists (e.g., pentazocine), exhibit agonist activity at KOR, but these drugs are not selective for KOR. A KOR-selective agonist has not been successfully marketed for clinical use, in large part due to the dysphoria and sedation resulting from activation of KOR in the CNS (see (13,14) for reviews).

Because of the activity of KOR agonists at peripheral receptors, especially in cases where the pain is associated with inflammation, there has been considerable interest in the development of peripherally selective KOR agonists to avoid the centrally mediated side effects (14,38). The first such peripherally selective compound evaluated clinically was fedotozine (Fig. 1) (60), which initially showed positive results in irritable bowel syndrome and dyspepsia. In subsequent studies, however, the drug was apparently less effective (61) and further clinical development was suspended. Asimadoline (EMD 61753, Fig. 1) has been reported to be a peripherally selective KOR agonist (14) whose transport across the blood–brain barrier (BBB) is limited by the efflux protein P-glycoprotein (62). Asimadoline has undergone clinical trials for the treatment of functional dyspepsia and irritable bowel syndrome, and analysis of the results suggested that asimadoline has some efficacy in subgroups of patients with these diseases (see (63) for a review). Asimadoline activity shows a bell-shaped dose–response curve, however, and a higher dose (10 mg p.o.) has been reported to increase pain in patients after knee surgery (64), although the adverse hyperalgesic and proinflammatory effects were apparently not mediated by opioid receptors. The KOR agonist ADL 10-0101 (Adolor Corp.) has been reported to be peripherally selective and to decrease pain scores in a preliminary study of patients with chronic pancreatitis (65). However, in the initial phase II clinical trial, patients did not report statistically significant decreases in pain following treatment with ADL 10-0101, a result that was attributed to an insufficient dose of the drug (Adolor Corp., news release, April 1, 2002, http://www.adolor.com). Recently, novel tetrapeptides with KOR agonist activity have been identified that are peripherally selective (66–68) and have entered clinical trials (see below).

The main active ingredient in the hallucinogenic plant *Salvia divinorum*, the nonnitrogenous heterocyclic compound salvinorin A (Fig. 1), was unexpectedly found to be a selective KOR agonist (69). While originally used as an ethnomedicinal plant by the indigenous Mazatecs of Mexico,

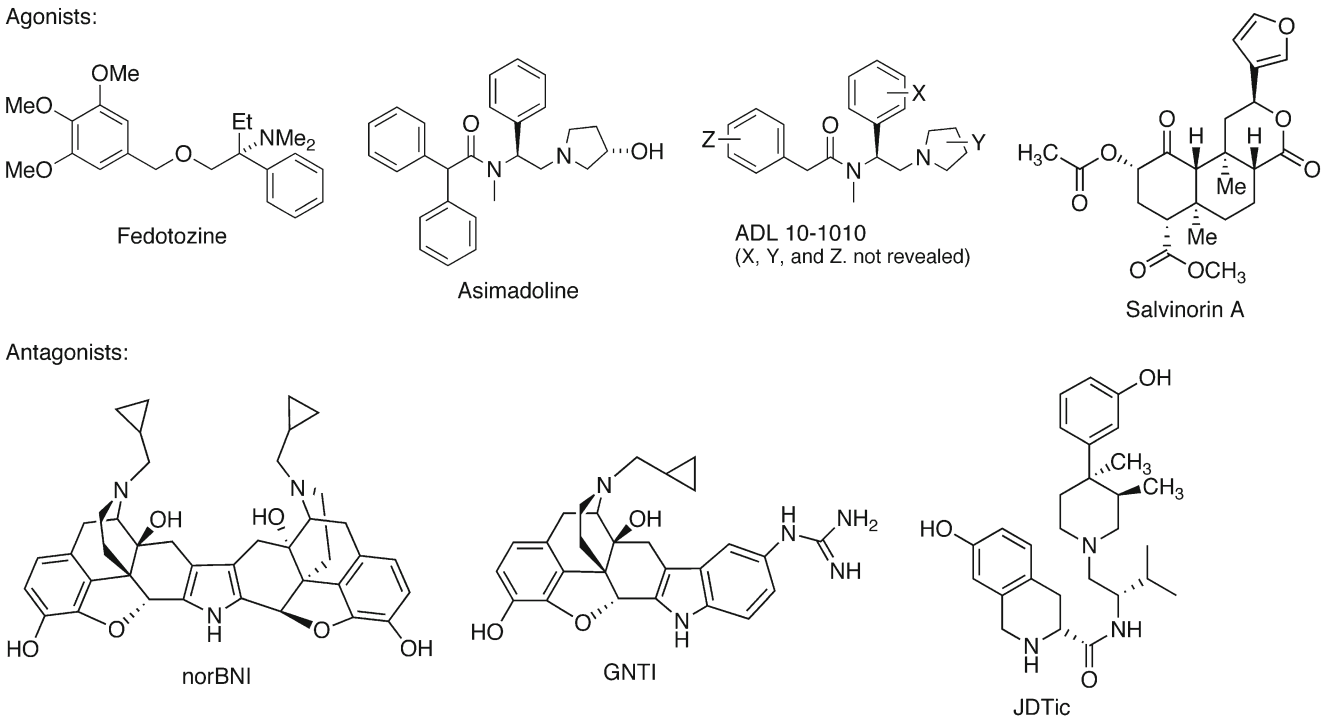


Fig. 1. Nonpeptide KOR-selective ligands discussed in the text. Fedotozine, asimadoline, and ADL 10-1010 are reported to be peripherally selective

in recent years, *S. divinorum* has become widely available, especially via the Internet, and has gained popularity for its hallucinogenic properties. Tests of salvinorin A in a controlled clinical setting in humans are not yet available, but preclinical studies in primates have confirmed that this compound is a potent KOR agonist (70,71).

Selective nonpeptide KOR antagonists have been used extensively in studies of these receptors *in vivo*. The morphinans norBNI and 5′-guanidinonaltrindole (72) and the phenylpiperidine JDTic ((3*R*)-7-hydroxy-*N*-((1*S*)-1-[[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl] methyl]-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide) (73) (Fig. 1) are well established for use in these studies, but all three compounds show exceptionally long durations of action, lasting weeks to more than a month, depending on the species, after a single dose (see (5) for a detailed review). This unusual prolonged antagonism of KOR does not appear to be a function of the receptors, as the nonselective opioid antagonist naloxone antagonizes KOR-mediated antinociception for less than a day (McLaughlin and Aldrich, unpublished results), but the mechanisms responsible for the prolonged activity of the selective KOR antagonists are not yet understood. This prolonged duration of action complicates the use of these small-molecule antagonists as tools in pharmacological studies and could hamper their development as therapeutic agents.

PEPTIDE LIGANDS FOR KOR

Endogenous Peptides and Their Metabolism

The endogenous heptadecapeptide Dyn A (Fig. 2) and the shorter fragment Dyn A-(1-13), which accounts for essentially all of the activity of the larger peptide (74), have been examined *in vivo* in primates (75–77) and humans (see (44,45,78–81) as well as in rodents (see (82)). These peptides elicit a variety of responses in humans, including analgesia (78), suppression of opioid withdrawal symptoms (44,45), and increases in prolactin levels (80). Dyn A-(1-13) has also shown neuroprotective effects *in vivo*, improving the survival in cats with cerebral ischemia (83).

Dyn A and its fragments are rapidly metabolized by a variety of peptidases. Several reports have appeared concerning the metabolism of Dyn A *in vitro* in rat brain (84,85), rhesus monkey blood (86), human blood (86,87), and *in vivo* in rats (88,89). The metabolism of the Dyn A-(1-13) fragment has also been studied in human plasma, blood (87), and cerebrospinal fluid (see (90) for a review) and *in vivo* in rats (91). The metabolism of full-length Dyn A differs from that of Dyn A-(1-13) in human blood (87) and can be altered in disease states (as demonstrated in a model of Parkinson's disease in rats (89)), illustrating the complexity of the metabolic pathways.

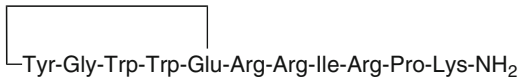
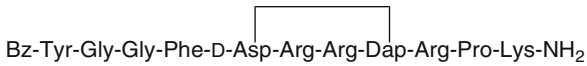
The metabolism of Dyn A results in fragments that have different activity profiles, complicating the interpretation of the results from *in vivo* studies. A major metabolic pathway is the removal of the N-terminal tyrosine residue by aminopeptidases, which abolishes opioid activity (92). However, the pharmacological profile is complicated because the des-Tyr Dyn A fragments, along with Dyn A, can produce toxic effects through nonopioid mechanisms, i.e., neurotoxic effects thought to be mediated primarily though glutamate receptors (principally NMDA (*N*-methyl-D-aspartate)) but also AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate)/kainate receptors (see (93–96) for reviews).

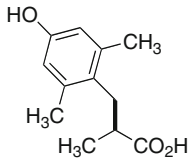
Metabolism in the C terminus of Dyn A yields peptides which retain affinity for opioid receptors, but the receptor

Agonists:

Dyn A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys^{13}-Trp-Asp-Asn-Gln
N-alkyl[D-Pro^{10}]Dyn A-(1-11) (R = allyl, cyclopropyl-methyl or benzyl)	R-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-pro-Lys
[L/D-Ala^3]Dyn A-(1-11)NH_2	Tyr-Gly-L/D-Ala-Phe-Leu-Arg-Arg-Ile-Arg-Pro-LysNH_2
[NMeTyr^1]Dyn A-(1-13)NH_2	MeTyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-LysNH_2
E-2078	MeTyr-Gly-Gly-Phe-Leu-Arg-NMeArg-leuNHEt
SK-9709	MeTyr-ala-Gly-Phe-Leu-Arg$\Psi(CH_2NH)$ArgNH_2

Antagonists:

JVA-901	AcTyr-Lys-Trp-Trp-Leu-Arg-Arg-ala-Arg-Pro-LysNH_2
Arodyn	Ac-Phe-Phe-Phe-Arg-Leu-Arg-Arg-ala-Arg-Pro-LysNH_2
Dynantin	(2*S*)-Mdp-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-LysNH_2
[Pro^3]- and [Pro^3,Arg^8]-Dyn A-(1-11)NH_2	Tyr-Gly-Pro-Phe-Leu-Arg-Arg-X-Arg-Pro-LysNH_2 (X = Ile or Arg)
Cyclodyn	
Zyklophin	



(2*S*)-Mdp

Fig. 2. Peptide KOR ligands discussed in the text. D-Amino acids in the sequences are in lower case. Dap = 2,3-diaminopropionic acid

selectivity profile can be altered. There are several bonds in the middle of the sequence of Dyn A that can be cleaved by different endopeptidases (see (88,89) and references cited therein). Notably, the C-terminal sequence of the peptide can also influence the nonopioid neurotoxicity exhibited; thus, in mouse spinal cord neurons the longer peptides Dyn A-(1-17), Dyn A-(1-13), and their des-Tyr fragments cause significant loss of neurons, while shorter fragments (e.g., Dyn A-(1-11)) do not (97).

DYNORPHIN A ANALOGS AND OTHER KOR PEPTIDES STUDIED *IN VIVO*

A variety of Dyn A analogs, mostly derivatives of Dyn A-(1-13) and Dyn A-(1-11), have been synthesized, including peptides with amino acid substitutions, cyclic analogs, etc. (see (2,98) for reviews). Interestingly, the most KOR-selective agonist derivatives have resulted from modifications in the N-terminal "message" sequence (92) common to most mammalian opioid peptides, rather than in the unique C-terminal "address" sequence of Dyn A. N-Terminal monoalkylation of [D-Pro^{10}]Dyn A-(1-11) with an allyl, cyclopropylmethyl, or benzyl group results in marked enhancement in KOR selectivity by decreasing MOR affinity (99), whereas N,N-dialkylation results in analogs with antagonist activity (99–101). Substitution of either Ala or D-Ala in position 3 of Dyn A-(1-11) amide markedly enhances KOR selectivity (102). Incorporation of other amino acids in this position, however, is generally less well tolerated (102). Interestingly, the Pro^3 derivative was subsequently reported to be a highly selective KOR antagonist (see below) (103).

Of interest to drug discovery, a number of modifications have been made to Dyn A analogs that increase metabolic stability. These have mostly involved incorporation of D-amino acids into the sequence, such as D-Ala^2 (which stabilizes the peptide to aminopeptidase degradation but decreases KOR selectivity (92)), D-Ala^8, and D-Pro^{10} (104). Other modifications introduced to stabilize the peptides to

metabolism (e.g., N-MeTyr (92) and reduced amide bond derivatives (105,106)) have also been reported.

However, very few Dyn A analogs have been studied *in vivo*. The Dyn A derivative that has been studied most extensively is the Dyn A-(1-8) analog E-2078 ([NMeTyr^1, NMeArg^7,D-Leu^8]Dyn A-(1-8) N-ethyl amide (Fig. 2) (107) which contains several modifications to impart metabolic stability. This peptide has been studied *in vivo* in both monkeys (77,108–110) and humans (111,112) in addition to in rodents (113–116). Although the selectivity of E-2078 for KOR over MOR in binding assays is low (only twofold) (107), studies with antagonists suggest that the E-2078-induced analgesic effects in mice are primarily due to activation of KOR (114). Following intramuscular administration in humans, E-2078 exhibits analgesic activity comparable to pentazocine (112). While this peptide crosses the blood–brain barrier in monkeys (108), it produces some (but not all) of the effects exhibited by nonpeptide KOR agonists (109,110). E-2078 potently increases serum prolactin levels and induces diuresis, but effects that are attributed to activation of KOR in the CNS, e.g., discrimination of the subjective effects of KOR agonists, were only detected in monkeys following systemic administration of high doses (109,110). The potent elevation of serum prolactin levels by E-2078 compared to the minimal generalization by subjects trained to discriminate U69,593 led Butelman *et al.* (77) to propose that both Dyn A and E-2078 are potent peripherally selective KOR agonists following systemic administration in primates.

Another stabilized Dyn A-(1-8) analog that has been evaluated *in vivo* is SK-9709 ([D-Ala^2,Arg$^6\Psi$(CH$_2$NH)Arg^7] Dyn A-(1-8) amide) (Fig. 2), which contains a reduced amide bond between Arg^6 and Arg^7 (106). This peptide exhibits threefold lower KOR affinity, but 15-fold higher selectivity for KOR over MOR, than E-2078. This peptide exhibits antinociceptive activity in mice in both the acetic acid writhing and hot-plate assays after systemic (subcutaneous, s.c.) as well as after i.c.v. or intrathecal (i.t.) administration. While it is less potent than E-2078 in these assays, SK-9709 possesses equal or greater potency following s.c. administration as the nonpeptide KOR agonist U50,488. The antinociceptive activity of SK-9709 appears to be mediated by both KOR and MOR since both the KOR antagonist norBNI (administered i.t.) and the MOR antagonist β-FNA (administered i.c.v.) reversed the antinociceptive effects of SK-9709 administered subcutaneously.

Dyn A-(1-13) analogs that have been stabilized to metabolism at the N- and C-termini by incorporation of either an N-MeTyr and/or C-terminal amide, respectively, have also been evaluated *in vivo* (117,118). These analogs enhanced morphine analgesia in morphine-tolerant rats following both intravenous and pulmonary delivery, with effects observed for up to 3.4 and 4.4 h, respectively, after administration of [N-MeTyr^1]Dyn A-(1-13) amide by these two routes. However, N- and C-terminal modifications were not sufficient to prevent metabolism of Dyn A-(1-11) amide analogs in rat brain homogenate (119). Endopeptidases capable of metabolizing Dyn A are also present on red blood cells (120) and can rapidly metabolize some linear Dyn A-(1-11) amide analogs in whole blood (119).

The activity profile of these peptides is dependent on whether or not they cross the BBB. The ability of Dyn A peptides to cross the BBB is species dependent and can also be influenced by disease state (121). The penetration of Dyn A-(1-13) across the BBB was detected in normal cats, but not rats, in a brain perfusion study, and the penetration of this peptide across the BBB was higher in cats following focal cerebral ischemia (121). Among the Dyn A fragments and analogs, only the transport of E-2078 has been studied in detail. Radioiodinated E-2078 was detected in rat brain parenchyma during a brain perfusion study (113), and E-2078 was detected in the cerebrospinal fluid by mass spectrometry following systemic administration in monkeys (108). Analysis of the transport of [^{125}I-Tyr]E-2078 *in vitro* using the bovine brain microvessel endothelial cell (BBMEC) model of the BBB indicated that this peptide was transported by absorptive-mediated endocytosis (122) (see (123,124) for general reviews of peptide transport across the BBB). A number of larger Dyn A-(1-11) amide analogs have also been shown to cross the BBMEC model of the BBB (119).

Peptides ligands unrelated to Dyn A that exhibit agonist activity at KOR have been identified from combinatorial libraries. A library of tetrapeptides was screened to identify peptides with affinity for each opioid receptor type (125). The most unusual results were obtained for KOR, where the peptides identified consisted entirely of D-amino acids. One of these peptides D-Phe-D-Phe-D-Nle-D-ArgNH$_2$ (FE20041, Fig. 2) was studied in detail and exhibits exceptional selectivity for KOR over MOR and DOR (30,000- and 68,000-fold selective, respectively) (66). This peptide appears to be primarily restricted to the periphery after peripheral administration, as the systemic administration of FE20041 at a dose tenfold higher than that required for antinociception did not cause centrally mediated sedation or locomotor impairment (66). Further modification of the C-terminal amide of these tetrapeptides resulted in FE200665 and FE20066 (Fig. 2) (67,68), which exhibit exceptional separation (548- and 182-fold, respectively) of antinociceptive activity (as measured in the writhing assay) from centrally mediated side effects, as measured by sedation. This separation of doses for peripheral *vs.* central activity is markedly higher than that found for the nonpeptide asimadoline (fivefold) in the same assays (see (14) for a discussion of the determination of peripheral selectivity and a comparison of the peripheral selectivity of different KOR agonists). FE200665 (now known as CR 665) completed phase Ia clinical trials (Cara Therapeutics, press release, July 28, 2005, http://www.caratherapeutics.com/press-releases.php#press2) and a second generation peptide CR 845 (126), which is reported to be orally bioavailable, has just completed Phase I clinical trials and begun phase II clinical trials for the management of acute post-operative pain (Cara Therapeutics, press release, January 12, 2009, http://www.caratherapeutics.com/press-releases.php#press2).

PEPTIDE ANTAGONISTS FOR KOR

Early attempts to prepare KOR-selective antagonists by modification of Dyn A resulted in limited success, with analogs exhibiting weak antagonist activity, residual agonist activity, and/or low selectivity for KOR over MOR (2), but in recent years antagonist analogs with improved pharmacological profiles have been identified. These include analogs without a basic amine terminus (127–129), the Pro^3 derivatives (103),

and recently cyclic derivatives with antagonist activity (130,131) (see Fig. 2). These analogs generally contain modifications in the N-terminal "message" sequence of Dyn A (92) that alter efficacy, although in the case of [*N*-benzylTyr^1,*cyclo*(D-Asp^5, Dap^8)]Dyn A-(1-11) amide (131) (now called zyklophin) cyclization between residues 5 and 8 in the C-terminal sequence resulted in loss of efficacy and KOR antagonist activity. In addition, a cyclic tetrapeptide with a structure unrelated to Dyn A has been reported to be a KOR antagonist in the rabbit vas deferens isolated tissue assay (132).

Peptide KOR antagonists have only recently begun to be examined *in vivo* (58,119). We have shown that the linear peptide arodyn can antagonize the antinociceptive effects of the KOR agonist U50,488 in the 55°C warm water tail withdrawal assay following i.c.v. administration in mice (58). Importantly, arodyn also blocks stress-induced reinstatement of cocaine-seeking behavior in a CPP model in mice, demonstrating that peptide KOR antagonists could have therapeutic application in the treatment of drug abuse. Consistent with previous results for the nonpeptide KOR antagonist JDTic (57), arodyn did not block reinstatement of cocaine-primed drug-seeking behavior. This peptide, however, is rapidly metabolized in blood, presumably by endopeptidases (119), therefore precluding its systemic administration. In contrast, the cyclic KOR antagonist zyklophin exhibits enhanced metabolic stability compared to arodyn and antagonizes central as well as peripheral KOR following systemic (s.c.) administration (119). The ability of peripherally administered zyklophin to antagonize the antinociceptive activity of centrally administered U50,488 suggests that this peptide crosses the BBB to act on KOR in the CNS (119). Further *in vivo* studies are currently underway in our laboratories examining additional potential applications of this and other peptide KOR antagonists.

While the metabolic lability of peptides can complicate their *in vivo* evaluation, this property could be an advantage in selected cases, particularly for KOR antagonists. As noted above, all of the established small molecule KOR-selective antagonists exhibit exceptionally long durations of action (5) that could hamper their development as therapeutic agents. In contrast, the metabolism of peptides by peptidases is expected to limit their duration of action. While arodyn exhibits longer activity *in vivo* (days) than expected (58), its duration of action is substantially shorter than those of norBNI and JDTic in the same animal model (1–2 weeks).

CONCLUSIONS AND FUTURE DIRECTIONS

While a wide variety of KOR peptide ligands have been synthesized and characterized for their interactions with opioid receptors *in vitro*, only a very small number have been examined *in vivo*. A limiting factor has been the challenge of sufficiently stabilizing these peptides to metabolism to permit systemic administration. In contrast to the shorter opioid peptides, e.g., the enkephalins, modification at or near the termini of the longer Dyn A analogs may not be sufficient to prevent rapid metabolism of the peptides (119), limiting their distribution to their sites of action. Thus, the incorporation of multiple modifications into Dyn A analogs, as is the case with E-2078 and zyklophin, may be necessary to produce significant activity following systemic administration. (The novel tetrapeptide agonists contain only D-amino acids, so these peptides are expected to be metabolically stable.) In the case of KOR antagonists, some metabolic lability of the peptides could be a distinct advantage, permitting the development of peptide antagonists that do not exhibit the exceptionally long durations of action found for the nonpeptide KOR-selective antagonists.

As discussed above, both KOR peptides that can cross the BBB (e.g., E-2078 and zyklophin) as well as peptides that appear to be restricted to the periphery (e.g., FE200665) have been identified. In the case of agonists used in the treatment of pain, agents that interact with KOR only in the periphery have distinct advantages because they should not exhibit the undesirable centrally mediated side effects (e.g., dysphoria) that have severely limited the use of KOR agonists as clinical analgesics. In contrast, for treatment of diseases such as depression, anxiety, and drug abuse, the ligands need to cross the BBB to reach KOR in the CNS. Thus, the demonstration that Dyn A analogs can cross the BBB is an important advancement for the development of peptide ligands, particularly KOR antagonists, as pharmacological tools and potentially as therapeutic agents.

Peptides have several advantages as drugs, including high activity, high specificity, low toxicity, and the minimization of drug–drug interactions (133), but the delivery of peptides as therapeutic agents remains a challenge. A recent report demonstrating that the tetrapeptide Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt^1]DALDA) can cross the Caco-2 cell model of the intestinal barrier (134) raises the intriguing possibility that some opioid peptide analogs could be active following oral administration, but it is likely that oral bioavailability will be limited to very few opioid peptide derivatives. Therefore, alternative methods for systemic administration of opioid peptides will be important for their development as potential therapeutic agents. The demonstrated activity of Dyn A analogs following inhalation (118) indicate that it is possible to administer these peptides by methods other than injection that could increase the acceptance of such therapeutic agents that are not taken orally.

These recent results with peptide KOR ligands *in vivo* are very promising, but clearly additional studies are required to determine the scope and limitations of such agents. These studies will require a multidisciplinary approach involving the design and synthesis of modified peptides, pharmacological evaluation both *in vitro* and *in vivo*, and examination of pharmacokinetic properties, including metabolism and distribution into the brain, in order to understand both how these peptides function and to fully exploit their potential.

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