Opioid peptides in peripheral pain control

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Opioids have a long history of therapeutic use as a remedy for various pain states ranging from mild acute nociceptive pain to unbearable chronic advanced or end-stage disease pain. Analgesia produced by classical opioids is mediated extensively by binding to opioid receptors located in the brain or the spinal cord. Nevertheless, opioid receptors are also expressed outside the CNS in the periphery and may become valuable assets in eliciting analgesia devoid of shortcomings typical for the activation of their central counterparts. The discovery of endogenous opioid peptides that participate in the formation, transmission, modulation and perception of pain signals offers numerous opportunities for the development of new analgesics. Novel peptidic opioid receptor analogs, which show limited access through the blood brain barrier may support pain therapy requiring prolonged use of opioid drugs.

Key words: immune cells, opioid peptides, pain, peripheral analgesia

Abbreviations:

β-FNA - β-funaltrexamine, **BBB** - blood-brain-barrier, **CGRP** - calcitonin gene-related peptide, **CFA** - complete Freund adjuvant, **CNS** - central nervous system, **CRF** - corticotropin releasing factor, **CYP** - cyprodime, **DAGO** - [Tyr-D-Ala-Gly-Me-Phe-Gly-ol]-enkephalin, **DAMGO** - [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin, **DOR** - delta opioid receptor, **DPDPE** - [D-Pen2,5]-enkephalin, **DRG** - dorsal root ganglion, **EM-1** - endomorphin 1, **EM-2** - endomorphin 2, **KOR** - kappa opioid receptor, **MOR** - mu opioid receptor, **NLZ** – naloxonazine, **NTI** - naltrindole, **NLXM** - naloxone methiodide; **nor-BNI** – nor-binaltorphimine, **PDYN** - prodynorphin, **PENK** - proenkephalin, **PNS** - peripheral nervous system, **POMC** - proopiomelanocortin

INTRODUCTION

The opioid system is one of the main system engaged in strongly conserved evolutionary mechanisms like pain perception and modulation, reward, addiction and fear behaviors (Herz 1998, Inturrisi 2002, Petrovic et al. 2008, Lehner et al. 2010). Opioid receptors and their corresponding agonists are key players in the inhibition and modulation of pain. It has long been postulated that sufficient clinically-relevant analgesia is obtained exclusively *via* activation of central opioid receptors (Lipkowski and Carr 2002). In addition to inducing an analgesic effect, typical centrally-active opioids are the source of undesirable side effects which may limit their therapeutic use in chronic pain. This effect of opioids is a consequence of their expression patterns in the areas of the CNS responsible for sensorimotor integration and cognitive functioning. Nevertheless, in some pain states including nerve damage, painful inflammation, tissue destruction by cancer expansion opioid receptors located in the periphery play a significant role in the development of analgesia. Besides their profound expression in the CNS, opioid receptors are also present in the PNS on peripheral sensory nerve terminals as well as on other nonneural tissues such as the vascular epithelium or keratinocytes (see Table I).

The expression of opioid receptors and their enhanced transport to sensory nerve terminals becomes prominent especially in the presence of inflammation. Immune cells are then recruited to the damaged tissue and secrete opioid peptides which bind to peripheral opioid receptors reducing pain. This endogenous pain-relief mechanism became an inspiration for the exog-

Table I

Localization of opioid receptor expression

Opioid receptor type	Expression		
	CNS	PNS	Non-neural tissues
MOR	neocortex, caudate – putamen, nucleus accumbens, thalamus, hippocampus, amygdala, nucleus tractus solitarius	peripheral sensory neuron DRG, stomach, duodenum, jejunum, oleum, proximal and distal colon	vascular endothelium, cardiac epithelium, keratinocytes, vas deferens, Sertoli cells
DOR	olfactory-related areas, neocortex, caudate – putamen, nucleus accumbens, amygdala	peripheral sensory neuron DRG	
KOR	caudate – putamen, nucleus accumbens, amygdala, neural lobe of the pituitary gland	sensory neuron DRG, stomach, duodenum, jejunum, oleum, proximal and distal colon	

enous administration of endogenous, synthetic opioid peptides and novel peptidomimetics in the hope to conquer pain.

OPIOID RECEPTOR TYPES

Opioid receptors belong to the rhodopsin-like subfamily of GPCR seven-transmembrane domain metabothropic receptors. Ligand binding facilitates coupling to the inhibitory $G_{i/o}$ protein decreasing cAMP levels by inhibiting Ca^{2+}/Na^+ influx. This results in a decrease of releasing of proalgesic mediators such as substance P, CGRP and nociceptor excitability. The existence of opioid receptors was first reported in 1973 in a series of radioligand binding experiments where radiolabeled opioid ligands bind to a receptor in the rat brain membrane homogenate (Pert and Snyder 1973, Simon et al. 1973, Terenius 1973). The first attempt to classify "opiate receptors" into three types was proposed by Gilbert and Martin (1976) and Lord and coworkers (1977) based on the efficacy of opiate binding in chronic spinal dogs and mouse vas deferens. Later in the early nineties the DOR (Evans et al. 1992), MOR (Chen et al. 1993, Thompson et al. 1993) and KOR (Li et al. 1993, Yasuda et al. 1993, Meng et al. 1993) genes were cloned. Although all three types of opioid receptors are encoded by a different gene they share high homology but can also exist in several splice variants due to a differential mRNA processing (Knapp et al. 1995, Wei et al. 2004, Wei and Loh 2011). Different pharmacological profiles of the existing opioid receptors may be a result of posttranslational modifications and dimerization (Bouvier 2001, Levac 2002, Rios et al. 2001, Gupta et al. 2006).

PERIPHERAL OPIOID RECEPTORS

Peripheral opioid receptors are synthetized in cell bodies of primary afferent neurons and intra-axonally transported to peripheral sensory nerve endings where they can interact with both endogenous and exogenous opioid agonists (Rau et al. 2005, Wang et al. 2010). Many studies have shown that the role of peripheral opioid receptors is more pronounced during peripheral inflammation due to a number of inflammation-driven processes. Under normal conditions nerve fibers are encapsulated by a perineurial barrier preventing the diffusion of high molecular weight particles and hydrophilic opioid receptors ligands. In pathological conditions such as inflammation easier access of opioid agonists through the perineurium of sprouting peripheral nerve fibers is observed (Olsson 1990, Antonijevic et al. 1995). Moreover, the expression of peripheral opioid receptors and their axonal transport

to the sensory nerve terminals is enhanced in the course of inflammation (Hassan et al. 1993, Ji et al. 1995, Schäfer et al. 1995, Pol and Puig 2004). The most extensively studied upregulation of MOR expression was observed in animal inflammatory pain models (Zhang et al. 1998, Ballet et al. 2003, Puehler et al. 2004, Taguchi et al. 2010) and in human inflammatory bowel disease (Philippe et al. 2004) but downregulated in bone cancer pain in mice (Yamamoto et al. 2008).

In parallel, data supporting DOR and KOR overexpression in inflammation has also been published but some results remain contradictory (Ji et al. 1995, Maekawa et al. 1996, Zhang et al. 1998, Shen et al. 2005, Puehler et al. 2006). In addition to opioid receptor upregulation, inflammation also changes the environment in local tissue *via* pH decrease rendering opioid receptors more active due to an increased G-protein coupling and cAMP level (Rasenik and Childers 1989, Reddy and Bhargava 1996, Zöllner et al. 2003, Shaqura et al. 2004).

Neuropathic pain resulting from mechanical nerve damage is another condition which may involve opioid receptor expression changes in peripheral sensory neurons. In many different studies employing different neuropathic pain models involving entrapment of the peripheral nerve MOR, DOR either KOR expression was observed to be unregulated (Sung et al. 2000, Truong et al. 2003, Kabli and Cahill 2007, Walczak et al. 2005, Obara et al. 2009). Some studies however found especially MOR expression to be downregulated in neuropathic pain (Rashid et al. 2004, Pol et al. 2006, Obara et al. 2010).

Despite some discrepancies there is overall more evidence of opioid receptor overexpression or increased binding affinity which makes them more susceptible and more accessible for both engogenous and exogenous opioid agonists.

ENDOGENOUS PERIPHERAL OPIOID PEPTIDE ANALGESIA

Endogenous opioid peptides are natural ligands which bind to opioid receptors during painful inflammation, neuropathy or cancer invasion. In mammals three types of opioid peptides endorphins, enkephalins and dynorphins are synthetized *via* cleavage of the precursor proteins. Endorphins derived from POMC exhibit a high affinity for MOR and DOR and low affinity for KOR. PENK is a enkephalin precursor and

is characterized by highest affinity to DOR, moderate to MOR and very low to KOR. Dynorphins show preferable binding to KOR but possess some MOR and DOR specificity. Two endogenous opioid peptides EM-1 and EM-2 also activate opioid receptors but their precursors are not yet known (Terskiy et al. 2007, Perlikowska et al. 2009). Some postulate a *de novo* synthesis of those peptides (Rónai et al. 2009). Several other non-mammalian opioid peptides which show affinity to opioid receptors have been discovered to date. This includes amphibian dermorphin and deltorphin (Broccardo et al. 1981, Glaser et al. 1981, Barra et al. 1994, Negri et al. 2000, Auvznet et al. 2006, Sinha et al. 2009), food opioid peptides formed in the process of milk digestion like casomorphins (Rüthrich et al. 1992) or gluten digestion such as gluteomorphin (Sun and Cade 2003) and exorphin (Takahashi et al. 2000).

Endogenous opioid peptides are released from immune cells, which in the presence of inflammatory mediators migrate to inflamed tissue in a process referred to as "homing" a centrally-mediated mechanism (Mousa et al. 2001, Shmitt et al. 2003, Heurich et al. 2007). Leukocytes roll along the blood vessel wall in a process mediated predominantely by L-selectins and E- and P-selectins present on endothelial cells (Machelska et al. 1998). Adhesion of the leukocytes to endothelial cells is mediated by integrins, for example ICAM-1. After a firm adhesion immune cells squeeze through the endothelium and migrate to the inflammation site (Butcher and Picker 1996), release opioid peptides which in turn bind to peripheral opioid receptors.

Endorphins and enkephalins are postulated to play a leading role in endogenous antinociception. In β-endorphin deficient mice a short stressful swim challenge failed to produce analgesia (Parikh et al. 2011) whereas antibodies against these opioid peptides abolished analgesia upon CRF stimulation of immune cells secreting β-endorphin and enkephalin (Cabot 2001).

The importance of endogenous peripheral analgesia has been examined in various animal pain models including chronic inflammatory and cancer pain giving hope for a potential therapeutic application of this natural pain-relief mechanism. In rats with an unilateral CFA-induced hindpaw inflammation, stress induced by a cold water swim produced analgesia in the inflamed but not in the contralateral non-inflamed paw. This analgesic effect was abolished by BBB-

permeable naloxone and naloxone methiodide a non-selective peripherally restricted opioid receptor antagonist. Nevertheless in chronic CFA inflammation (four days after the injection of CFA) analgesia was exclusively naloxone methiodide reversible. These results support a notion that acute inflammation is both centrally and peripherally mediated whereas during chronic inflammatory pain analgesia is a result of opioid peptide binding to peripheral opioid receptors (Machelska et al. 2003).

At the early stages of the development of mouse osteosarcoma β-endorphin containing immune cells were detected inside and surrounding the tumoral mass. The local administration of non-selective NLXM, MOR-selective CYP and DOR-selective NTI produced hyperalgesia in the ipsilateral paw. Further confirmation of the involvement of immune cell-derived opioid peptides in tumor pain attenuation was the injection of a CRF receptor antagonist. Blockade of the CRF receptor inhibits opioid peptide release from immune cells and promoted the development of pain (Baamonde et al. 2006).

The study conducted by Stein and coworkers (1993) aimed to target intra-articular opioid peptides present in synovia of human patients who underwent arthroscopic knee surgery. Synovia samples were found to contain immune cells abundant in β-endorphin and Met-enkephalin as shown by immunohistochemistry staining. The blockage of synovial opioid receptors by intra-articular naloxone resulted in higher pain scores in numerical rating scale and increased the demand for supplementary analgesics. These findings further confirm the future application of opioid peptides in pain control not only in animal models but also in human patients.

EXOGENOUS PERIPHERAL OPIOID PEPTIDE ANALGESIA

One of the first clinical attempts to target the peripheral opioid receptor system for pain control was a study which aimed to examine the local analgesic effect of intra-articular morphine (Stein et al. 1991). Low-dose intra-articular morphine produced more pronounced postoperative analgesia than in patients injected with intravenous morphine.

The findings confirming the clinically-relevant and selective analgesic effect of opioids in inflamed peripheral tissue triggered ideas to administer low, systemi-cally inactive doses of opioid peptides and their analogs locally into pathologically changed tissue. In the visceral pain mouse model an intraperitoneal or subcutaneous injection of a cyclic EM-1 analog reduced the number of acetic acid induced writhes. The analgesic effect was reversed by MOR specific antagonists β-FNA and NLZ and peripherally active NLXM but not by nor-BNI and NTI suggesting a predominant involvement of peripheral MOR. Interestingly, EM-1 failed to abolish pain behavior presumably due to a low stability of the parent compound (Bedini et al. 2010).

Morphiceptin [Tyr-Pro-Phe-Pro-NH$_2$] is yet another example of an opioid peptide showing high opioid receptor affinity and peripheral activity in visceral pain. It is an amide fragment of bovine β-casein and highly specific for MOR but not for DOR (Chang et al. 1981, 1983, Vogel et al. 1996). Morphiceptin has been shown to elicit strong analgesia after intracerebroventricular injection but not when injected peripherally due to rapid degradation. However, a more stable morphiceptin analog [Tyr-Pro-NMePhe-Pro-NH$_2$] acted peripherally inhibiting diarrhea in mice (Shook et al. 1989). Chemical modifications of the morphiceptin structure led to a synthesis of analogs resistant to peptidase degradation displaying a potent supraspinal and peripheral MOR-mediated analgesia (Hau et al. 2002). Along with their peripheral analgesic effect new morphiceptin analogs inhibited gastrointestinal transit *in vivo* making them interesting novel therapeutics in the treatment of gastrointestinal mobility disorders e.g., the irritable bowel syndrome (Gach et al. 2010).

Biphalin [(Tyr-D-Ala-Gly-PheNH-)$_2$], dimeric peptide analog of enkephalin, is another example of an opioid peptide that has limited permeability of an intact BBB (Silbert et al. 1991). Biphalin expresses synergic activities with current drugs used in AIDS therapy. Therefore, biphalin has been proposed to be applied as a component of antiviral HIV multidrug therapies in combination with chronic pain treatment in AIDS patients as a primary therapeutic target (Tang et al. 2008).

Allodynia is a frequent condition in multiple clinical conditions (Peyron et al. 1998, 2004, Becerra et al. 2006, Moller et al. 2006, Witting et al. 2006) when patients perceive normally innocuous tactile or thermal stimuli as painful. Over the years numerous rodent models of neuropathy have been developed to help to understand the mechanisms governing persistent pain resulting from peripheral nerve injury. Animal models

of neuropathic pain include: partial ligation of the sciatic nerve (Seltzer et al. 1990), chronic constriction injury (Bennett and Xie 1988), ligation of the L5/L6 spinal nerves (Kim and Chung 1992) or the spared nerve injury model (Decosterd and Wolf 2000). Although these models differ in the type of injury or the magnitude or duration of pain symptoms they all mimic pain conditions seen in humans (Martin et al. 2003).

In an experimental rat model of neuropathy developed by loosely tying four ligatures around the sciatic nerve, locally injected opioid peptides alleviated mechanical allodynia in the von Fray test (Obara et al. 2004). In the study it was demonstrated that MOR agonists: morphine, DAMGO, EM-1 and EM-2, were successful in relieving neuropathic pain after intraplantar injection as opposed to a subcutaneous injection. DAMGO had an antiallodynic effect on the injured paw at a relatively low dose compared with endomorphins which required much higher doses to induce a similar magnitude of analgesia. EM-2 produced a similar effect as EM-1 at a similar dose range but its effect started to diminish 10 min after injection. Interestingly, opioid peptides were more effective in pain relief than a prototipical alkaloid – morphine, which analgesic effect was delayed in time. The reason for these differences in effectiveness between opioid peptides and morphine are still not cleat-cut. Several possible explanations have been raised like the involvement of different subtypes of MOR (Labuz et al. 2002), unequal receptor binding and a change in receptor binding parameters (Patel et al. 2002). The latter arguments may be supported by the role of activated macrophages which gather around the site of injury and take part in the regeneration process in this model (Stoll et al. 1992). The change in the specific milieu may in turn affect opioid receptor binding properties and their availability for various opioid peptides. Another plausible explanation is the fact that morphine readily crosses the BBB exerting profound central analgesia but rudimentary analgesia in the periphery due to a rapid uptake by the CNS and escape from local tissue.

Cancer pain can also be in the area of interest when discussing antihyperalgesic effect of opioid peptides. The peritumoral injection of a selective MOR agonist - DAGO inhibits thermal hyperalgesia in a mouse model of cancer pain produced by intratibial implantation of NCTC 2472 cells. The analgesic effect of DAGO was only observed in tumor-bearing animals and completely abolished by NLXM strongly support the hypothesis that this agonists action is peripherally and not centrally mediated (Menéndez et al. 2003). Very similar results were obtained by Baamonde (2006) where the study confirmed peripheral analgesia elicited additionally by a DOR agonist - DPDPE and KOR agonist - U50,488H. The greatest analgesic effect was achieved by stimulation of MOR although analgesia produced by DAGO as well as DPDE was shorter than that after U50,488H administration.

The potential use of peripheral KOR agonists as novel analgesics was encouraged by the observation that, unlike MOR agonists, KOR agonists do not cause typical morphine-like aversive side effects. The first generation KOR agonists however were burdened with dose limiting neuropsychiatric side effect like dysphoria as a result of dopamine release inhibition (Donzanti et al. 1992; Pande et al. 1996). The second generation KOR agonists were targeted towards peripheral KORs to decrease brain penetration (Barber et al. 1994). Asimadoline a prototypic peripherally active KOR agonist has been researched for possible treatment for human irritable bowel syndrome and was shown effective in clinical trials (Delvaux et al. 2004, Szarka et al. 2007). Unfortunately, apart from peripheral activity, oral asimadoline was not devoid of central psychotomimetic effects (Machelska et al. 1999). In order to overcome these therapy-limiting difficulties, a third generation of peptidic KOR agonists has been established. A hydrophilic structure of these peptides prevented from passive transport through biological membranes acting primarily on the periphery. The novel D-amino acid tetrapeptide CR665 which is now under clinical development by Cara Therapeutics when injected intravenously in nanomolar range elicited potent analgesia in the mouse writhing test without producing motor impairment (Vanderah et al. 2008).

CONCLUSIONS

In conclusion, growing knowledge of peripheral endogenous pain pathways opens a new chapter in pain control which may bring substantial benefit for pain sufferers. Many animal and human studies have shown a justification for such claims. The development of novel peptidomimetics characterized by increased plasma stability, lower toxicity, high affinity for opioid receptors is a milestone for researchers aiming for satisfactory long-lasting pain relief in patients.

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