REVIEW

Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Fl, USA

Metabolism of dynorphin A(1-13)

B. Brugos, G. Hochhaus

Received October 29, 2003, accepted November 12, 2003

Guenther Hochhaus, Ph.D., Department of Pharmaceutics, College of Pharmacy, Gainesville, FL., 32610 USA
Hochhaus@ufl.edu

Pharmazie 59: 339–343 (2004)

Dynorphin A(1–13), a tridecapeptide of the endogenous opioid peptides, has modest effects in reducing mild opiate withdrawal in humans. Previous studies revealed that dynorphin also potentiates the analgesic effect of morphine in morphine-tolerant rats and mice. The therapeutic potential of dynorphin A(1–13) is limited due to extensive metabolism by human metabolic enzymes resulting in an *in vivo* half-life of less than one minute. Chemical modifications of dynorphin A(1–13), such as N-methylation of Tyr^1 and amidation of the C-terminus have been shown to be effective in protecting against the proteolytic enzymes in human plasma. This article is a general review of the metabolism of dynorphin A(1–13) in human plasma and CSF.

1. Introduction

Dynorphin A(1–13) is a tridecapeptide (Fig. 1) belonging to the family of endogenous opioid peptides. It has been shown to modulate the effects of other opioids (Smith and Lee 1988). Dynorphins (Dyn) are derived from a common precursor peptide called prodynorphin (Goldstein et al. 1979), by endoproteolysis. These include Dyn A(1–17) and its active fragments Dyn A(1–13), Dyn A(1–8), all with potent opioid activity and binding preference for the κ-opioid receptors (Terasaki et al. 1989). The first 5 amino acids are believed to constitute the message sequence for interaction with opioid receptors (Chavkin and Goldstein 1981). Beside the opioid receptors Dyn A(1–13) has been reported to bind to other non-opioid receptors such as muscarinic receptors (Hu and El-Fakahany 1993), orphanin receptor (Zhang and Yu 1995), melanocortin receptors (Quillan and Sadee 1997), and N-methyl-D-aspartate-receptors (NMDA) (Shukla and Lemaire 1994).

It was shown that dynorphin is not analgesic itself when given in the mammalian brain (Chavkin et al. 1982; Friedman, et al. 1981), however, it is analgesic when administered intrathecally (Herman and Goldstein 1985). It was also reported that dynorphin has modulatory effects on morphine induced analgesia, antagonizes it in naive and potentiates it in morphine tolerant animals after different route of administration (Friedman et al. 1981; Hooke and Lee 1995; Hooke et al. 1995). Hooke et al. found Dyn A(1–13) effective in suppressing morphine tolerance in mice in the writhing test after intrathecal, intracerebroventricular and intravenous administration. They stated in agreement with Nakazawa et al. that there might be two distinct sites of action for dyn A(1–13) one central and one peripheral (Hooke et al. 1995; Nakazawa et al. 1985). However, the spinal administration is hampered by the observation, that the peptide has neurotoxic effects as it produces hindlimb paralysis and necrosis of the spinal cord close to the catheter tip after administration of high doses (Caudle and Isaac 1987). This effect is not antagonized by naloxone (Caudle and Isaac 1987). This is the reason that most of the studies evaluating the opioid modulatory actions of dynorphins have been performed after intravenous administration or direct injection into the brain. In conclusion the evidence that dynorphin has modulatory effects in opioid analgesia and withdrawal suggests that it might be beneficial in the treatment of morphine addicted patients.

Of significant therapeutic interest, Dyn A(1–13) has been reported to modulate morphine tolerance and attenuate the effects of opiate withdrawal symptoms in mice, rats, mon-

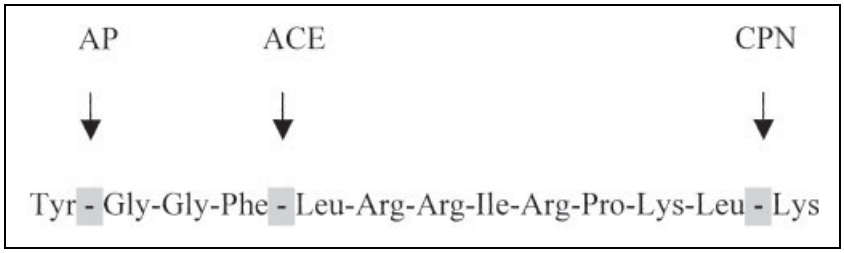

Fig. 1
Structure of Dynorphin A(1–13) with main enzymatic cleavage points. AP = Aminopeptidase, ACE = Angiotensin converting enzyme, CPN = Carboxypeptidase N

keys and humans (Aceto et al. 1982; Green and Lee 1988; Hooke and Lee 1995; Specker et al. 1998; Takemori et al. 1992; Wen and Ho 1982). Wen et al. observed a significant lowering in withdrawal symptoms in heroin addict patients after intravenous administration [60 μg/kg Dyn A(1–13)] (Wen and Ho 1982; Wen et al. 1984). Specker et al. also found Dyn A(1–13) effective in ameliorating withdrawal syndrome in opiate addicts and the peptide was well tolerated even in high doses (1000 μg/kg) (Specker et al. 1998). The exact mechanism of action is still unknown, but dynorphins seem to exert these modulatory effects through non-opioid receptors. This statement is based partly on the insensitivity of these actions to naloxone, and the retention of those actions by non-opioid fragments like des-Tyr Dyn A(1–17) (Hooke et al. 1995). Walker et al. have postulated the existence of opioid and non-opioid effects (Walker et al. 1980). NMDA receptors have been suggested to be involved in these non-opoid effects of Dyn A since NMDA receptor antagonist prevent hindlimb paralysis (Shukla and Lemaire 1994). Moreover Dyn A was shown to bind directly to these receptors (Chen et al. 1995) and interact with the glutamate site (Muller et al. 1998). It is believed that dynorphin's interaction with NMDA receptors lead to toxic effects, like hyperalgesia, allodynia, neurodegeneration observed in many animal models (Hauser et al. 1999; Laughlin et al. 2001). NMDA-related mechanisms may also be involved in suppression of opiate tolerance and withdrawal (Shukla and Lemaire 1994). It is difficult to understand the pharmacological effect of the peptide due to the rapid metabolism in human plasma by proteolytic enzymes and it might be speculated that the effects in humans might be enhanced by increasing the pharmacokinetic stability of dynorphin derivatives in humans while maintaining the pharmacodynamic activity. A proper understanding of the underlying metabolic pathways is important in order to design a more stable derivative. This review focuses on the metabolism of Dyn A(1–13) in plasma and cerebrospinal fluid, with respect to some chemical modification to improve the stability.

2. Inactivation of dynorphin in plasma

Recent clinical studies have determined the pharmacokinetics of Dyn A(1–13) *in vivo* and have found an initial half life of 0.56 min for the peptide (Gambus et al. 1998), which agrees well with the half-life of less then 1 min determined from *in vitro* studies conducted in our lab (Muller and Hochhaus 1995) and is consistent with the statement that more stable derivatives are necessary for therapy.

Plasma has been described as a primary site for peptide degradation (Powell et al. 1992). Determination of *in vitro* stability of peptides in plasma is therefore relevant for predicting the overall stability of a peptide, selecting a therapeutical drug candidate which would be more stable than its peptide precursor and as a result would be expected to be more stable *in vivo*.

Dyn A(1–13) is rapidly broken down in human plasma to metabolites mainly by exopeptidases. Exopeptidases, which cleave amino acids from either end of a peptide chain, include aminopeptidases and carboxypeptidases. Aminopeptidases cleave the N-terminal amino acid from a peptide chain, while carboxypeptidases cleave the C-terminal amino acid. Aminopeptidase M is a member of this group and responsible for the cleavage of the Tyr^1-Gly^2 bond of different neuropeptides [enkephalins, Dyn A(1–17)] (Csuhai et al. 1995). Another group of enzymes are endopeptidases. These enzymes generally are specific for a particular amino acid side chain or discrete sequence (Csuhai et al. 1995). Exopeptidases are responsible for more than 95% of the total metabolism of Dyn A(1–13), specifically carboxypeptidases and aminopeptidases account for 80% and 15% respectively (Muller and Hochhaus 1995). The major metabolites for Dyn A(1–13) were DynA(1–12), A(2–12), A(4–12) and A(4–8) (Fig. 2). Dyn A(1–12) is formed by carboxypeptidases N, known to act on peptides with basic C-terminal amino acids as the C-terminal Lys in Dyn A(1–13) (McCleave et al. 1996). Formation of Dyn A(2–13) is believed to be mediated by aminopeptidase M by cleavage of the N-terminal amino acid (Safavi and Hersh 1995). Further metabolites are Dyn A(3–13), A(3–12), A(5–12), A(6–12), A(7–12), A(1- 10), A(2–10), A(2–8), A(3–8) (Muller and Hochhaus 1995; Muller et al. 1997).

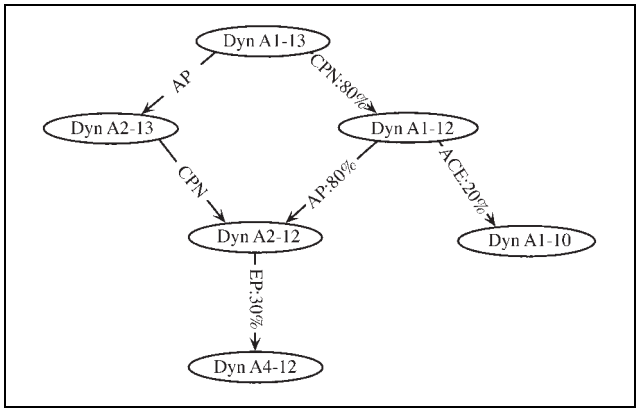

Fig. 2: Major plasma metabolites of Dyn A(1–13) and the proteolytic enzymes involved in the metabolism in human plasma. AP = Aminopeptidase, ACE = Angiotensin converting enzyme, CPN = Carboxypeptidase N, EP = Endopeptidase (Muller, Hochhaus 1995)

Chou et al. compared the metabolism of natural peptide Dyn A(1–17) to synthetic analogues like Dyn A(1–13) and Dyn A(1–10) amide (Chou et al. 1996). They found that the natural peptide has a half-life of 180 min, and they believe that the four carboxyl-terminal amino acid present in Dyn A(1–17) protect the peptide from enzymes. In good agreement with Muller et al. Dyn A(1–13) was processed rapidly in human blood (half-life < 1 min), while the carboxyl-terminally protected Dyn A(1–10) amide has a half-life of 10 min (Chou et al. 1996). Young et al. suggested the existence of specific aminopeptidase which could convert Dyn A(1–17) to Dyn A(2–17) *in vitro*, but not Dyn A(1–13) (Young et al. 1987). Safavi et al. have shown that aminopeptidase M has a high affinity to longer peptides like Dyn A(1–17) (Safavi and Hersh 1995). Dyn A(2–17), as a non-opioid fragment was also effective in suppressing withdrawal symptoms (Hooke and Lee 1995; Hooke et al. 1995). Relying on these observations truncated non-opioid peptides could have clinical significance in treating addicts.

3. Inactivation of dynorphin in cerebrospinal fluid (CSF), importance of blood-brain barrier

While the potential site of action might be also in the central nervous system, metabolism in these areas might be of interest as well. In addition, endogenous dynorphins are widely distributed in most areas of the central nervous system (Chavkin and Goldstein 1981), they are present in the hypothalamus, in the nucleus tractus solitarii and in

the areas of spinal cord involved in transmission of pain (Przewlocki and Przewlocka 2001). Opioid administration increases the expression of dynorphin in spinal cord, where this peptide is a mediator of sustained pain (Vanderah et al. 2001). Occurrence of these peptides in several regions of the brain and spinal cord suggests that dynorphins have important functions, however these functions are still not well understood. In order to induce its actions the peptide should have the ability to cross blood-brain barrier (BBB). BBB protects the brain from hydrophilic molecules greater than 400 Da (Borlongan and Emerich 2003). BBB is poorly permeable for Dyn A(1–13) due to large molecular weight and hydrophilicity. However, the passage of Dyn A(1–13) through BBB into hippocampus, cortex, cerebellum has been demonstrated in cats, especially in induced focal cerebral ischaemia (Turner et al. 1998). Small peptides with an N-terminal tyrosine residue have been found to cross BBB by carrier-mediated transport mechanism (Banks et al. 1986). E-2078, a stable Dyn A(1–8) analog has also been shown to be transported by absorptive-mediated endocytosis through BBB (Terasaki et al. 1989).

It was suggested that the peptide concentrations in the CSF might reflect the extrasynaptically available peptide activity in the brain and spinal cord (Terenius and Nyberg 1988). CSF has also been studied for the metabolism of different peptides, including dynorphins. The absence of carboxypeptidase N and the presence of a serine protease, identified by Nyberg et al., which is specific for cleaving neuropeptides at monobasic sites, called dynorphin converting enzyme (DCE), suggests a different way of Dyn degradation than observed in plasma (Nyberg et al. 1986). DCE exhibits a very high specificity towards DynA, DynB, α-neo endorphin (Csuhai et al. 1995). The activity of DCE in CSF has been reported to be correlated to opioid tolerance (Persson et al. 1989). Other enzymes such as angiotensin converting enzyme, substance P-converting endopeptidases, α-amidating monooxygenase, neprilysin (enkephalinase), aminopeptidases and acethylcholinesterase have also been identified in CSF (Csuhai et al. 1995). Studies conducted by Muller et al. indicate that 85% of Dyn A(1–13) in CSF is metabolized by exopeptidases, more specifically 50% by carboxypeptidase and 35% by aminopeptidases (Muller et al. 1996) (Fig. 3). This agrees well with previous studies on metabolism of Dyn A(1–13) in plasma (Muller and Hochhaus 1995) and rat cranial membranes (Leslie and Goldstein 1982). It was also shown that Dyn A(1–6), the major metabolite resulting from DCE action, does not represent a major metabolic fragment of Dyn A(1–13) in CSF, which agrees with earlier reports of DCE being more efficient with larger dynorphins [Dyn A(1–17)] than with smaller ones (Nyberg et al. 1986). The major metabolites found in CSF were Dyn A(2–13), Dyn A(1–12) and Dyn A(1–6) (Muller et al. 1996). Results from the metabolism studies of Dyn A(1–13) in human CSF agree well with studies dealing with the metabolism of Dyn A(1–13) in human plasma and suggest that derivatives with improved plasma stability are also likely to show improved CSF stability.

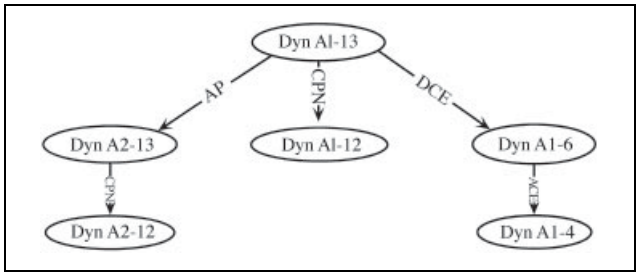

Fig. 3: Major plasma metabolites of Dynorphin A(1–13) and the proteolytic enzymes involved in the metabolism in CSF. AP = Aminopeptidase, ACE = Angiotensin converting enzyme, CPN = Carboxypeptidase N, DCE = dynorphin converting enzymes (Muller, et al. 1996)

4. Approaches to stabilize dynorphin derivatives

As discussed above, Dyn A(1–13) is rapidly broken down by peptidases. Consequently for the peptide to be of relevant clinical potential, the half-life needs to be prolonged. In development of neuropeptides for clinical use, several factors should be considered: 1) the stability of the peptide in the systemic circulation; 2) the permeability of the peptide through BBB (if effect is in the central nervous system); 3) the stability of the peptide in cytoplasm (Terasaki et al. 1989).

Previously a variety of analogs were synthesized to stabilize Dyn A(1–13) against enzymatic degradation. Introduction of a steric bulk along the peptide backbone by placing an amide group at the C-terminal end has been used to generally reduce the rate of enzymatic degradation by carboxypeptidases and has shown to be effective in improving the stability of different peptides (Heavner et al. 1986; Wettergren et al. 1998; Brinckerhoff et al. 1999). Dyn A(1–10) amide (Chou et al. 1996), Dyn A(1–8) amide (Yoshino et al. 1990), [N-methyl-Tyr^1, N-methyl-Arg^7-D-Leu^8] Dyn A(1–8) ethylamide, designated as E-2078 (Yu et al. 1997), Tyr-D-Ala-Phe-Leu-ArgΨ (CH_2NH) Arg$-NH_2$ (Hiramatsu et al. 2001) have been suggested to be show protection against C-terminal attack. However, the stability gain observed after amidation is not the same for all peptides and depends on the C-terminal peptide sequence. As an example amidation of dynorphin A(1–13) resulted in a 24-fold gain in stability (shifting the half-life in plasma from 0.9 to 24 min, while the half-life Dyn A(1–10) amide increased from 1.3 min to only 3.3 min). These data together with enzyme inhibition studies on the above peptides indicated that amidation might block attack from the C-terminal end of the peptide in the case of carboxydase attack, but will only be partially successful if other enzymes such as ACE (not being affected by amidation) are involved. Thus, the protective effect of amidation might differ among opioid peptides. Modulation of the amino-terminus, for example, by using D-amino acids or N-methylation of the amino group in position 1 reduces the attack by aminopeptidases and increases peptide stability. Studies conducted in our lab have shown that blocking of C and N-terminal attack by exopeptidases using N-methylated tyrosine in position 1 (or the use of D-tyrosine in position 1) in combination with C-terminal amidation of Dyn A(1–13) increased significantly the peptide stability, while the pharmacological activity of the derivatives is retained as shown by the effectiveness of these derivatives in suppressing tolerance in morphine-dependent rats (data not published). Such derivatives which show enhanced stability in human plasma and retain pharmacological activity would therefore be expected to be of pharmacological benefit, although further protection, e.g. by blocking endopeptidase attack might be beneficial. In addition, encapsulation of these derivatives, such as [N-Met-Tyr^1]-Dyn A(1–13) in stealth liposomes might be further beneficial in prolonging the half-life of the drug (data not published).

While it is not fully clear whether the modulation of morphine tolerance and attenuation of the effects of opiate withdrawal symptoms represent central or peripheral effects, potential approaches to increase the uptake of these derivatives across the blood brain barrier might be helpful. With respect to the permeability of the peptide through BBB there are some delivery techniques available like absorptive-mediated endocytosis (Terasaki et al. 1989), sustained-release polymer systems, use of active transporters systems and chemical delivery systems for peptides which allow passive diffusion after lipophilization and subsequent trapping after oxidation. Such approaches might be incorporated into the design if beneficial.

5. Conclusion

In conclusion, the therapeutic use of endogenous peptides is often challenging because these peptides have been designed to be short-lived in order to function within a complex neuronal signal transduction pathway. Applying rational drug design approaches, based on a detailed understanding of metabolic degradation pathways and a full knowledge of the structure activity relationships and its incorporation in pharmacokinetic/pharmacodynamic, development approach is the basis for potentially overcoming these obstacles and improving drug therapy for this important class of drugs.

This research paper was presented during the 4th Conference on Retrometabolism Based Drug Design and Targeting, May 11–14, 2003, Palm Coast, Florida, USA.

References

Aceto MD, Dewey WL, Chang JK, Lee NM (1982) Dynorphin (1–13) effects in nontolerant and morphine-dependent rhesus monkeys. Eur J Pharmacol 83: 139–142.

Banks WA, Kastin AJ, Fischman AJ, Coy DH, Strauss SL (1986) Carrier-mediated transport of enkephalins and N-tyr-MIF-1 across blood-brain barrier. Am J Physiol 251: E477-E482.

Borlongan CV, Emerich DF (2003) Facilitation of drug entry into the CNS via transient permeation of blood brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist, Cereport. Brain Res Bull 60: 297–306.

Brinckerhoff L, Kalashnikov V, Thompson L, Yamshchikov G, Pierce R, Galavotti H, Engelhard V, Slingluff CJ (1999) Terminal modifications inhibit proteolytic degradation of an immunogenic MART-1(27–35) peptide: implications for peptide vaccines. Int J Cancer 83: 326–334.

Caudle RM, Isaac L (1987) Intratechal dynorphin (1–13) results in irreversible loss of tail flick reflex in rats. Brain Res 435: 1–6.

Chavkin C, Goldstein A (1981) Specific receptor for the opioid peptide dynorphin: structure-activity relationships. Proc Natl Acad Sci 78: 6543–6547.

Chavkin C, James IF, Goldstein A (1982) Dynorphin is a specific endogenous ligand of the ϰ receptor. Science 215: 413–415.

Chen L, Gu Y, Huang LYM (1995) The opioid peptide dynorphin directly blocks NMDA receptor channels in the rat. J Physiol 482: 575–581.

Chou JZ, Chait BT, Wang R, Kreek MJ (1996) Differential biotransformation of dynorphin A(1–17) and dynorphin A(1–13) peptides in human blood, ex vivo. Peptides 17: 983–990.

Csuhai E, Little SS, Hersh LB (1995) Inactivation of neuropeptides. Prog Brain Res 104: 131–141.

Friedman HJ, Jen MF, Chang JK, Lee NM, Loh HH (1981) Dynorphin: a possible modulatory peptide on morphine or β-endorphin analgesia in mouse. Eur J Pharmacol 69: 351–360.

Gambus PD, Schnider TW, Minto CF, Youngs EJ, Billard V, Brose WG, Hochhaus G, Shafer SL (1998) Pharmacokinetics of intravenous dynorphin A(1–13) in opioid-naive and opioid-treated human volunteers. Clin Pharmacol Ther 64: 27–38.

Goldstein A, Tachibana S, Lowney LI, M. H, L. H (1979) Dynorphin-(1–13), an extraordinary potent opioid peptide. Proc Natl Acad Sci 76: 6666–6670.

Green PG, Lee NM (1988) Dynorphin A-(1–13) attenuates withdrawal in morphine-dependent rats: effect of route of administration. Eur J Pharmacol 145: 267–272.

Hauser KF, Foldes JK, Turbek ST (1999) Dynorphin A(1–13) neurotoxicity *in vitro*: Opioid and non-opioid mechanisms in mouse spinal cord neurons. Exp Neurol 160: 361–375.

Heavner G, Kroon D, Audhya T, Goldstein G (1986) Biologically active analogs of thymopentin with enhanced enzymatic stability. Peptides 7: 1015–1019.

Herman BH, Goldstein A (1985) Antinociception and paralysis induced by intratechal dynorphin-A. J Pharmacol Exp Ther 232: 27–32.

Hiramatsu M, Inoue K, Ambo A, Sasaki Y, Kameyama T (2001) Long-lasting antinociceptive effects of a novel dynorphin analogue, Tyr-D-Ala-Phe-Leu-ArgΨ (CH_2NH) Arg—NH_2, in mice. Br J Pharmacol 132: 1948–1956.

Hooke LP, Lee NM (1995) Dynorphin A modulates acute and chronic opioid effects. J Pharmacol Exp Ther 273: 292–297.

Hooke LP, He L, Lee NM (1995) [Des-Tyr^1] Dynorphin A-(2–17) has naloxone-insensitive antinociceptive effect in the writhing assay. J Pharmacol Exp Ther 273: 802–807.

Hu J, El-Fakahany EE (1993) Allosteric interaction of dynorphin and myelin basic protein with muscarinic receptors. Pharmacol 47: 351–359.

Laughlin TM, Larson AA, Wilcox GL (2001) Mechanisms of induction of persistent nociception by dynorphin. J Pharmacol Exp Ther 299: 6–11.

Leslie FM, Goldstein A (1982) Degradation of dynorphin A1–13 by membrane-bound rat brain enzymes. Neuropep 2: 185–196.

McCleave MJ, Elliott RJ, Archbold GP (1996) Human plasma carboxypeptidases N; stability of enzyme activity following collection. Biochem Soc Trans 24: 42S.

Muller S, Hochhaus G (1995) Metabolism of dynorphin A 1–13 in human blood and plasma. Pharm Res 12: 1165–1170.

Muller S, Grundy BL, Hochhaus G (1996) Metabolism of dynorphin A1–13 in human CSF. Neurochem Res 21: 1213–1219.

Muller S, Pang CSP, Hochhaus G (1998) Displacement of [^{3}H]-L-glutamate in rat brain membranes by metabolic dynorphin fragments. Pharmazie 53: 142–143.

Muller S, Ho B, Gambus P, Millard W, Hochhaus G (1997) An HPLC/RIA method for dynorphin A1–13 and its main metabolites in human blood. J Pharm Biomed Anal 16: 101–109.

Nakazawa T, Ikeda M, Kaneko T, Yamatsu K (1985) Analgesic effects of dynorphin-A and morphine in mice. Peptides 6: 75–78.

Nyberg F, Lyrenas S, Terenius L (1986) Assay and biochemical characterization of a dynorphin converting enzyme in human cerebrospinal fluid. NIDA Res Monogr 75: 251–254.

Pentel PR, Wananukul W, Hooke LP, Jones CRN, Hatsukami D, Anderson WR, Lee NM (1995) Effects of high intravenous doses of dynorphin A(1–13) on tail flick latency and central nervous system histology in rats. Pharmacol Biochem Behav 51: 387–390.

Persson S, Post C, Alari L, Nyberg F, Terenius L (1989) Increased neuropeptide-converting enzyme activities in cerebrospinal fluid of opiate-tolerant rats. Neurosc. Lett 107: 318–322.

Powell MF, Grey H, Gaeta F, Sette A, Colon S (1992) Peptide stability in drug development: a comparison of peptide reactivity in different biological media. J Pharm Sci 81: 731–738.

Przewlocki R, Przewlocka B (2001) Opioids in chronic pain. Eur J Pharmacol 429: 79–91.

Quillan JM, Sadee W (1997) Dynorphin peptides: antagonists of melanocortin receptors. Pharm Res 14: 713–719.

Safavi A, Hersh LB (1995) Degradation of dynorphin-related peptides by the puromycin-sensitive aminopeptidase and aminopeptidase M. J Neurochem 65: 389–395.

Shukla VK, Lemaire S (1994) Non-opioid effects of dynorphins: possible role of the NMDA receptor. Trends Pharmacol Sci 15: 420–424.

Smith AP, Lee NM (1988) Pharmacology of dynorphin. Ann Rev Pharmacol Toxicol 28: 123–140.

Specker S, Wananukul W, Hatsukami D, Nolin K, Hooke L, Kreek MJ, Pentel PR (1998) Effects of dynorphin A(1–13) on opiate withdrawal in humans. Psychopharmacology 137: 326–332.

Takemori AE, Loh HH, Lee NM (1992) Suppression by dynorphin A-(1–13) of the expression of opiate withdrawal and tolerance in mice. Eur J Pharmacol 221: 223–226.

Terasaki T, Hirai K, Sato H, Kang YS, Tsuji A (1989) Absorptive-mediated endocytosis of a dynorphin-like analgesic peptide, E-2078, into the blood-brain barrier. J Pharmacol Exp Ther 251: 351–357.

Terenius L, Nyberg F (1988) Neuropeptide-processing, -converting, and -inactivating enzymes in human cerebrospinal fluid. Int Rev Neurobiol 30: 101–121.

Turner TD, Browning JL, Widmayer MA, Baskin DS (1998) Penetration of dynorphin 1–13 across the blood-brain barrier. Neuropeptides 32: 141–149

Vanderah TW, Ossipov MH, Lai J, Malan TP, Porreca F (2001) Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. Pain 92: 5–9.

Walker JM, Katz RJ, Akil H (1980) Behavioral effects of dynorphin$_{1-13}$ in the mouse and rat: initial observations. Peptides 1: 341–345.

Wen HL, Ho WKK (1982) Suppression of withdrawal symptoms by dynorphin in heroin addicts. Eur J Pharmacol 82: 183–186.

Wen HL, Ho WKK, Wen PYC (1984) Comparison of the effectiveness of different opioid peptides in suppressing heroin withdrawal. Eur J Pharmacol 100: 155–162.

Wettergren A, Pridal L, Wojdemann M, Holst J (1998) Amidated and non-amidated glucagon-like peptide-1 (GLP-1): non-pancreatic effects (cephalic phase acid secretion) and stability in plasma in humans. Regul Pept 77: 83–87.

Yoshino H, Nakazawa T, Arakawa Y, Kaneko T, Tsuchiya Y, Matsunaga M, Araki S, Ikeda M, Yamatsu M, Tachibana S (1990) Synthesis and structure-activity relationships of dynorphin A(1–8) amide. J Med Chem 33: 206–212.

Young EA, Walker M, Houghten R, Akli H (1987) The degradation of dynorphin A in brain tissue in vivo and in vitro. Peptides 8: 701–707.

Yu J, Butelman ER, Woods JH, Chait BT, Kreek MJ (1997) Dynorphin A(1–8) analog, E-2078, is stable in human and rhesus monkey blood. J Pharmacol Exp Ther 280: 1147–1151.

Zhang S, Yu L (1995) Identification of dynorphins as endogenous ligands for an opioid receptor-like orphan receptor. J Biol Chem 270: 22772–22776.