The Endomorphin System and Its Evolving Neurophysiological Role

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Abstract——Endomorphin-1 (Tyr-Pro-Trp-Phe-NH$_2$) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH$_2$) are two endogenous opioid peptides with high affinity and remarkable selectivity for the μ-opioid receptor. The neuroanatomical distribution of endomorphins reflects their potential endogenous role in many major physiological processes, which include perception of pain, responses related to stress, and complex functions such as reward, arousal, and vigilance, as well as autonomic, cognitive, neuroendocrine, and limbic homeostasis. In this review we discuss the biological effects of endomorphin-1 and endomorphin-2 in relation to their distribution in the central and peripheral nervous systems. We describe the relationship between these two μ-opioid receptor-selective peptides and endogenous neurohormones and neurotransmitters. We also evaluate the role of endomorphins from the physiological point of view and report selectively on the most important findings in their pharmacology.

I. Introduction

The three opioid receptors, designated μ, δ, and κ, which were found in the central and peripheral nervous systems, mediate the biological functions of opioids. After the discovery of δ-opioid receptor-selective enkephalins in 1975 (Hughes et al., 1975), other peptides have been characterized as endogenous ligands for the opioid receptors (Table 1) (Goldstein et al., 1979; Nakanishi et al., 1979; Bloom, 1983). Naturally occurring opioid peptides, which were shown to bind preferentially to the μ-opioid receptor, were β-casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) from the tryptic digests of β-casein (Henschen et al., 1979), hemorphin-4 (Tyr-Pro-Trp-Thr) from digests of hemoglobin (Brantl et al., 1986), Tyr-Pro-Leu-Gly-NH$_2$ (Tyr-MIF-1[1]) and Tyr-Pro-Trp-Gly-NH$_2$ (Tyr-W-MIF-1), both isolated from the brain (Horvath and Kastin, 1989; Erchegyi et al., 1992). However, until 1997, no mammalian peptide was identified that would show substantial μ-opioid receptor affinity and selectivity.

In 1997, Zadina's group (Zadina et al., 1997) synthesized a number of Tyr-W-MIF-1 analogs, containing all possible natural amino acid substitutions at position 4, which were subsequently screened for the opioid receptor binding. A biologically potent sequence, Tyr-Pro-Trp-Phe-NH$_2$, was discovered and then identified in the bovine brain (Zadina et al., 1997) and human cortex (Hackler et al., 1997). This new peptide, which was named endomorphin-1, showed remarkable affinity for the μ-opioid receptor (360 pM) and selectivity of 4000-

and 15,000-fold for the μ-opioid receptor over the δ- and κ-opioid receptors, respectively. Endomorphin-1 was extremely potent in the guinea pig ileum assay, a classic test for μ-opioid receptor agonist activity (Zadina et al., 1997). This peptide also had a potent and specific antinociceptive effect in vivo, as shown in the tail-flick test (Hackler et al., 1997; Zadina et al., 1997). A second peptide, endomorphin-2 (Tyr-Pro-Phe-Phe-NH$_2$), which differs by one amino acid from endomorphin-1, was also isolated. Endomorphin-2 was shown to be almost as potent as endomorphin-1 (Hackler et al., 1997; Zadina et al., 1997). Endomorphins were the first peptides isolated from brain that bind to the μ-opioid receptor with high affinity and selectivity and therefore were proposed as endogenous μ-opioid receptor ligands. However, their precursor(s) still remain(s) unidentified.

In this review we discuss the biological effects of endomorphin-1 and endomorphin-2 in relation to their distribution in the central and peripheral nervous systems. We describe the relationship between these μ-opioid receptor-selective peptides and endogenous neurohormones and neurotransmitters. We evaluate the role of endomorphins from the physiological point of view and report selectively on the most important findings in their pharmacology, rather than present all available data.

II. Structure-Activity Relationship Studies

Full descriptions of all the structure-activity relationships studies of endomorphins are beyond the scope of this review. For more data on endomorphin analogs, please refer to the articles by Janecka et al. (2004), Gentilucci and Tolomelli (2004), and Janecka and Kruszynski (2005).

The structures of endomorphin-1 and endomorphin-2 are quite distinct from those of the traditional opioid peptides (endorphins, enkephalins, and dynorphins), which all share the Tyr-Gly-Gly-Phe sequence at the N terminus. The N-terminal message sequence of endomorphin-1 is composed of two pharmacophoric amino acid residues, Tyr and Trp (in endomorphin-2 Trp is replaced by Phe), in which the amino and phenolic groups of Tyr and the aromatic ring of Trp (or Phe) are required for μ-opioid receptor recognition. The sequence of endomorphins also includes a spacer (Pro), which joins the pharmacophoric residues. Podlogar et al. (1998) presented a

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[1] Abbreviations: Tyr-MIF, Tyr-Pro-Leu-Gly-NH$_2$; Tyr-W-MIF, Tyr-Pro-Trp-Gly-NH$_2$; IR, immunoreactivity; CNS, central nervous system; PAG, periaqueductal gray; LC, locus coeruleus; NTS, nucleus of the solitary tract; Nac, nucleus accumbens; VTA, ventral tegmental area; DAMGO, Tyr-D-Ala-Gly-MePhe-Gly-ol; i.c.v., intracerebroventricular; i.t., intrathecal; ACh, acetylcholine; DPP IV, dipeptidyl-peptidase IV; Glu, glutamate; 5-HT, serotonin; NA, noradrenalin; 6-OHDA, 6-hydroxydopamine; 5,7-DHT, 5,7-dihydroxytryptamine; DA, dopamine; NO, nitric oxide; CTOP, D-Phe-c(Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH$_2$); HPA, hypothalamo-pituitary-adrenal; CRF, corticotrophin-releasing factor; AVP, arginine vasopressin; ACTH, adrenocorticotropin; FST, forced swimming test; BDNF, brain-derived neurotrophic factor; VMH, ventromedial hypothalamus; mPOA, medial preoptic area; MCG, mesencephalic central gray; MS-HDB, medial septum-horizontal limb of the diagonal band; PVN, paraventricular nucleus; mNTS, medial subnucleus of the nucleus of the solitary tract; NANC, nonadrenergic, noncholinergic; DRN, dorsal raphe nucleus; OT, oxytocin.

TABLE 1
Endogenous opioid peptides

Receptor	Precursor	Endogenous Peptide	Amino Acid Sequence	Source	References
μ (MOR, OP$_3$)	Pro-opiomelanocortin	β-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	Mammalian brain	Li and Chung (1976)
	β-Casein (bovine)	β-Casomorphin-5	Tyr-Pro-Phe-Pro-Gly	Bovine milk	Blanchard et al. (1987)
		β-Casomorphin-7	Tyr-Pro-Phe-Pro-Gly-Pro-Ile		
		Morphiceptin	Tyr-Pro-Phe-Pro-NH$_2$		
	β-Casein (human)	β-Casomorphin-5	Tyr-Pro-Phe-Val-Glu	Human milk	Blanchard et al. (1987)
		β-Casomorphin-7	Tyr-Pro-Phe-Val-Glu-Pro-Ile		
	Hemoglobin	Hemorphin-4	Tyr-Trp-Thr	Human blood	Nyberg et al. (1997); Zhao et al. (1997)
		Hemorphin-7	Tyr-Pro-Trp-Thr-Gln-Arg-Phe		
	Unknown	Dermorphin	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$	Frog skin	Bozu et al. (1997)
	Unknown	Endomorphin-1	Tyr-Pro-Trp-Phe-NH$_2$	Bovine brain, human brain cortex	Hackler et al. (1997); Zadina et al. (1997)
	Unknown	Endomorphin-2	Tyr-Pro-Phe-Phe-NH$_2$	Bovine brain, human brain cortex	Zadina et al. (1994)
		Tyr-MIF-1	Tyr-Pro-Leu-Gly-NH$_2$		
δ (DOR, OP$_1$)	Proenkephalin	Tyr-W-MIF-1	Tyr-Pro-Trp-Gly-NH$_2$	Mammalian brain	Hughes et al. (1975)
		[Met^5]enkephalin	Tyr-Gly-Gly-Phe-Met		
		[Leu^5]enkephalin	Tyr-Gly-Gly-Phe-Leu		
			Tyr-Gly-Gly-Phe-Met-Arg-Phe		
			Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu		
	Unknown	Dermenkephalin	Tyr-D-Met-Phe-His-Leu-Met-AspNH$_2$	Frog skin	Amiche et al. (1989); Kreil et al. (1989)
κ (KOR, OP$_2$)	Prodynorphin	Deltorphin-I	Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH$_2$	Mammalian brain	Chavkin et al. (1982)
		Deltorphin-II	Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH$_2$		
		Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln		
		Dynorphin A(1-8)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile		
		Dynorphin B	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr		
ORL$_1$ (NOP$_1$)	Pronociceptin	Nociceptin/orphanin FQ	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln	Mammalian brain	Meunier et al. (1995); Reinscheid et al. (1995)

structural and comparative study of endomorphin-1 to identify its bioactive conformation and attributes responsible for the μ-opioid receptor selectivity. Nuclear magnetic resonance data showed that Pro[2] provides the necessary stereochemical requirements for activity of endomorphin-1 at the μ-opioid receptor. The bioactive conformation of endomorphin-1 is characterized by a structure, in which the Tyr[1] and Trp[3] side chains have opposite orientations with respect to Pro[2] (Paterlini et al., 2000). Synthesizing pseudoproline-containing analogs of endomorphin-2, which are known to be quantitative inducers of the *cis* conformation, the group of Schiller (Keller et al., 2001) demonstrated that the Tyr-Pro amide bond in the bioactive conformation is *cis*.

III. Distribution

Radioimmunological and immunocytochemical analyses revealed that endomorphin immunoreactivities (IRs) are distributed throughout the human, bovine, and rodent central nervous systems (CNS) (Hackler et al., 1997; Martin-Schild et al., 1997, 1999; Pierce et al., 1998; Schreff et al., 1998; Pierce and Wessendorf, 2000). Both endomorphins are abundant in the areas such as the stria terminalis, the periaqueductal gray (PAG), the locus coeruleus (LC), the parabrachial nucleus, and the nucleus of the solitary tract (NTS) (Table 2). However, there are also important differences in the neuroanatomical localization of these peptides. Endomorphin-1 is widely and densely distributed throughout the brain and upper brainstem and is particularly abundant in the nucleus accumbens (Nac), the cortex, the amygdala, the thalamus, the hypothalamus, the striatum, and the dorsal root ganglia (Schreff et al., 1998; Martin-Schild et al., 1999). In contrast, endomorphin-2 is more prevalent in the spinal cord and lower brainstem (Martin-Schild et al., 1999; Pierce and Wessendorf, 2000); endomorphin-2-immunoreactive cell bodies were most prominent in the hypothalamus and the NTS, whereas endomorphin-2-immunoreactive varicose fibers were mainly observed in the substantia gelatinosa of the medulla and the spinal cord dorsal horn. More modest endomorphin-2 IR was seen in the Nac, substantia nigra, nucleus raphe magnus, ventral tegmental area (VTA), and pontine nuclei and amygdala. The differences in the distribution of endomorphins could indicate the existence of two distinct endomorphin precursors or two different processing pathways of the same precursor.

The distribution of endomorphin IRs in the CNS seems to be similar to that of the classic endogenous opioid peptides (Hokfelt et al., 1977; Simantov et al., 1977; Johansson et al., 1978; Sar et al., 1978; Uhl et al., 1979). However, in the case of endomorphin-2 two major differences were found. Unlike δ-opioid receptor-selective enkephalin (Watson et al., 1977; Sar et al., 1978) and κ-opioid receptor-selective dynorphin (Gramsch et al., 1982), endomorphin-2 IR was sparse in the hippocampus and striatum (Schreff et al., 1998; Martin-Schild et al., 1999). In this way the distribution of endomorphin-2 was analogous to that of β-endorphin, an endogenous μ-opioid receptor-selective ligand (Finley et al., 1981).

The reports on the presence of endomorphins outside the CNS are scarce. Jessop et al. (2000) used a combination of specific radioimmunological assays and reversed phase high-performance liquid chromatography techniques to characterize the level of endomorphin IR in rat and human peripheral tissue samples. They found that endomorphin-1 IR and endomorphin-2 IR are present in significant amounts in human spleen; relatively high levels of endomorphin IR were also detected in the rat spleen, thymus, and blood. Because very low amounts of endomorphin IR were found in anterior and posterior rat pituitaries, secretion from these glands could not account for the significant plasma level of endomorphins. The authors of that report suggested that endomorphins are secreted into the general circulation from the nerve fibers and terminals of the spinal cord. Interestingly, in the same study a number of peaks of endomorphin-1 IR and endomorphin-2 IR, which do not coelute with their respective synthetic standards, were also detected. These might represent precursor polypeptides or degradation products of endomorphin-1 and endomorphin-2, or their post-translational modifications.

Endomorphins were also detected in immune cells in inflamed subcutaneous tissue, whereas they were almost absent in noninflamed tissue (Mousa et al., 2002). The endomorphin-positive cells could only be identified in the periphery of inflammatory foci and had morphological appearances consistent with macrophages/monocytes, lymphocytes, and polymorphonuclear leukocytes.

IV. Receptors

The μ-opioid receptors belong to the superfamily of heterotrimeric, guanine-nucleotide binding, G-protein-coupled receptors. The results of numerous in vitro studies clearly demonstrated that the μ-opioid receptors are exclusive binding sites for endomorphins. In the classic binding assays on the rat and mouse brain membrane preparations, both peptides displaced naloxone, Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO), and other μ-opioid receptor-selective ligands in a concentration-dependent manner (for review, see Horvath, 2000). Endomorphins stimulated [^{35}S]guanosine 5′-O-(3-thio)triphosphate binding through the μ-opioid receptors in rat thalamus membrane preparations (Kakizawa et al., 1998; Sim et al., 1998; Fichna et al., 2006a), in the PAG (Narita et al., 2000), and in the pons/medulla of μ-opioid receptor-nondeficient mice (Mizoguchi et al., 2000). Both peptides were potent μ-opioid receptor agonists in the aequorin luminescence-based calcium assay performed on recombinant Chinese hamster ovary cell lines (Fichna et al.,

TABLE 2

Distribution of endomorphins in the selected structures of the central nervous system

Data from Martin-Schild et al. (1997, 1998, 1999), Schreff et al. (1998), Barr and Zadina (1999), Pierce et al. (1998), and Pierce and Wessendorf (2000).

Region	Structure	Endomorphin-1[a]	Endomorphin-2[a]
Brain			
Telencephalon	Amygdala	++/+ (particularly abundant in central and intercalated amygdaloid nuclei)	+
	Cortex	++/+ (particularly abundant in medial frontal regions: cingulate, prelimbic, infralimbic, and dorsal peduncular cortices)	−
	Globus pallidus	−	−
	Hippocampus	−	−
	Lateral septum	++	+
	Nucleus accumbens	++ (dorsal and ventral)	+
	Stria terminalis	++	++ (particularly abundant in the bed nucleus of stria terminalis)
	Striatum	++	
Diencephalon	Hypothalamus	++ (particularly abundant in posterior hypothalamic area)	++ (immunoreactive cell bodies found)
	Thalamus	++/+ (particularly abundant in centromedial and paraventricular thalamic nuclei)	++/−[c] (particularly abundant in paraventricular thalamic nuclei)
Mesencephalon	Superior colliculus	+	+
	Inferior colliculus	++	++
	Periaqueductal gray	+	+
	Ventral tegmental area	+	
	Cerebellum	−	+/−[b]
Metencephalon and myelencephalon	Kölliker-Fuse nucleus	+	++
	Locus coeruleus	++	++
	Nucleus of the solitary tract	++ (located mostly in the dorsomedial subnucleus)	++/−[c] (immunoreactive cell bodies found)
	Parabrachial nucleus	++ (particularly abundant in lateral and external medial parabrachial nuclei)	++ (particularly abundant in lateral, medial, and external medial parabrachial nuclei)
	Raphe nucleus	+	+
	Spinal trigeminal tract	−	++
Spinal cord	Cervical, thoracic, lumbar, and sacral segments	−	++ (particularly abundant in superficial layers of the dorsal horn: substantia gelatinosa, marginal zone, and nucleus proprius, as well as in the lateral spinal nucleus and ventral to the spinal central canal)
	Dorsal root ganglia	−	++
	Substantia gelatinosa of the medulla	+	++

[a] ++, dense endomorphin-like immunoreactivity; +, moderate endomorphin-like immunoreactivity; −, no endomorphin-like immunoreactivity observed.
[b] No endomorphin-2-like immunoreactivity detected by Martin-Schild et al. (1999).
[c] No endomorphin-2-like immunoreactivity detected by Schreff et al. (1998).

2006b). The autoradiographic methods showed that in the CNS endomorphins labeled the same binding sites as DAMGO, a classic μ-opioid receptor-selective ligand (Goldberg et al., 1998; Kakizawa et al., 1998; Sim et al., 1998). Results of the in vivo studies also demonstrated that endomorphins are μ-opioid receptor ligands. The intracerebroventricular (i.c.v.) administration of endomorphins produced a potent antinociceptive effect in wild-type mice (for review, see Horvath, 2000) and no significant effect in μ-opioid receptor knockout mice (for reviews, see Sakurada et al., 2002; Narita et al., 1999; Zadina et al., 1999; Tseng, 2002). Intrathecal (i.t.) administration produced significant antinociception in the tail-flick, paw-withdrawal, tail pressure, and flexor-reflex tests in adult rodents (Stone et al., 1997; Zadina et al., 1997; Goldberg et al., 1998; Horvath et al., 1999; Sakurada et al., 1999, 2000, 2001; Ohsawa et al., 2001; Grass et al., 2002).

Endomorphin-1 and endomorphin-2 are regarded as partial agonists of μ-opioid receptors. The efficacy of endomorphins in many bioassays, such as guanosine $5'$-O-(3-thio)triphosphate binding, is slightly lower than that of DAMGO but higher than that of morphine (Alt et al., 1998; Harrison et al., 1998; Sim et al., 1998). The extent to which these relative efficacies apply to the biological activity of endomorphins has yet to be elucidated.

The endomorphins are principal, but not exclusive, endogenous ligands of μ-opioid receptors. In the CNS, endomorphins, although anatomically positioned to activate the μ-opioid receptors, are not selectively associated with the regions expressing these binding sites. In several telencephalic and limbic structures, μ-opioid receptors and endomorphin-immunoreactive fibers are colocalized. These include septal nuclei, the bed nucleus of the stria terminalis, Nac, the amygdaloid complex, and many hypothalamic nuclei (for reviews, see Zadina et al., 1999, Zadina, 2002; Martin-Schild et al., 1999). There are also brain regions that contain low concentrations of endomorphins, in which significant numbers of μ-opioid receptors can be found, namely the amygdala (telencephalon), the thalamus, the hypothalamus (diencephalon), and the PAG (mesencephalon). Of note is the negligible amount of endomorphin IR in the striatum, a region known to express high levels of μ-opioid receptors (Pierce and Wessendorf, 2000). μ-Opioid receptors have also been detected outside the CNS, in the enteric nervous system (for review, see Olson et al., 1998) and throughout the immune tissues (Sharp et al., 1998), where they were found to be colocalized with endomorphins (Jessop et al., 2000).

Recent studies indicated that endomorphin-1 and endomorphin-2 produce their biological effects by stimulating functionally diverse subtypes of μ-opioid receptors, μ_1 and μ_2, which might be responsible for their distinct pharmacological activity (Sakurada et al., 1999, 2000; Tseng et al., 2000). The μ_1-opioid receptor antagonist naloxonazine was shown to block the antinociception induced by i.c.v. administration of endomorphin-2 more effectively than endomorphin-1, whereas β-funaltrexamine inhibited both. Spinal pretreatment with antisense oligodeoxynucleotides against different exons in the μ-opioid receptor gene differentially attenuated the antinociception induced by endomorphin-1 and endomorphin-2 (Wu et al., 2002; Garzon et al., 2004). These results showed that μ_2-opioid receptors would be stimulated by both endomorphin-1 and endomorphin-2, whereas μ_1-opioid receptors would be stimulated only by endomorphin-2. Further studies revealed that μ_1-opioid receptors mediate supraspinal analgesia and modulate acetylcholine (ACh) and prolactin release, whereas μ_2-opioid receptors mediate spinal analgesia, respiratory depression, and inhibition of gastrointestinal transit (Pasternak, 1993).

V. Enzymatic Degradation

The majority of opioid peptides undergo rapid enzymatic degradation (Egleton et al., 1998). Most of the extracellular peptide-degrading enzymes are membrane-bound exo- and endopeptidases, integral membrane proteins that have active sites facing the extracellular space. An intracellular cleavage of opioid peptides by cytosolic peptidases is also possible.

Endomorphins seem to be vulnerable to enzymatic cleavage, and several enzymes have been proposed as participants in endomorphin degradation (Tomboly et al., 2002) (Fig. 1). Aminopeptidase M (EC 3.4.11.2) and aminopeptidase P (EC 3.4.11.9) could degrade N-terminal Tyr-Pro peptide bonds, release an N-terminal amino acid, and generate tripeptides from endomorphins (Dua et al., 1985; Harbeck and Mentlein, 1991). The literature data also indicate that, when the N-terminal hydrophobic residue is followed by a Pro residue, the two amino acids may be released by aminopeptidase M as an intact dipeptide (Bairoch, 1996). Carboxypeptidase Y (serine peptidase, EC 3.4.16.5) and proteinase A (nonpepsin-type acid endopeptidase, EC 3.4.23.6) in the first step could convert the C-terminal amide group into a carboxyl group and then catalyze the hydrolysis of the Xaa^3–Phe^4 peptide bond, where Xaa indicates a natural amino acid) (Berne et al., 1990; Peter et al., 1999). Dipeptidyl-peptidase IV (DPP IV) (EC.3.4.14.5), a membrane-bound serine proteinase, removes dipeptides from the amino terminus of peptides containing proline as the penultimate amino acid and has also been proposed to participate in endomorphin degradation (Kato et al., 1978).

The experimental data are in good agreement with the above theoretical assumptions. The in vitro assays revealed that aminopeptidase M cleaves endomorphins at the Pro^2–Trp^3 and Pro^2–Phe^3 peptide bonds and the C-terminal dipeptides are then hydrolyzed into amino acids (Tomboly et al., 2002; Janecka et al., 2006). Car-

Tyr-Pro-Trp-OH   Phe   Tyr-Pro-Phe-OH

Carboxypeptidase Y
or
Proteinase A

Tyr-Pro-Trp-Phe-OH   Tyr-Pro-Phe-Phe-OH

Carboxypeptidase Y
or
Proteinase A

Tyr ← Tyr-Pro-Trp-Phe-NH_2 Tyr-Pro-Phe-Phe-NH_2

[Endomorphin-1]   [Endomorphin-2]

Pro-Trp-Phe-NH_2

Dipeptidylpeptidase IV
or
Aminopeptidase M

Pro ←

Trp-Phe-NH_2 Tyr-Pro-OH   Phe-Phe-NH_2

Aminopeptidase   Aminopeptidase

Trp   Phe-NH_2 Tyr   Pro   Phe   Phe-NH_2

FIG. 1. Enzymatic degradation pathways of endomorphin-1 and endomorphin-2.

boxypeptidase A does not degrade endomorphins, because they are amidated peptides, whereas carboxypeptidase Y and proteinase A reveal deamidase activity; they hydrolyze endomorphins into peptide acids, releasing ammonia, and then cleave off the C-terminal Phe. The studies in vivo showed that there are two groups of enzymes mainly responsible for the degradation of endomorphins: DPP IV, which triggers the process, and aminopeptidases, which are involved in secondary cleavage (Shane et al., 1999; Sakurada et al., 2003). Consequently, endomorphins are degraded by similar pathways. The first step in their catabolism is the cleavage of Pro^2–Trp^3 and Pro^2–Phe^3 peptide bonds, respectively, and the dipeptides formed are then hydrolyzed into amino acids. However, the degradation of endomorphin-1 contains an additional minor route: the Tyr^1–Pro^2 peptide bond might also be cleaved in the first step of the enzymatic degradation pathway.

Interestingly, some authors claim that endomorphin-1 is more resistant to enzymatic degradation in vivo than endomorphin-2 (Fujita and Kumamoto, 2006). This is in good agreement with the observation that the duration of spinal antinociceptive effects was significantly longer for endomorphin-1 than for endomorphin-2 (Grass et al., 2002) and that endomorphin-1 required a longer pretreatment time than endomorphin-2 before tolerance

was observed (Stone et al., 1997). This might also explain a relatively shorter duration of the antidepressant-like activity of i.c.v. administered endomorphin-2 in mice (Fichna et al., 2007).

One reason the elucidation of the degradation pathways of endomorphins was necessary was to isolate their effects from these produced by the degradation products. Because of structural similarities to the parent compound, the degradation products might compete with endomorphins for the receptor binding site and could influence their biological activity. However, Szatmari et al. (2001) demonstrated that the primary degradation products of endomorphin-1, Tyr-Pro-Trp-Phe-OH and Pro-Trp-Phe-OH, possess low μ-opioid receptor binding affinity, do not activate G-proteins and have no antinociceptive activity.

The effect of peptidase inhibitors on endomorphin degradation has also been studied. Spetea et al. (1998) examined the influence of peptidase inhibitors on the binding characteristics of [^{3}H]endomorphin-2 in rat brain membrane preparations and showed that in the absence of peptidase inhibitors 40% of the radioligand in the incubation mixture was destroyed. Actinonin, but not thiorphan, was found to be effective in inhibiting the degradation of endomorphin-1 in a 6-day-old rat spinal cord homogenate (Sugimoto-Watanabe et al., 1999). Endomorphin-2-induced antinociception was remarkably enhanced in the paw withdrawal test when diprotin A (Ile-Pro-Ile), a DPP IV inhibitor, was simultaneously injected (Sakurada et al., 2003): the peptide in combination with diprotin A was 5-fold more potent than endomorphin-2 alone in producing antinociceptive effects. Shane et al. (1999) reported that i.c.v. administered Ala-pyrrolidonyl-2-nitrile, another specific inhibitor of DPP IV, increased the magnitude, duration, and potency of endomorphin-2-induced antinociception in the rat tail-flick test.

VI. Neurophysiological Role

A. Biological Effects of Endomorphins

The neuroanatomical distribution of endomorphins and the μ-opioid receptors in the CNS reflects their potential endogenous role in many major biological processes. These include perception of pain, responses related to stress, and complex functions such as reward, arousal, and vigilance, as well as autonomic, cognitive, neuroendocrine, and limbic homeostasis (Fig. 2). Some of these phenomena, summarized in Tables 3 and 4, will be discussed in the following sections.

1. Pain. An important role of endomorphins in pain modulation is indicated by their presence in well-characterized nociceptive pathways (Fields and Basbaum, 1994; Guilbaud et al., 1994): endomorphin-containing neuronal elements were found in most regions of the spino(trigemino)-ponto-amygdaloid pathway (Fig. 3). These regions, which include the caudal nucleus of spi-
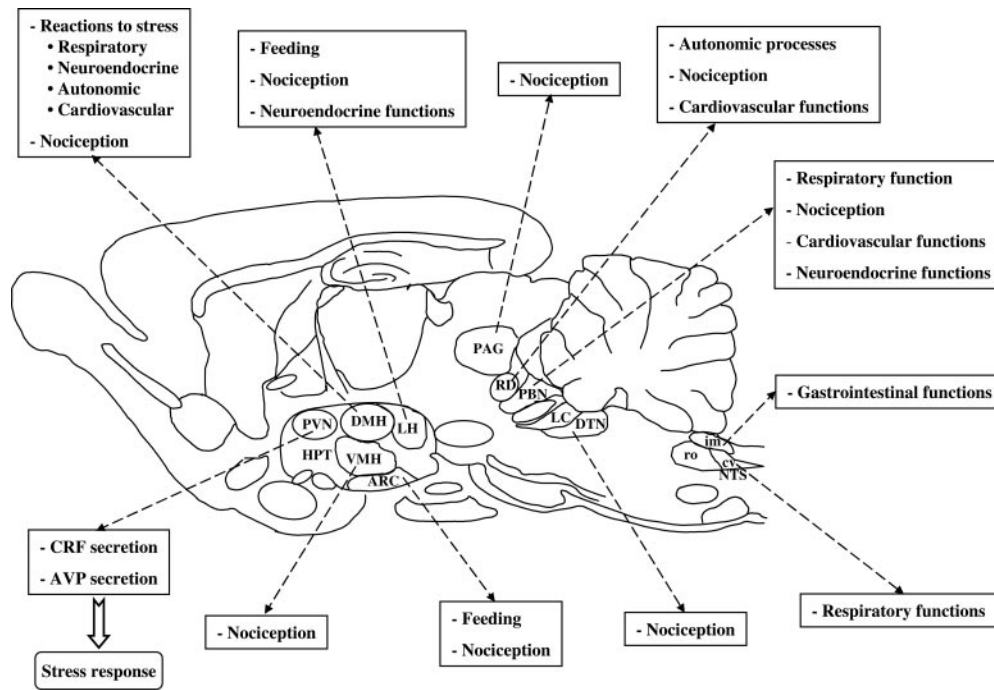
FIG. 2. Major structures in the central nervous system implicated in endomorphin-dependent effects. ARC, arcuate nucleus; DMH, dorsomedial nucleus; DTN, dorsal nucleus; HPT, hypothalamus; LC, locus coeruleus; LH, lateral nucleus; NTS, nucleus of the solitary tract (cv, caudal ventrolateral; im, intermediate, ro, rostral); PAG, periaqueductal gray; PBN, parabrachial nucleus; PVN, paraventricular nucleus; RD, caudal dorsomedial part of NTS; VMH, ventromedial nucleus

nal trigeminal tract, parabrachial nucleus, NTS, PAG, nucleus ambiguous, LC, and midline thalamic nuclei, are known to be involved in the transmission of the nociceptive information by direct input from the primary afferents and/or as relay nuclei to other pain-processing circuits (for reviews, see Fields and Basbaum, 1978; Basbaum and Fields, 1984; Przewlocki et al., 1999; Przewlocki and Przewlocka, 2001). Endomorphins were also found in amygdala, which has been proposed to play an important role in nociception (Manning and Mayer, 1995), particularly with regard to the affective influences on and responses to noxious events (Bernard et al., 1992; Helmstetter and Bellgowan, 1993; Watkins et al., 1993).

Zadina (2002) suggested that endogenous endomorphin-2, due to its specific neuroanatomical localization, might be involved in the earliest stages of nociceptive information processing. To begin with, endomorphin-2 IR was found to be predominantly present in the spinal cord, in the superficial layers of the dorsal horn, and in the primary afferent fibers (including small diameter primary afferent neurons, i.e., most likely nociceptors), whose cell bodies are localized within the dorsal root ganglia (Mousa et al., 2002). These are the regions with the highest densities of μ-receptors in the nervous system (Martin-Schild et al., 1997) and are thought to play an important role in the modulation of nociceptive transmission by various endogenous substances, including opioids (for review, see Fürst, 1999). Moreover, the electrical stimulation of the dorsal roots was shown to promote the release of endomorphin-2 from dense-cored

vesicles, situated in dorsal horn axons (Williams et al., 1999; Wang et al., 2002). Thus, it was hypothesized that endomorphin-2 could serve both a regulatory function, by hyperpolarizing the membranes on intrinsic neurons of the dorsal horn and decreasing the excitability of postsynaptic μ-opioid receptors (Wu et al., 1999), and an autoregulatory function, by limiting the release of excitatory transmitters, glutamate (Glu), substance P, γ-aminobutyric acid, glycine, and calcitonin gene-related peptide through the activation of presynaptic μ-opioid autoreceptors on primary afferent fibers in the spinal cord (Yajiri and Huang, 2000; Wu et al., 2003) (Fig. 4). Endomorphin-2 might also modify pain sensations from the visceral organs and alter the efferent components of the autonomic nervous system by interacting with their preganglionic neurons (Martin-Schild et al., 1997). This latter hypothesis was recently confirmed by Silverman et al. (2005).

a. Central administration of endomorphins. Supraspinally and spinally administered endomorphins are believed to influence neurotransmitter systems similar to those influenced by the μ-opioid receptor agonists, morphine and DAMGO (Fig. 5). The neurochemical substrates mediating mesencephalic morphine analgesia in the rostral ventromedial medulla include, among others, serotonin (5-HT) (Kiefel et al., 1992a,b), GABA (Heinricher et al., 1991; McGowan and Hammond, 1993a,b), the excitatory amino acid transmitters, L-Glu or N-methyl-D-aspartate (Aimone and Gebhart, 1986; Van Praag and Frenk, 1990; Spinella et al., 1996), and neurotensin (Urban and Smith, 1993). The antinociception

TABLE 3
Biological actions of endomorphins (in vivo studies)

Phenomenon	Effect	Species	Site of Administration	References
Pain	Antinociception	Rat, mouse	i.c.v., i.t.	Chapman et al. (1997); Stone et al. (1997); Zadina et al. (1997); Goldberg et al. (1998); Loh et al. (1998); Yamaguchi et al. (1998); Shane et al. (1999); Horvath et al. (1999); Przewlocki et al. (1999); Ronai et al. (1999); Sakurada et al. (1999); Sanchez-Blanquez et al. (1999)
		Rat	i.p.	Li et al. (2001)
Tolerance	Development of tolerance to nociceptive stimulation	Rat, mouse	i.c.v./i.t. pretreatment followed by i.c.v. or i.t. administration	Stone et al. (1997); Higashida et al. (1998); Horvath et al. (1999); Wu et al. (2001, 2003); Hung et al. (2002); Labuz et al. (2002)
Dependence	Withdrawal, development of physical dependence	Rat	i.c.v.	Chen et al. (2003)
Locomotor activity	Hyperlocomotion	Rat, mouse	i.c.v., injection to VTA	Latimer et al. (1987); Calenco-Choukroun et al. (1991); Bujdoso et al. (2001, 2003)
Behavioral sensitization, drug addiction, reward	Orofacial dyskinesia	Rat	Injection to globus pallidus	Delfs et al. (1994); Obeso et al. (2000)
	No significant effect	Mouse	i.c.v.	Fichna et al. (2007)
	Stimulation of sensitization to amphetamine	Rat	Injection to VTA	Chen et al. (2001)
	Establishment of conditioned place preference	Rat, mouse	i.c.v., injections to posterior VTA	Narita et al. (2001a,b); Zangen et al. (2002); Huang et al. (2004)
	Establishment of place aversion	Mouse	i.c.v.	Narita et al. (2001a,b)
	No significant effect on conditioned place preference	Rat	i.c.v., injections to anterior VTA and Nac	Wilson et al. (2000); Zangen et al. (2002)
Stress response	No significant effect on HPA axis	Rat	i.c.v.	Coventry et al. (2001)
	No significant effect on corticosteroid release	Rat	i.c.v.	Coventry et al. (2001)
	Stimulation of NO release followed by the stimulation of HPA axis	Rat	i.v.	Champion et al. (1999a)
Anxiety	Anxiolytic	Mouse	i.c.v.	Asakawa et al. (1998)
Depression	Antidepressant	Mouse	i.c.v.	Fichna et al. (2007)
	No significant effect	Rat	i.c.v.	Zhang et al. (2006)
Social defeat	Inhibition of expression of conditioned defeat	Syrian hamster	i.c.v.	Whitten et al. (2001)
	No effect on consolidation of conditioned defeat	Syrian hamster	i.c.v.	Whitten et al. (2001)
Food intake	Orexigenic	Mouse	i.c.v.	Asakawa et al. (1998)
Sexual behavior	Inhibition of lordosis	Rat	Infusion into the mPOA or MS-HDB	Sinchak and Micevych (2001); Acosta-Martinez and Etgen (2002)
	No significant effect	Rat	Infusion into VMH, mPOA, or MCG	Acosta-Martinez and Etgen (2002)
		Mouse	i.c.v.	Ukai et al. (2000)
Learning	Impairment of spontaneous alternation performance (short-term memory)	Mouse	i.c.v.	Itoh et al. (1994); Ragozzino et al. (1994); Ukai et al. (1995b); Ukai and Lin (2002b)
	Impairment of passive avoidance learning (long-term memory)			
Cardiovascular	Decrease of systemic arterial pressure	Rat, mouse, cat, rabbit	i.v., i.c.v.	Champion et al. (1997a, 1998b, 1999a); Czapla et al. (1998); Kwok and Dun (1998); Makulska-Nowak et al. (2001)
	Decrease of heart rate	Rat, mouse	i.v.	Champion et al. (1998c, 1999b)
	No significant effect on cardiac output	Rat	i.v.	Champion et al. (1999b)
	Biphasic effects (initial rapid ventilatory depression, followed by an increase in ventilation)	Rat	i.v.	Czapla et al. (2000)
Respiration	Depression of ventilation by attenuation of ventilatory response to hypercapnia	Rat	i.v.	Czapla and Zadina (2005)
	Increase of oxygen consumption	Mouse	i.c.v.	Asakawa et al. (2000)
Neurotransmitter turnover	Increase of DA level in Nac	Rat	Microdialysis into Nac	Okutsu et al. (2006)
	Stimulation of NA release in the spinal cord	Rat	i.t.	Hao et al. (2000)
	Increase of 5-HT efflux	Rat	Microdialysis into DRN	Tao and Auerbach (2002)
	Inhibition of the serotoninergic activity in medial prefrontal cortex and ventral striatum	Rat	Injection into VTA	Chen et al. (2001)
	Inhibition of OT and AVP cells	Rat	i.c.v.	Doi et al. (2001)

TABLE 4
Biological actions of endomorphins (selected in vitro studies)

Phenomenon	Effect	Tissue Preparation	References
Tolerance	Development of tolerance after chronic treatment	μ-Opioid receptor-expressing Chinese hamster ovary cells and African green monkey kidney cells	Nevo et al. (2000)
Stress response	No significant effect on corticosterone secretion	Mouse adrenal slices	Bujdoso et al. (2003)
Respiration	Inhibition of cholinergic contractile responses	Guinea pig trachea	Patel et al. (1999)
	Inhibition of electrically evoked ACh release	Guinea pig trachea	Patel et al. (1999)
	Inhibition of electrically evoked contractions	Guinea pig bronchus	Fischer and Undem (1999)
Gastrointestinal	Inhibition of electrically evoked contractions	Guinea pig ileum longitudinal muscle-myenteric plexus preparations	Tonini et al. (1998)
	Inhibition of ACh release	Rat stomach	Yokotani and Osumi (1998)
	Inhibition of smooth and striated muscle activation	Rat esophagus	Storr (2000a,b)
	Inhibition of gastrointestinal transit	Guinea-pig colon	Storr et al. (2002a)
	Inhibition of contractile response	Rat small intestine	Storr et al. (2002b)
Neurotransmitter turnover	Hyperpolarization of membrane potential in LC, inhibition of spontaneous firing, decrease of excitability	Rat LC slices	Yang et al. (1999)
	Inhibition of 5-HT$_{2A}$ postsynaptic currents	Rat brain slices	Marek and Aghajanian (1998)
	Inhibition of ACh-evoked currents	Frog and rat inner hair cells	Lioudyno et al. (2002)
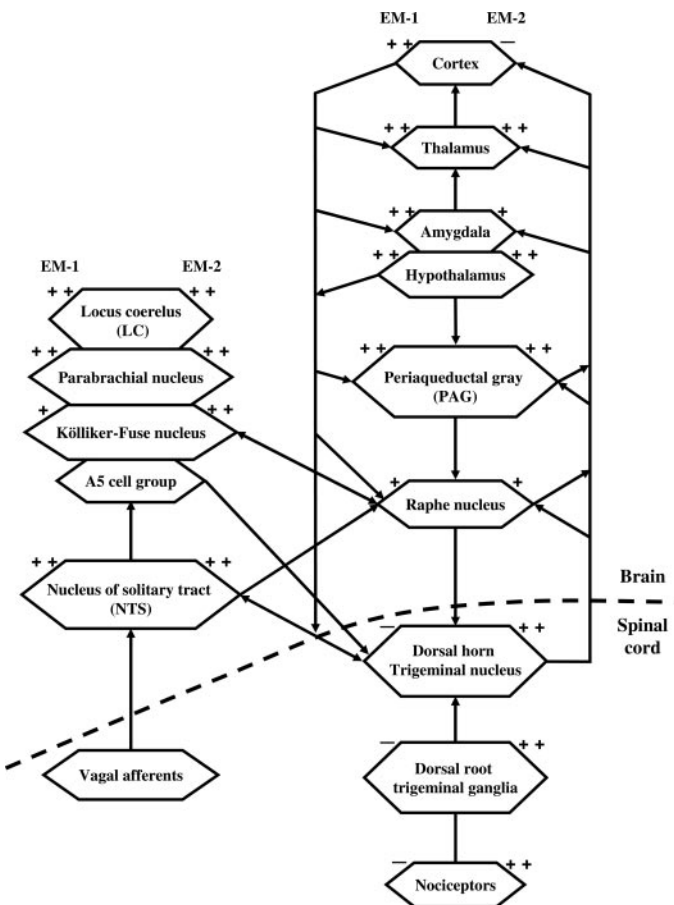
FIG. 3. Neuroanatomical distribution of endomorphin-1 (EM-1) and endomorphin-2 (EM-2) in major structures involved in the sensation, transmission, and modulation of pain. ++, dense endomorphin-like immunoreactivity; +, moderate endomorphin-like immunoreactivity; −, no endomorphin-like immunoreactivity observed. Arrows represent major ascending and descending pain pathways. Adapted from Kanjhan (1995) with permission from Blackwell Publishing.

induced by i.c.v. administered DAMGO is mediated by the release of noradrenaline (NA) and 5-HT, which act on α_2- and 5-HT receptors, respectively, in the spinal cord (Tseng and Tang, 1990; Tseng and Collins, 1991). The depletion of NA and 5-HT, induced by i.t. pretreatment with 6-hydroxydopamine (6-OHDA) and 5,7-dihydroxytryptamine (5,7-DHT), respectively, or the blockade of α_2-adrenoreceptors and 5-HT receptors by i.t. pretreatment with yohimbine and methysergide, respectively, attenuates the antinociception induced by morphine given supraspinally (Zhong et al., 1985; Rodriguez and Rodriguez, 1989; Suh et al., 1989, 1992; Sawynok et al., 1991).

Hung et al. (2003) determined the effects of i.t. pretreatment with 6-OHDA and 5,7-DHT to deplete NA and 5-HT, respectively, on the antinociception induced by supraspinally administered endomorphins. They found that 3-day i.t. pretreatment with 6-OHDA, which depleted spinal NA (>90%), completely abolished the antinociception induced by i.c.v. administration of endomorphins. These results strongly indicate that the release of NA in the spinal cord plays an essential role in the endomorphin-induced antinociception. The 3-day i.t. pretreatment with 5,7-DHT, which depleted both 5-HT (>90%) and NA (up to 25%) attenuated, but did not block, the endomorphin-induced antinociception. These data demonstrate that the adrenergic pathway is more important than the serotonergic pathway in mediating the antinociception activated by supraspinally administered endomorphins.

In the same study the antinociception induced by spinally administered endomorphins was blocked by i.t. pretreatment with the μ-receptor antagonists, but not with 6-OHDA or 5,7-DHT (Ohsawa et al., 2001; Hung et
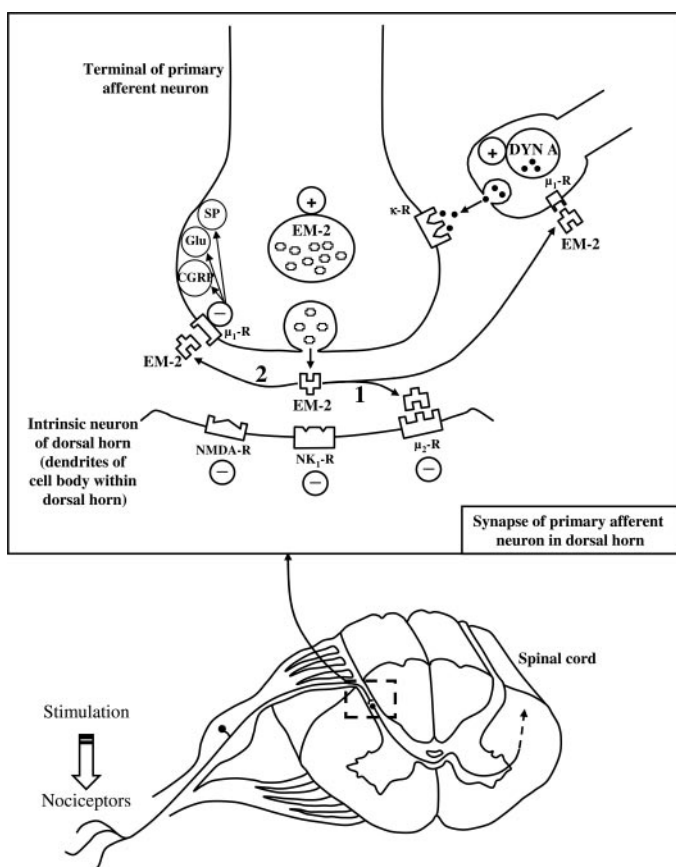
FIG. 4. Hypothetical involvement of endogenous endomorphin-2 (EM-2) in the earliest stage of pain sensation, transmission, and modulation. 1, regulatory function. Decrease of excitability of postsynaptic μ-opioid receptors. 2, autoregulatory function. Inhibition of the release of excitatory transmitters [calcitonin gene-related peptide (CGRP), glutamate (Glu), substance P (SP)]. DYN A, dynorphin A.

FIG. 5. Simplified representation of pain modulatory pathways activated by supraspinally and spinally administered endomorphin-1 and endomorphin-2. DYN A, dynorphin A; MET-ENK, Met-enkephalin. Adapted from Tseng (2002).

al., 2003). These observations clearly indicated that direct stimulation of the μ-opioid receptors, located in the dorsal horn of the spinal cord, mediates the antinociception induced by spinally administered endomorphins, without the involvement of NA or 5-HT transmission.

Supraspinal endomorphin-2- but not endomorphin-1-induced tail-flick inhibition was blocked by i.c.v. or i.t. pretreatment with an antiserum against dynorphin A(1-17) or norbinaltorphimine. Similarly, it was blocked by i.t. pretreatment with an antiserum against Met-enkephalin or naltriben (Tseng et al., 2000). These findings indicated that the supraspinal endomorphin-2-induced antinociception also involved additional components, corresponding to the release of dynorphin A(1-17), as well as Met-enkephalin, acting on the κ- and δ_2-opioid receptors, respectively. This accounts for the differences in the antinociceptive effects between endomorphin-1 and endomorphin-2 (Fig. 4).

b. Peripheral administration of endomorphins. Several studies have shown that peripherally administered opioids produce analgesic effects mediated through peripheral μ- and κ-opioid receptors located on primary afferent neurons (Craft et al., 1995; Kolesnikov and Pasternak, 1999; Nozaki-Taguchi and Yaksh, 1999). How-

ever, detailed pathways and exact mechanisms, through which peripherally administered opioids produce antinociception, have not been elucidated.

As for endomorphins, there is only one report on their analgesic effect after peripheral administration. Li et al. (2001) used various nociceptive tests to demonstrate that endomorphin-1 produced a dose-dependent, naloxone-reversible analgesia after i.p. administration in rats. The peak analgesic effect appeared later in the time course and was less pronounced than that induced by centrally (i.c.v. and i.t.) administered peptide. Because it is generally believed that peripherally administered endomorphins, because of their rapid enzymatic degradation in peripheral tissues and low permeation through the brain-blood barrier, cannot reach the CNS in an amount sufficient to elicit analgesia (Hau et al., 2002; Spampinato et al., 2003), these observations need to be further investigated. The question whether the analgesic effect resulting from this route of administration has a peripheral or a central origin needs to be answered as well.

2. Tolerance. The development of tolerance, as well as physical dependence limits the clinical use of the opioids as pharmacological agents. Numerous studies have shown that the μ-opioid receptor ligands are responsible for the emergence of both phenomena (Schiller et al., 1999; Shen et al., 2000; Spreekmeester and Roch-

ford, 2000). Recently, much emphasis has been put on the μ-opioid receptor-mediated intracellular signal transduction mechanisms and long-lasting molecular and cellular adaptations after chronic treatment with the μ-opioid receptor ligands (Defer et al., 2000; Heyne et al., 2000; Law et al., 2000). The ability of endomorphins, being potent μ-opioid receptor-selective agonists, to develop tolerance and dependence has also been investigated.

As it was shown in the in vitro assays, acute treatment with or chronic administration of endomorphins may stimulate the development of tolerance (Higashida et al., 1998; McConalogue et al., 1999). Nevo et al. (2000) demonstrated that forskolin-stimulated adenylyl cyclase activity was elevated above control levels after naloxone in μ-opioid receptor-expressing Chinese hamster ovary cells chronically treated with endomorphin-1 and endomorphin-2. Similarly, chronic exposure to endomorphins stimulated adenylyl cyclase activity in μ-opioid receptor-expressing African green monkey kidney cells cotransfected with type I and V isozymes (Nevo et al., 2000).

In vivo investigations also showed that pretreatment with endomorphins may develop tolerance. First, it was demonstrated that i.t. pretreatment with endomorphin-1 and endomorphin-2 attenuated the antinociceptive response in mice induced by i.t. administration of endomorphin-1 and endomorphin-2, respectively, and developed acute tolerance to endomorphins (Stone et al., 1997; Higashida et al., 1998; Horvath et al., 1999). Further studies showed that i.c.v. or i.t. administration in mice and rats of a single high dose of endomorphin-1 or endomorphin-2 induced an acute antinociceptive tolerance to the subsequent challenging dose of i.c.v. or i.t. administered endomorphin-1 or endomorphin-2, respectively (Wu et al., 2001, 2003; Hung et al., 2002; Labuz et al., 2002). Interestingly, endomorphins induced the development of tolerance much faster than morphine, although endomorphin-1 required a longer pretreatment time than endomorphin-2 before the acute antinociceptive tolerance was observed. It was proposed that these diverse effects might result from different peptide half-lives or differences in the μ-opioid receptor selectivity or divergent neuronal mechanisms and not from the degree of receptor stimulation by endomorphins or differences in the opioid receptor desensitization (Wu et al., 2001).

Labuz et al. (2002) reported on results obtained in a cross-tolerance study, in which the antinociceptive effects of endomorphins and morphine were compared in tail-flick and paw pressure tests in rats. The animals made tolerant to endomorphin-2 exhibited a partial antinociceptive cross-tolerance to endomorphin-1, whereas rats tolerant to endomorphin-1 showed no cross-tolerance to endomorphin-2. The study also described the cross-tolerance between morphine and endomorphin-1, suggesting a common target, which was probably the μ_2-opioid receptor subtype. Because no cross-tolerance was observed between morphine and endomorphin-2, it

was suggested that endomorphin-2 acts via another μ-opioid receptor subtype, apparently μ_1, which could also be a different splice variant or a physical state of the μ-opioid receptor.

As discussed above, so far only animal models of pain have been used to characterize the influence of endomorphins on the development of tolerance. However, tolerance to other endomorphin effects needs to be elucidated. This study might be particularly interesting, as tolerance did not develop to all of the effects of morphine: for example, it did not occur to morphine-induced locomotor activity, which is linked to the physical dependence (Spanagel et al., 1998).

3. Physical Dependence. Chronic administration of opioids usually results in physical dependence, as measured in terms of the appearance of withdrawal symptoms after cessation of the drug or when an opioid antagonist is administered. In animals, the opioid withdrawal symptoms include abnormal posture (Pineda et al., 1998), diarrhea (Miranda and Pinardi, 1998; Pineda et al., 1998), hypothermia (Thornton and Smith, 1998), changes in blood pressure (Zhang and Buccafusco, 1998), and several others (for reviews, see Vaccarino et al., 1999; Vaccarino and Kastin, 2000, 2001). In some cases single, but not chronic, administration of opioids is necessary for the development of dependence (Kest et al., 1998). The μ-opioid receptor ligands are regarded as being mainly responsible for the development of physical dependence and drug addiction (Reisine and Pasternak, 1996; Rockhold et al., 2000).

McConalogue et al. (1999) demonstrated that endomorphins specifically activated the μ-opioid receptor and induced its endocytosis in cells transfected with the μ-opioid receptor cDNA, as well as in the enteric neurons that naturally express the μ-opioid binding sites. Endomorphin-induced endocytosis and trafficking of the μ-opioid receptor may mediate receptor desensitization, resensitization and down-regulation, mechanisms that regulate cellular responsiveness to ligand stimulation and that might be important in the development of opioid tolerance and addiction (Bohm et al., 1997).

To examine the possible effects of endomorphins on physical dependence in vivo, Chen et al. (2003) investigated the ability of both peptides to induce naloxone-precipitated withdrawal in rats. Using a previously established scoring system, 12 withdrawal signs (chewing, sniffing, grooming, wet-dog shakes, stretching, yawning, rearing, jumping, teeth grinding, ptosis, diarrhea, and penile erection) were observed and scored after a naloxone (4 mg/kg i.p.) challenge. Endomorphins (20 μg i.c.v., b.i.d. for 5 days) were shown to induce physical dependence, but they displayed different potency for certain signs. The severity of the endomorphin-induced withdrawal was similar to that induced by the same dose of morphine.

Unfortunately, no data on the interactions between endomorphins and the nonopioid systems are available,

although dopamine (DA) (El-Kadi and Sharif, 1998; Samini et al., 2000; Tokuyama et al., 2000), NA (Milanes et al., 1998; Fuentealba et al., 2000; Fuertes et al., 2000; Laorden et al., 2000), ACh (Zhang and Buccafusco, 1998; Buccafusco et al., 2000), benzodiazepine (Tejwani et al., 1998), N-methyl-D-aspartate (Popik et al., 1998), imidazoline (Su et al., 2000), Glu (Rockhold et al., 2000), and GABAergic (Sayin et al., 1998) pathways were suggested to play an important role in the development of physical dependence. Nitric oxide (NO) may also be involved in endomorphin-induced dependence, both directly, as systemic injections of NO synthase inhibitors attenuated some signs of naloxone-precipitated withdrawal and hyperactivity of the LC (Pineda et al., 1998), and indirectly, as the i.t. infusion of morphine increased N-methyl-D-aspartate binding activity and up-regulated neuronal NO synthase expression (Wong et al., 2000).

4. Effects on Locomotor Activity. The effects of opioid receptor ligands on locomotor activity are influenced by a number of variables, including the dose and the paradigm used in the experiment. However, in most studies the μ- and δ-opioid receptor ligands stimulated locomotor activity by increasing the synthesis and the release of DA from dopaminergic neurons in the nigrostriatal and the mesolimbic dopaminergic system (Chesselet et al., 1981; Urwyler and Tabakoff, 1981; Broderick, 1985; Locke and Holtzman, 1986; Cunningham and Kelley, 1992). In contrast, κ-opioid receptor agonists were shown to decrease both vertical and horizontal locomotion (Kuzmin et al., 2000).

The opioid alkaloid morphine, a μ-opioid receptor ligand with low affinities to δ- and κ-opioid receptors, was shown to increase locomotion under most conditions (Latimer et al., 1987; Austin and Kalivas, 1990; Calenco-Choukroun et al., 1991; Aguilar et al., 1998; Kimmel et al., 1998; Schildein et al., 1998; Stinus et al., 1998), but in some cases had no effect (Waddell and Holtzman, 1998). Acute injections of morphine stimulated horizontal locomotion through the μ-opioid receptors and grooming through the δ-opioid binding sites, localized in the VTA, Nac, and PAG (Babbini and Davis, 1972; Joyce and Iversen, 1979; Katz, 1979; Havemann et al., 1983; Morgan et al., 1998; Schildein et al., 1998). A single administration of morphine was shown to increase behavioral sensitivity to the DA agonist, apomorphine (de la Baume et al., 1979), whereas chronic treatment resulted in the development of supersensitive DA receptors, as determined by induction of the stereotypic behaviors by the DA agonists (Ritzmann et al., 1979). To elucidate the relationship between morphine and DA, Jang et al. (2001) compared the effect of morphine on the modulation of the apomorphine-induced climbing behavior in wild-type and μ-receptor knockout mice. Treatment with morphine potentiated apomorphine-induced climbing behavior in wild-type mice, whereas it did not produce any significant effect in the μ-receptor knockout mice. These results indicated that μ-receptors play an important role in potentiation of the climbing behavior induced by DA receptor agonists (Jang et al., 2000) and proved that μ-receptor ligands influence the dopaminergic system.

Effects of centrally administered endomorphin-1 and endomorphin-2 on locomotor activity were evaluated and seem confusing. In some studies endomorphins, like morphine, were shown to increase horizontal and vertical activity (i.e., hyperlocomotion) and not to alter grooming activity (Bujdoso et al., 2001, 2003). Interestingly, the concentration-response curves for endomorphin-1 and endomorphin-2 in mice were of a bell-shaped type (Bujdoso et al., 2001). However, relatively lower concentrations of endomorphin-2 than of endomorphin-1 were used to obtain the same response (Bujdoso et al., 2001), suggesting differences in activation of the μ-opioid receptor subtypes (Sakurada et al., 1999, 2000) or the involvement of the enkephalinergic or dynorphinergic system (Sanchez-Blazquez et al., 1999; Tseng et al., 2000).

In contrast, some studies showed that endomorphins did not influence locomotor activity. Our group (Fichna et al., 2007) investigated the effect of i.c.v. administration of endomorphin-1 and endomorphin-2 on locomotor activity in mice. The peptides did not modify horizontal locomotor activity in any of the time periods of the test. Furthermore, endomorphin-1, at the highest dose (30 μg/mouse), and endomorphin-2, at the lowest doses (0.3 and 1 μg/animal), produced weak inhibition of vertical locomotor activity.

Our results are consistent with those obtained by Mehta et al. (2001), who examined the influence of endomorphins on the activity of basal ganglia, specific subcortical brain structures that play an important role in the control of movement (Delfs et al., 1994; Peckys and Landwehrmeyer, 1999). Dysfunction of the basal ganglia, resulting from specific degeneration of neurons, as in Parkinson's and Huntington's diseases, or from the administration of pharmacological agents, leads to severe motor disorders (Albin et al., 1991; Chesselet and Delfs, 1996). Within the basal ganglia, a subpopulation of neurons of the globus pallidus expresses particularly high levels of μ-opioid receptor mRNA (Delfs et al., 1994; Obeso et al., 2000). Morphine injections into the globus pallidus produced a robust increase in locomotor activity (Anagnostakis et al., 1992), whereas endomorphin-1 induced orofacial dyskinesia (Mehta et al., 2001). The authors of that report suggested that stimulation of the locomotor activity by morphine could be mediated by δ- and κ-opioid receptors and the inhibitory activity of endomorphin-1 might involve μ-opioid receptors. Previous studies showed that stimulation of GABA receptors induced catalepsy in rats (Egan et al., 1995). Endomorphin-1 and GABA could therefore induce opposite behavioral effects in the globus pallidus and alterations in their equilibrium could play a crucial role in control of movement and development of dyskinesia.

5. Behavioral Sensitization. Repeated administration of psychoactive drugs can lead either to a decrease (tolerance) or an increase (sensitization) in their behavioral effects. The term "behavioral sensitization" was first used to describe the augmented motoric stimulant effect produced by a given dose of a psychomotor-stimulant drug, such as amphetamine or cocaine, after repeated intermittent injections (Segal and Kuczenski, 1997). This phenomenon could persist for a long period after drug abstinence (Robinson and Becker, 1986). The term is now more readily used for the phenomenon of increased behavioral and neurochemical responsiveness of any type to the administration of the same or lower doses of drug after repeated drug injections (Spyraki et al., 1983).

Behavioral sensitization plays an important role in the development and maintenance of drug addiction (Gaiardi et al., 1991; Hunt and Lands, 1992; Robinson and Berridge, 1993); simple "preference" for a drug becomes "sensitized" (i.e., increases) up to the point where the urge to take the drug becomes overwhelming (i.e., loss of behavioral control or drug craving) (Wise and Bozarth, 1987; Robinson and Berridge, 1993). Virtually all human drugs of abuse that show positive results in animal models of reward and addiction produce behavioral sensitization (Stewart and Badiani, 1993).

In the search for neuroanatomical and neurochemical bases of drug-induced sensitization, research has focused on the mesolimbocortical dopaminergic pathway, which arises in the VTA and projects mainly to the Nac (Spanagel, 1995). There are several transmitter systems in the VTA known to evoke behavioral sensitization, including DA (Hooks et al., 1993, Hooks and Kalivas, 1994'02; Vezina, 1996), GABA (Johnson and North, 1992), Glu/aspartate (Karler et al., 1990), and tonically active endogenous opioid systems (Spanagel et al., 1992).

In vivo DA release measurements (microdialysis) clearly demonstrated that systemic administration of μ-opioid receptor agonists increases DA release in the Nac (Di Chiara and Imperato, 1988a,b; Spanagel et al., 1990; Pentney and Gratton, 1991). DAMGO and morphine increased DA release in the Nac after injection into the VTA (Leone et al., 1991; Longoni et al., 1991; Spanagel et al., 1992; Devine et al., 1993), whereas D-Phe-c(Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH$_2$) (CTOP)], a highly specific μ-opioid receptor antagonist, produced a significant decrease of DA release in the Nac (Spanagel et al., 1992). In contrast, infusion of either ligand into the Nac was without any effect.

Chen et al. (2001) reported that repeated administration of endomorphins in the VTA has a significant effect on the development of sensitization to amphetamine in rats. To understand the neural basis of the behavioral outcome of the chronic endomorphin treatment, the tissue contents of several neurotransmitters and their metabolites in the limbic forebrain (ventral striatum and medial prefrontal cortex) and extrapyramidal area (dor-

sal striatum) of rats treated with amphetamine or endomorphin were analyzed. It has been demonstrated that Glu concentrations increased significantly in all tested brain areas in both groups of animals. It was suggested that μ-opioid receptor agonists could activate dopaminergic neurons through the activation of GABA or Glu neurons in the VTA (Karler et al., 1990, Wolf and Xue, 1998; Johnson and North, 1992).

6. Drug Addiction, Mechanism of Reward. Numerous studies, in which μ-selective agonists and antagonists were used, showed that the μ-opioid receptor is the primary factor in the development of drug addiction because of its central role in the mediation of reward (Negus et al., 1993, Devine and Wise, 1994; Pieppponen et al., 1997). μ-Opioid receptor ligands, including DAMGO, morphine, codeine, and sufentanyl, elicited strong rewarding effects in several brain structures, such as the VTA or Nac (for review, see Wise, 1989; Suzuki, 1996; Martin-Schild et al., 1999), whereas selective κ-opioid receptor agonists produced significant aversion (Becker et al., 2000). The μ-opioid receptor agonists were also shown to mediate the reward effects induced by stress (Zacharko et al., 1998).

It is generally assumed that the critical brain region for μ-opioid effects on motivation is the VTA. The rewarding effects of μ-opioid injections into the VTA are mediated by the μ-opioid receptors expressed on the GABA-containing cells. μ-Opioid receptor agonists inhibit GABAergic inputs to the dopaminergic VTA principal cells, projecting to the Nac, and disinhibit release of DA in the Nac, a major component of the endogenous reward circuitry of the brain (Johnson and North, 1992; Margolis et al., 2003).

Similarly to morphine and DAMGO, endomorphins injected into the posterior VTA established a conditioned place preference and produced significant psychomotor stimulating effects (Zangen et al., 2002). Endomorphin-1 injected into the posterior VTA gave also strong rewarding effects in the self-administration paradigm. Injection of endomorphin-1 and DAMGO into the Nac gave no rewarding effect (Zangen et al., 2002). The effect of DAMGO was not only weak but also generally delayed, which suggests the lack of sites of action for μ-opioid agonists in the Nac (Churchill and Kalivas, 1992; Johnson et al., 1996; Churchill et al., 1998).

The possible rewarding effect of i.c.v.-administered endomorphins has also been investigated in different species; however, the results obtained were inconsistent. Narita et al. (2001a,b) reported that endomorphin-1 produced a significant place preference and endomorphin-2 produced a significant place aversion in mice, which were inhibited by pretreatment with either an antiserum against the endogenous κ-opioid receptor agonist dynorphin A(1-17) or coadministration with a κ-opioid receptor antagonist (Wu et al., 2004). The authors suggested that endomorphins stimulate different subtypes of the μ-opioid receptor and that endomorphin-2 could

additionally stimulate the release of dynorphins, which are responsible for its aversive effect (Fig. 6).

In rats no significant rewarding effect of endomorphins, even at the doses producing significant antinociception, on conditioned place preference was found (Wilson et al., 2000). The dose-effect relationship studies revealed that both endomorphins, at a lower dose (15 μg), had no effect on the conditioned place preference (Huang et al., 2004). The animals treated with endo-morphin-1 at a higher dose (30 μg) showed severe barrel rotation of the body trunk, whereas endomorphin-2 induced a significant place preference. The discrepancy between the results obtained in rats and mice might be explained by the pharmacogenetic differences between animals, that is, the differential expression of target sites, as well as species differences in the endomorphin-modulated rewarding. As suggested by Wilson et al. (2000), the length of conditioning trials in the conditioned place preference test might also have been a critical factor for the absence of the rewarding effect in rats.

Recently, an important role of the μ-opioid receptors located in the PAG in morphine-induced aversion has been suggested (Sante et al., 2000). Moreover, an integrative model of addiction, which emphasized the relationship between chronic opioid administration, disrup-

tion of the hypothalamo-pituitary-adrenal (HPA) axis, and dysregulation of brain reward systems, has been proposed (Kreek and Koob, 1998). However, the possible involvement of endomorphins in both systems has not been yet characterized.

7. *Psychiatric disorders.*

a. Stress. So far, the precise role of endogenous opioid peptides and opioid receptors in the response to stress stimuli has not been fully elucidated. However, many stressors were shown to interact with the endogenous opioid system (Baumann et al., 2000; Huang et al., 2000; Jamurtas et al., 2000; LaBuda et al., 2000; Matsuzawa et al., 2000; Spreekmeester and Rochford, 2000; Takahashi et al., 2000; Van den Berg et al., 2000), and therefore a close relationship between the level of the opioid receptor ligands, corticosteroids, and related hormones has been proposed.

The HPA axis seems to play the major role in the stress response. The activation of the HPA axis by different stimuli (stress, immune challenge, and others) causes increased secretion of corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) from the median eminence of the hypothalamus. In turn, these neuropeptides stimulate the secretion of adrenocorticotropin (ACTH) from the anterior lobe of the pituitary (Coventry et al., 2001). Elevated levels of ACTH stimulate the production and release of glucocorticoids from the adrenal glands into the circulation, which then exert negative feedback actions on the pituitary and other target areas in the CNS, thus regulating the HPA axis activation (Harbuz and Lightman, 1989).

The role of the μ-opioid receptor ligands in the control of the HPA axis remains ambiguous. Some authors claim that the stress-responsive HPA axis is under tonic inhibition via endogenous β-endorphin and the μ-opioid receptors in the hypothalamus (Nikolarakis et al., 1987; Kreek et al., 2002). In contrast, it has been demonstrated in rat that acute administration of morphine increased ACTH and corticosterone levels in plasma (Ignar and Kuhn, 1990) and that morphine acts primarily through μ-receptors to activate the HPA axis (Mellon and Bayer, 1998).

Centrally administered endomorphins did not stimulate the HPA axis and had no effect on corticosterone release, although it was injected at a dose of 10 μg, which is sufficient to activate other physiological systems (Coventry et al., 2001). In addition, endomorphin-1 failed to block the stimulatory effects of morphine on the plasma corticosterone level. Furthermore, activation of the HPA axis did not influence the plasma level of endomorphins in rats exposed to the chronic inflammatory stress of adjuvant-induced arthritis, the psychological stress of restraint, and the immunological stress of lipopolysaccharide, although plasma corticosterone, ACTH, and β-endorphin increased. These results suggested that endomorphins do not play an important role in mediating the stress response or regulating the HPA
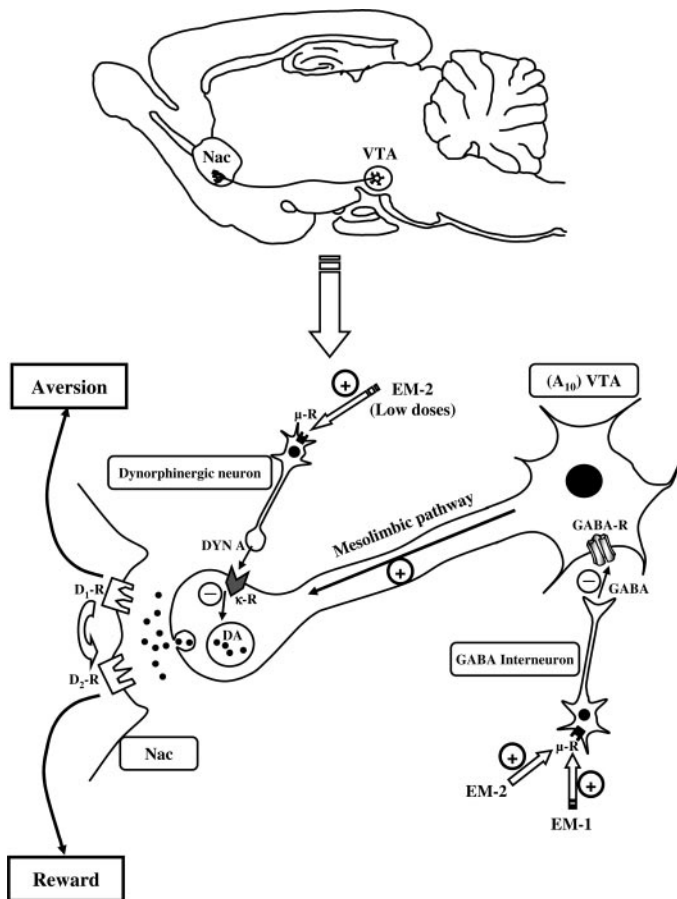
FIG. 6. Different motivational effects of endomorphin-1 (EM-1) and endomorphin-2 (EM-2). DYN A, dynorphin A. Adapted from Narita et al. (2002).

axis. If activation of the HPA axis is indeed mediated by μ-opioid receptors, the lack of an endomorphin-induced effect might be due to the differences in diffusion and metabolism of these peptides, compared with morphine. Another possible explanation is that the μ-receptor agonists have different patterns for the stimulation of the G-proteins coupled to the μ-binding sites, as was shown in animal models of pain (Sanchez-Blazquez and Garzon, 1988; Sanchez-Blazquez et al., 1999).

In contrast, subsequent studies showed a close relationship between endomorphins and the HPA axis. Champion et al. (1999a) provided strong evidence that the release of the gaseous neurotransmitter, NO, which plays an important role in mediation of the physiological actions of morphine (Granados-Soto et al., 1997) and other opioids (Gholami et al., 2002), is responsible for the vasodilatory action of the endomorphins. Bujdoso et al. (2003) suggested that a much broader range of endomorphin actions is mediated by NO, including activation of the HPA system, and introduced the term of "endomorphin-NO-HPA axis" (Calignano et al., 1993; Bujdoso et al., 2003) (Fig. 7).
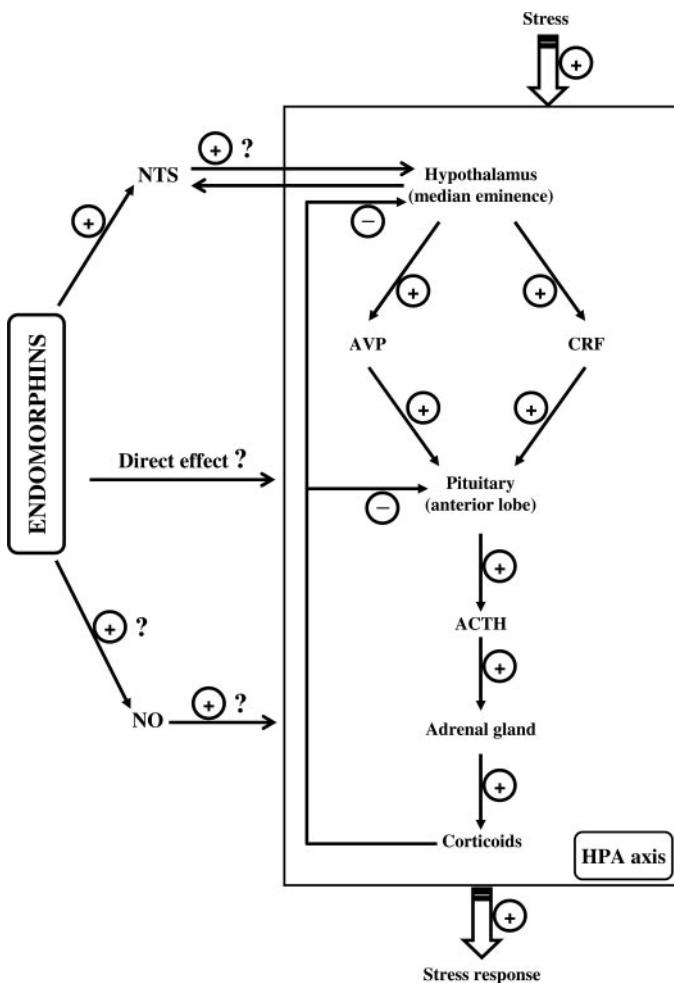
FIG. 7. Possible implications of endomorphins in the activation and the control of the HPA axis in the stress response.

Hui et al. (2006) demonstrated that the NTS, through endomorphin-containing ascending fibers, might exert modulatory functions in the hypothalamus, a critical element of the HPA axis (Ter Horst et al., 1989; Boscan et al., 2002). Likewise, the hypothalamus might directly influence the NTS via endomorphinergic descending fibers. Therefore, endomorphins might be involved in activation of the HPA axis and release of CRF, a major mediator of the stress response (Swanson, 1987; Saper, 1995).

The PAG is yet another brain structure likely to be implicated in the stress response, as it was shown to mediate stress-induced immobility and analgesia in adult rats and rat pups (Wiedenmayer and Barr, 2000). Experiments with the general opioid receptor antagonist naltrexone and the selective μ-opioid receptor antagonist CTOP demonstrated that the analgesic effect was produced by endogenous opioids that bind to μ-opioid receptors in the ventrolateral PAG. Perhaps endomorphins might also be involved in the PAG-mediated stress-induced analgesia.

b. Anxiety. There are several reports on the anxiolytic action of morphine and μ-opioid receptor agonists, injected both centrally (File and Rodgers, 1979; Fanselow et al., 1988; Costall et al., 1989; Motta et al., 1995) and peripherally (Millan and Duka, 1981; Koks et al., 1999; Zarrindast et al., 2005), and the anxiogenic effect produced by μ-opioid receptor antagonists (Tsuda et al., 1996). The effects of morphine are dose- and site-dependent: morphine injected into the midbrain tectum of rats at low doses produced anxiolytic-like effects, but at high doses displayed an anxiogenic-like activity (Brandao et al., 1999); when injected to the rat dorsal PAG (Nobre et al., 2000) and lateral septum (Le Merrer et al., 2006), morphine produced dose-dependent aversive effects.

The anxiolytic action of μ-opioid ligands is mediated by their interaction with the GABAergic system in some specific brain areas (Sasaki et al., 2002), the amygdala being one of these (Kang et al., 2000; Sasaki et al., 2002). Other studies on anxiety-related behavior in animals suggested the involvement of serotonergic, benzodiazepine, and neuropeptide receptors (for reviews, see Rodgers and Cole, 1993; Griebel, 1995, 1999; Menard and Treit, 1999).

A high degree of homology between the neuroanatomical distribution of endomorphins and the localization of μ-opioid receptors in the areas important for motivation, arousal, and vigilance, namely the paraventricular nucleus, septum, lateral hypothalamus, dorsomedial nucleus of the hypothalamus, LC, and amygdala (Stanley et al., 1988), suggests that they might be involved in the modulation and expression of anxiety- or stress-induced behaviors. The only report on endomorphin and anxiety, published by Asakawa et al. (1998), showed that i.c.v. administered endomorphin-1 increased the preference for the open arms of the elevated plus maze and pro-

duced an anxiolytic effect in mice. This observation is in good agreement with previous reports stating that μ-opioid receptor agonists produce drowsiness and feelings of warmth and well-being (Tsuda et al., 1996).

c. Depression and other psychiatric disorders. According to Vaccarino et al. (1999), interest in the possible role of opioid systems in modulation of mental illness and mood continues to decline. A likely contributing factor is the failure to demonstrate any clear global therapeutic benefit from treatment with opioid ligands, thus obscuring the role of endogenous opioid systems in mediation of mental illnesses. However, the available data clearly demonstrate that the μ-opioidergic system is considerably involved in the etiology of mental disorders, thus providing a rationale for the use of μ-opioid ligands in behavioral therapies.

High concentrations of μ-opioid receptors and their endogenous ligands were observed in the limbic areas involved in regulation of mood and stress responses (Belenky and Holaday, 1979; Waksman et al., 1986; Mansour et al., 1988; Bodnar and Klein, 2004). Moreover, μ-opioid receptor knockout mice were shown to have an altered emotional state, consistent with depressed mood (Filliol et al., 2000). Clinical studies revealed that there is an increased level of β-endorphin and μ-opioid receptors in plasma and brain of the patients suffering from depression and schizophrenia (Lindstrom et al., 1978; Gross-Isseroff et al., 1990; Scarone et al., 1990; Darko et al., 1992; Gabilondo et al., 1995).

However, no differences in β-endorphin plasma levels between manic, depressed, or neurotic patients and healthy volunteers were found (Emrich et al., 1979). Therefore, the theory, based on the observation that opiates exert euphorogenic effects in healthy volunteers (Byck, 1976; Kline et al., 1977), suggesting that the endogenous depression would represent a state of dysfunction of the opioidergic system (e.g., in limbic structures), whereas mania would be considered as a state of opioidergic hyperactivity (Byck, 1976; Belluzzi and Stein, 1977), is no longer in use.

Numerous reports showed that a wide variety of μ-opioid receptor agonists may act as antidepressant agents (Kraepelin, 1905; Kline et al., 1977; Kastin et al., 1978; Mansour et al., 1988; Darko et al., 1992; Bodkin et al., 1995; Tejedor-Real et al., 1995; Besson et al., 1996; Stoll and Rueter, 1999; Makino et al., 2000a,b; Vilpoux et al., 2002). Since the work of Kraepelin (1905), opium has been recommended for the treatment of depressed patients. Kline et al. (1977) performed clinical trials on patients with different types of psychiatric disorders (schizophrenia, depression, and neuroses) and observed an antidepressant effect of β-endorphin. The μ-opioid receptor agonists, oxycodone and oxymorphone, administered chronically for several months, were shown to act as antidepressants without causing opioid tolerance or dependence (Stoll and Rueter, 1999). Similarly, mor-

phine produced a significant antidepressant-like effect in the forced swimming test after s.c. injections (Eschalier et al., 1987). Naloxone, a universal opioid antagonist, was shown to reverse the effect of anticonvulsive therapy in depressed patients, one of the most effective treatments in severe depression (Belenky and Holaday, 1979). However, treatment with naloxone administered alone produced neither a significant change in healthy volunteers (Grevert and Goldstein, 1978) nor an effect on manic or depressive disorders (for review, see Emrich, 1982).

In view of the fact that endomorphins and the μ-opioid receptors are colocalized in the brain regions involved in the physiopathology of depression, such as the limbic system (the septum, Nac, and amygdala), the thalamic nuclei, LC, and some regions of the brainstem (Schreff et al., 1998; Martin-Schild et al., 1999; Zadina, 2002), it was suggested that these peptides could play an important role in the etiology of depressive disorders. However, although various studies associated the μ-opioidergic system and the antidepressant actions, it was only recently that the antidepressant effect of endomorphins was clearly verified.

In our study (Fichna et al., 2007), endomorphin-1 and endomorphin-2 (0.3–30 μg/animal i.c.v.) produced significant antidepressant-like activity in the forced swimming test (FST) and the tail suspension test in mice. These effects were dose-dependent and short-lasting; a significant response was observed only 10 and 15 min after i.c.v. administration. The magnitude of the effect induced by endomorphins was comparable to that observed after the treatment with well-known antidepressants and the compounds with potential antidepressant-like activity (Cryan et al., 2005; Petit-Demouliere et al., 2005). In contrast with the effect of other μ-opioid receptor agonists (Babbini and Davis, 1972), the antidepressant-like effect of endomorphins did not result from stimulation of animal motor activity: the peptides did not increase the horizontal locomotor activity evaluated in a new environment. We have even observed a weak tendency to decrease vertical movements. This result is in good agreement with the classic observations that antidepressants reduce immobility in the forced swimming test at doses that do not cause stimulation of locomotion (Porsolt et al., 1977) or even tend to decrease locomotor activity (Duterte-Boucher et al., 1988; Bourin, 1990).

We also demonstrated that both naloxone, a reference opioid receptor antagonist with a relatively high affinity toward μ-opioid receptors, and β-funaltrexamine, a selective μ-opioid receptor antagonist, significantly antagonized the antidepressant-like effect of endomorphins. In contrast, neither naltrindole, a selective δ-opioid receptor antagonist, nor nor-binaltorphimine, a selective κ-opioid receptor antagonist, was able to block the antidepressant-like effect of endomorphins. These results

indicated that the effect of endomorphins is mediated, in great part, through central μ-opioid receptors.

There is one more recent study on the possible involvement of endomorphins in the pathophysiology of depression. Zhang et al. (2006) examined the effects of i.c.v. administered endomorphins, at doses active in other behavioral studies (30 and 90 nmol/animal, i.e., ~20 and 50 μg/animal), on animal behavior in the FST and on brain-derived neurotrophic factor (BDNF) gene expression in rats. BDNF, a neurotrophic factor from the nerve growth factor family, regulates neuronal survival, differentiation, and plasticity (Binder and Scharfman, 2004). Several lines of evidence suggest that the BDNF plays an important role in the pathophysiology of depression and in the mechanism of therapeutic actions of antidepressants (Siuciak et al., 1997; D'Sa and Duman, 2002; Shirayama et al., 2002; Shimizu et al., 2003; Castren, 2004; Hashimoto et al., 2004). Studies revealed that endomorphins do not influence the time of the animal immobility in the FST in rats. However, centrally administered endomorphins significantly increased BDNF gene expression in the frontal cortex, hippocampus, and amygdala in a dose-dependent manner. Moreover, the opioid receptor antagonist naltrexone completely blocked the increase in BDNF mRNA expression produced by endomorphin-1 in all brain regions and blocked the effect of endomorphin-2 in the frontal cortex. In contrast, the δ-opioid receptor antagonist naltrindole showed little or no antagonist effect against both peptides. Taken together, these results show that endomorphins produced contradictory effects: they were shown to up-regulate BDNF mRNA expression in the rat brain through the activation of the central μ-opioid receptor and to lack antidepressant-like behavioral effects.

Both this inconsistency and the disagreement with our results may be easily explained by observation of the time course of endomorphin effects. Zhang et al. (2006) performed the FST 30 min after central administration of endomorphins, which is far too late. As was shown in our report, endomorphins, because of rapid enzymatic degradation, produced a significant antidepressant effect only 10 to 15 min after i.c.v. administration. For this reason, no antidepressant activity was observed beyond that period of time.

8. Social Defeat.   In a number of animal models of social defeat, the subordinate animal shows signs of stress as indicated by physiological, neuroendocrine, neurochemical, and behavioral changes (Martinez et al., 1998). Physiological changes after defeat include increases in blood pressure and heart rate (Bohus et al., 1990; Meehan et al., 1995), as well as activation of the HPA axis (Miczek et al., 1991; Blachard et al., 1993). Behaviorally, defeat leads to decreases in activity (Raab et al., 1986; Kudryavtseva et al., 1991) and social interaction (Van de Poll et al., 1982; Kudryavtseva, 1994; Avgustinovich et al., 1996), as well as aggression (Potegal et al., 1993; Albonetti and Farabollini, 1994; Avgusti-

novich et al., 1996), and increases in defense (Andrade et al., 1989; Potegal et al., 1993), anxiety (Andrade et al., 1989; Heinrichs et al., 1992), and drug seeking (Haney et al., 1995). These behavioral changes are thought to be related to fear, anxiety, depression, and panic (Blanchard et al., 1998a,b).

The μ-opioid agonists might play a critical role in the behavioral response to defeat. It was demonstrated that μ-opioid receptor up-regulation in the VTA occurs after social defeat (Nikulina et al., 1999), and defeat-induced ultrasonic vocalizations are decreased by μ-receptor agonists (Vivian and Miczek, 1998). Whitten et al. (2001) tested the hypothesis whether endomorphin-1 inhibits the expression and/or consolidation of conditioned defeat in Syrian hamsters. Intracerebroventricular administration of endomorphin-1 reduced the expression of conditioned defeat without stimulating locomotor activity or inducing sedation. The following mechanisms of action were suggested: 1) inhibition of memory retrieval, which was observed for other μ-agonists, such as Tyr-D-Arg-Phe-β-Ala (Ukai et al., 1995a) and morphine (Saha et al., 1991); 2) modulation of normal animal response to fear or anxiety (Asakawa et al., 1998), similar to that elicited by morphine and DAMGO, which exert anxiolytic activity in rats (File and Rodgers, 1979; Motta and Brandao, 1993; Motta et al., 1995; Koks et al., 1999); and 3) inhibition of noradrenergic neurons, because an opposite effect was observed for the μ-receptor antagonist naloxone (Introini and Baratti, 1986). Intracerebroventricular administration of endomorphin-1 failed to inhibit the consolidation of conditioned defeat, whereas morphine was shown to impair the consolidation of newly acquired memories in rats and mice (Izquierdo, 1979; Introini et al., 1985; Castellano et al., 1994; Cestria and Castellano, 1997; Rudy et al., 1999). Again three possibilities were proposed: 1) the endomorphin-1 dose used was too low; 2) the time of exposure to endomorphin-1 was not long enough to develop consolidation; and 3) the interaction with the μ-receptor could result in secondary messenger cascade different from that activated by morphine.

9. Food Intake.   Central mechanisms regulating food intake are complex and only a few peptides, including neuropeptide Y, growth hormone-releasing factor, orexin, and 26RFa were shown to possess orexigenic activity (Morley et al., 1983; Clark et al., 1984; Vaccarino et al., 1985; Inui et al., 1991; Sakurai et al., 1998; Chartrel et al., 2003).

The opioid receptor agonists display well-documented effects on feeding behavior. They tend to increase food intake, namely that of sucrose (Higgs and Cooper, 1998), other carbohydrates (Zhang et al., 1998), or fat (Higgs and Cooper, 1998; Zhang et al., 1998) in rodents exposed to different environmental conditions (Giraudo et al., 1998a,b; Itoh et al., 1998; Leventhal et al., 1998a,b; Zhang et al., 1998). The μ-opioid receptor agonists, which also exhibit orexigenic activity (Morley et al., 1982; Woods and Leibowitz, 1985; Gosnell et al., 1986;

Glass et al., 1999), modulate feeding behavior and gustatory information through μ-opioid receptors located in the hypothalamus (Pfeiffer and Herz, 1984; Kuhn and Windh, 1989) and NTS (Matsuo et al., 1984; Moufid-Bellancourt and Velley, 1994).

As observed for the μ-opioid receptor ligands, morphine and DAMGO, i.c.v. injection, in nonfood-deprived mice of either endomorphin-1 or endomorphin-2 (0.03–30 nmol) increased food intake in a dose-related manner for up to 4 h after injection (Asakawa et al., 1998). Similarly to the morphine metabolite, morphine-6β-glucuronide, the effect of endomorphins was dose dependently attenuated by a μ-opioid receptor antagonist, β-funaltrexamine, and not by δ- or κ-antagonists (Leventhal et al., 1998a,b). However, a κ-opioid receptor-selective agonist, dynorphin A, was more potent than endomorphins in stimulating food intake. These observations confirmed that the orexigenic activity of endomorphins is mediated by μ-opioid receptors and suggested that μ-opioid receptor agonists play a rather minor role in this phenomenon.

10. Sexual Behavior. There is an extensive evidence for the involvement of the endogenous opioid system in the regulation of pregnancy, parturition, and reproductive functions in female rats (Pfaus and Gorzalka, 1987a; Argiolas, 1999; Gilbert et al., 2000). Both direct and indirect actions of opioids on gonadal hormones were demonstrated: endogenous opioid peptides activate specific receptors within the interconnected limbic-hypothalamic reproductive behavior-regulating circuits to mediate facilitatory and inhibitory effects of sex steroids (Sinchak and Micevych, 2001). Moreover, the administration of opiates and opioid peptides influenced gonadotropin release (Bakker and Baum, 2000) and altered female rat sexual behavior (Wiesner and Moss, 1984, 1986; Sirinathsinghji, 1985, 1986; Pfaus et al., 1986; Forsberg et al., 1987; Vathy et al., 1991).

The influence of μ-opioid receptor-selective ligands on female reproduction is complex. The immunocytochemical data showed that μ-opioid receptors are widely distributed in some areas involved in female reproductive behavior, such as the ventromedial hypothalamus (VMH), the medial preoptic area (mPOA), and the mesencephalic central gray (MCG) (Pfaff et al., 1994; Martin-Schild et al., 1999). Concurrently, μ-opioid receptor-selective ligands were shown to possess a dual effect on lordosis, a hormone-related behavior displayed by hormonally primed female rodents during mating (Pfaff et al., 1994), depending on the concentration used and the route of administration (Wiesner and Moss, 1984; Sirinathsinghji, 1986; Pfaus and Gorzalka, 1987a,b; Vathy et al., 1991; Pfaff et al., 1994; Van Furth et al., 1995). For example, low doses of β-endorphin inhibited lordosis in gonadectomized, steroid-primed female rats when infused into the third ventricle (Wiesner and Moss, 1984, 1986), lateral ventricles (Pfaus et al., 1986; Pfaus and Gorzalka, 1987b), the MCG (Sirinathsinghji, 1984,

1985), or mPOA (Sirinathsinghji, 1986). On the contrary, β-endorphin and morphiceptin at high doses facilitated lordosis in estrogen- or estrogen- and progesterone primed rats after infusions into the third or lateral ventricles (Pfaus et al., 1986; Pfaus and Gorzalka, 1987b; Torii and Kubo, 1994; Torii et al., 1997, 1999).

Reports on the influence of endomorphins on the reproduction behavior are sparse. Sinchak and Micevych (2001) investigated the role of μ-opioid receptors and their ligands in the progestin-induced lordosis in nonreceptive female rats treated with estrogen. They showed that endomorphin-1, infused into the mPOA, inhibited lordosis through the activation of the μ-opioid receptors in the mPOA.

In another study, Acosta-Martinez and Etgen (2002) used endomorphins to investigate at which brain site(s) μ-opioid receptor activation affects lordosis behavior in hormonally primed female rats. Administration of endomorphin-1 and endomorphin-2 into the third ventricle resulted in a dose- and time-dependent, naloxone-reversible attenuation of lordosis behavior in rats. None of the tested peptides inhibited lordosis when infused into the VMH, mPOA, or MCG. Interestingly, endomorphins significantly inhibited lordosis behavior in animals, whose bilateral infusion cannulae were located at the ventral part of the medial septum-horizontal limb of the diagonal band (MS-HDB). Because both endomorphin IR and μ-opioid receptors were found in the MS-HDB (Loughlin et al., 1995; Martin-Schild et al., 1999) and the fibers from the MS-HDB were shown to innervate the mPOA and several hypothalamic nuclei (Jakab and Leranth, 1995), brain regions previously linked to the inhibition of lordosis by opiates, it was proposed that the MS-HDB is the major site of action of endomorphins on lordosis behavior. Moreover, because luteinizing hormone releasing hormone-, ACh-, and GABA-containing cells are present in the MS-HDB (for review, see Pfaff et al., 1994), it is possible that endomorphins could inhibit lordosis by modulating the release of any of these factors.

11. Learning and Memory. A variety of endogenous systems are considered to be involved in learning and memory. These include the opioid system, which has been demonstrated in operant and classic conditioning, as well as in various cognitive tasks to play an important role in the memory processes and memory storage. The μ-opioid receptor agonists, DAMGO and Tyr-D-Arg-Phe-β-Ala, and the δ-selective opioid receptor agonists [D-Pen^2,L-Pen^5]enkephalin and [D-Ala^2]deltorphin II, were demonstrated to impair short-term memory and passive avoidance learning, associated with long-term memory processes (Ukai et al., 1993b, 1997; Itoh et al., 1994). Activation of μ-opioid receptors in the septal nuclei has been reported to affect spatial memory (Bostock et al., 1988). The κ-agonist dynorphin A(1-13) reversed the disturbance of learning and memory in aversive and nonaversive tasks (Ukai et al., 1993a). The opioid recep-

tor antagonists enhanced memory retention (Ferry and McGaugh, 2000).

There is a growing body of evidence on the involvement of endomorphin-1 and endomorphin-2 in learning and memory. However, to the best of our knowledge, only a single report on the role of both peptides in short-term memory processes has been published so far. Ukai et al. (2000) demonstrated that endomorphins impair spontaneous alternation performance in mice, which is associated with short-term memory.

The effects of endomorphin-1 and endomorphin-2 on impairment of passive avoidance learning, based on the long-term memory processes, were also established. Ukai and Lin (2002a,b) demonstrated that both peptides, after i.c.v. administration, significantly shortened the step-down latency in the passive avoidance learning task in mice. Endomorphin-induced inhibitory activity was attenuated by the selective μ-opioid receptor antagonist, β-funaltrexamine, whereas the μ_1-receptor antagonist, naloxonazine, fully reversed the effect of endomorphin-1 but not that of endomorphin-2. These data demonstrated that endomorphin-1 impaired long-term memory through μ_1-opioid and endomorphin-2 through μ_2-opioid receptors.

Freeman and Young (2000) demonstrated that passive avoidance learning in chicks disrupted by endomorphin-2 may involve cytosolic and mitochondrial protein synthesis within the lobus paraolfactorius, which constitutes a structure homologous to the caudate putamen in mammals (Veenman et al., 1995; Metzger et al., 1996; Durstewitz et al., 1999). Endomorphin-2 was shown to reverse the amnesic effects of anisomycin and blocked cytosolic protein inhibition in this structure. However, Ukai et al. (2001) suggested that endomorphin-induced inhibition of passive avoidance learning resulted from functional disconnection of the hippocampus, which is believed to be important for converting new memories into long-term memories.

There could be at least two neurotransmitter systems, which could contribute to the endomorphin-induced impairment of long-term memories, namely cholinergic and dopaminergic, although their precise role has not been elucidated. For example, it was shown that both peptides significantly decreased ACh release in the brain areas associated with memory processes, and this effect was mediated by μ-opioid receptors (Ragozzino et al., 1994), which is in good agreement with the observation that spatial working memory involves an interaction between opioid and ACh systems (Kameyama et al., 1998). Moreover, endomorphin-induced impairment of passive avoidance learning was reversed by physostigmine, a classic cholinesterase inhibitor (Itoh et al., 1994; Ukai and Lin, 2002b). Alternatively, Ukai and Lin (2002a) demonstrated that the dopamine D_2-receptor antagonist attenuated endomorphin-2-induced impairment of passive avoidance learning and suggested that the inhibitory effect of endomorphin-2 resulted from the

stimulation of D_2-receptors, probably in the dopaminergic pathways projecting into the striatum or Nac during the process of acquisition and/or consolidation of memory. They also proposed that there is a similarity between endomorphin-2- and scopolamine-induced memory impairment, as the effects of both are mediated through the D_2-receptors.

Recently, centrally administered endomorphin-1 and -2 were shown to increase BDNF mRNA expressions in hippocampus and amygdala (Zhang et al., 2006). BDNF participates in synaptic plasticity mechanisms, such as long-term potentiation, learning, and memory (Huang and Reichardt, 2001; Malcangio and Lessmann, 2003). These observations may suggest a link between endomorphins, μ-opioid receptors, and BDNF in learning and memory.

12. *Effects on Cardiovascular System.* Many neuroanatomical regions within the brain, which regulate cardiovascular activity, contain opioid receptors and/or endogenous opioid peptides. These include the ventral lateral medulla, NTS, lateral hypothalamus, paraventricular nucleus (PVN), dorsal hippocampus, and integral parts of the limbic system. Endogenous opioid systems play an important role in mediating cardiovascular responses. However, the reported effects of opioid receptor ligands on blood pressure and heart rate are confusing. Direct injections of μ-, δ-, and κ-opioid receptor agonists into the PVN, dorsal hippocampus, and rostral ventrolateral medulla of normotensive and spontaneously hypertensive rats demonstrated that, in general, μ- and δ-agonists reduced heart rate and blood pressure (Sun et al., 1996). Intracerebroventricular administration of the μ-opioid receptor agonists, morphine, β-endorphin, and DAMGO, also induced hypotension in a variety of species (Holaday, 1983; Johnson et al., 1985; Unal et al., 1997; Champion et al., 1998a,b; Olson et al., 1998; Vaccarino et al., 1999).

Similarly, endomorphin-1 and endomorphin-2 lowered heart rate and decreased blood pressure in normotensive and spontaneously hypertensive rats (Champion et al., 1997b; Czapla et al., 1998; Kwok and Dun, 1998; Makulska-Nowak et al., 2001), mice (Champion et al., 1998c), and rabbits (Champion et al., 1997a). Interestingly, both peptides produced dose-dependent biphasic changes in systemic arterial pressure in cats (Champion et al., 1998b). The cardiovascular responses to endomorphins were not altered by their repeated administration (Champion et al., 1999b). All of these effects were reversed by the μ-opioid receptor antagonists, naloxone and β-funaltrexamine, and were not blocked by the δ- or κ-opioid receptor antagonists, suggesting that the cardiovascular activity of endomorphins is primarily mediated by μ-opioid binding sites (Champion et al., 1998a; Czapla et al., 1998; Kwok and Dun, 1998; Makulska-Nowak et al., 2001). Surprisingly, the hypotensive effect of endomorphin-2 after i.c.v. administration in rats was less pronounced than that after i.v. injections (Czapla et

al., 1998; Kwok and Dun, 1998; Makulska-Nowak et al., 2001). This suggests that the peripheral μ-opioid receptors might play an important role in the endomorphin-2-induced hypotensive effect (Rialas et al., 1998).

The mechanisms underlying the cardiovascular activity of endomorphins are not clear. It is commonly acknowledged that bradycardia is a consequence of vagus activation. Because atropine or bilateral vagotomy abolished the effects of endomorphin-1 on heart rate in rats, it was suggested that endomorphins might activate the vagal afferents. On the other hand, endomorphins are known to have inhibitory actions on neurons (Baraban and Stornetta, 1995; Hayar and Guyenet, 1998; Wu et al., 1999; Dun et al., 2000; Guyenet et al., 2002), and this effect is expected to elicit an increase in blood pressure and heart rate on the basis of known circuitry of cardiovascular regulatory areas in the medulla (Dampney, 1994; Sapru, 2002). Viard and Sapru (2006) demonstrated that microinjections of endomorphin-2, into the medial subnucleus of the NTS (mNTS) in rat attenuated reflex responses to stimulation of the carotid sinus and aortic baroreceptors. In this way both the inhibitory effect on the baroreflex and the excitatory effect on the mNTS neurons, resulting in depressor and bradycardic responses, were observed. The authors of this report hypothesized that the activity of the mNTS neurons might be under the dual control of the inhibitory influence of GABAergic neurons from the mNTS (Maqbool et al., 1991; Izzo and Sykes, 1992) and the excitatory influence of the glutamatergic projections from other areas of the brain, e.g., the insular cortex (Torrealba and Müller, 1996; Owens et al., 1999), to the mNTS (Fig. 8). The inhibitory effect of the GABA neurons in the mNTS (Fig. 8B) may be presynaptic (i.e., via inhibition of the release of Glu from the terminals) (Fig. 8C), as well as postsynaptic (i.e., via hyperpolarization of the postsynaptic neuron) (Fig. 8A). Inhibition of the GABAergic neurons by exogenously injected endomorphin-2 via the μ-opioid receptors located on the GABAergic neurons would result in increased release of Glu from presynaptic terminals, a reduction in the inhibitory influence on the postsynaptic neurons (disinhibition) and, in consequence, excitation of postsynaptic mNTS neurons. Endomorphin-2 might also act through the μ-opioid receptors located on the glutamatergic baroreceptor afferent terminals in response to baroreceptor stimulation (Fig. 8D), by decreasing Glu release and attenuating the baroreflex. All of these effects would explain the mechanism of the depressor and bradycardic responses to endomorphin-2 (Kasamatsu and Chitravanshi, 2004).

Similarly, the pathways that lead to the vasodilative action of endomorphins still remain ambiguous. Some authors suggested that the hypotensive effects of endomorphins might be secondary to bradycardia (Kwok and Dun, 1998). The vasodepressor effect of endomorphins might also be mediated, in large part, by stimulation of NO synthesis (Champion and Kadowitz, 1998;
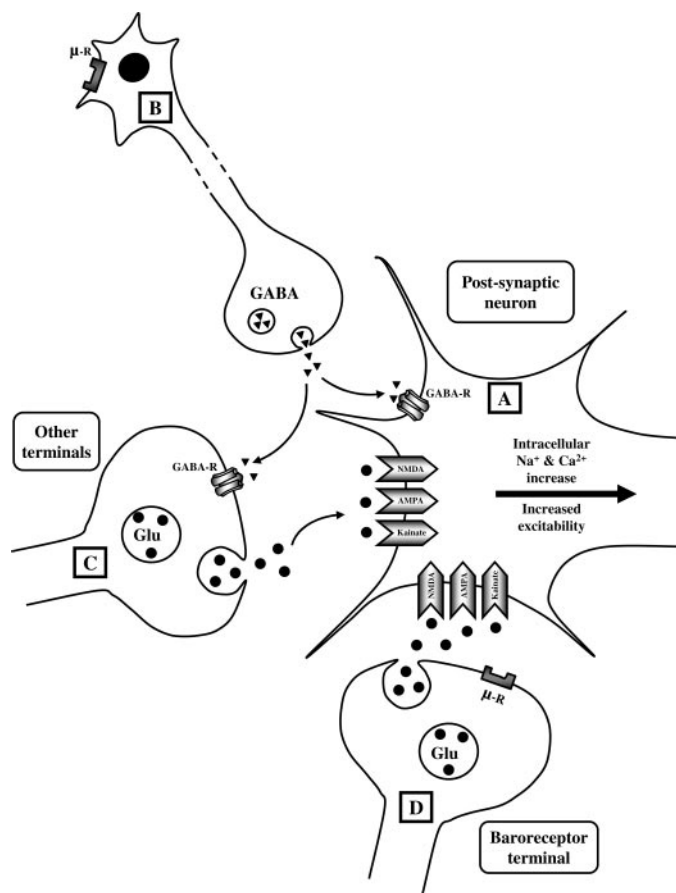
FIG. 8. Hypothetical model showing possible effects of exogenously administered endomorphin-2 in the mNTS. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid. Adapted from *Brain Research*, volume 1073–1074, Viard E and Sapru HN, "Endomorphin-2 in the Medial NTS Attenuates the Responses to Baroflex Activation," pages 365–373, copyright 2006, with permission from Elsevier.

Champion et al., 1998a) and NO release from the endothelium (Champion et al., 2002). These observations were not confirmed by Rialas et al. (1998), who proposed that the vasodilatative action of endomorphins results from inhibition of NA release from nerve endings in the vessel walls. Other mechanisms involved in endomorphin-induced hypotensive effects were suggested, such as hyperpolarization of vascular smooth muscle cells by opening of K^+-ATP channels and increasing the potassium permeability or the release of vasodilator prostaglandins.

13. Effects on Respiratory System. The opioid receptor ligands significantly influence the respiratory system (Bartho et al., 1987; Belvisi et al., 1990, 1992). In general, the opioid agonists produce respiratory depression (Haji et al., 2000), as it was observed for morphine (Kato, 1998; Takita et al., 1998; Weinger et al., 1998; Gwirtz et al., 1999; Mendelson et al., 1999; Osterlund et al., 1999; Sleigh, 1999; Czapla et al., 2000; Dershwitz et al., 2000; Heishman et al., 2000; Herschel et al., 2000; Hill and Zacny, 2000; Kishioka et al., 2000a,b; Krenn et al., 2000; Owen et al., 2000; Takita et al., 2000; Teppema et al., 2000), heroin (Vivian et al., 1998; Comer et al.,

1999; Kishioka et al., 2000b), fentanyl (Gerak et al., 1998; Ma et al., 1998; Kishioka et al., 2000b), buprenorphine (Benedetti et al., 1999; Schuh et al., 1999), and DAMGO (Takita et al., 1998; Czapla et al., 2000), although some exceptions were reported (Greenwald et al., 1999; Mendelson et al., 1999; Angst et al., 2000; Czapla et al., 2000; Preston and Bigelow, 2000). Whereas numerous mechanisms are involved in respiratory regulation, the primary mechanism thought to be involved in opioid-induced respiratory depression is a general decrease in the sensitivity of brainstem respiratory centers to carbon dioxide, followed by a decrease in respiratory rate (for review, see Martin, 1983; Reisine and Pasternak, 1996; Shook et al., 1990).

Although endomorphin IR (Pierce and Wessendorf, 2000) and μ-opioid receptors (Moss, 2000; Moss and Laferriere, 2000) are colocalized in brain areas associated with respiratory control, such as the NTS and parabrachial nuclei (Martin-Schild et al., 1999), little is known about the effects of endomorphins on respiratory activity (Fig. 9). Intravenous injection of endomorphin-1 and endomorphin-2 at higher supra-analgesic doses produced biphasic effects in rats, characterized by an initial rapid ventilatory depression, lasting 4 to 6 s, followed by an increase in ventilation, lasting 10 to 12 min (Czapla
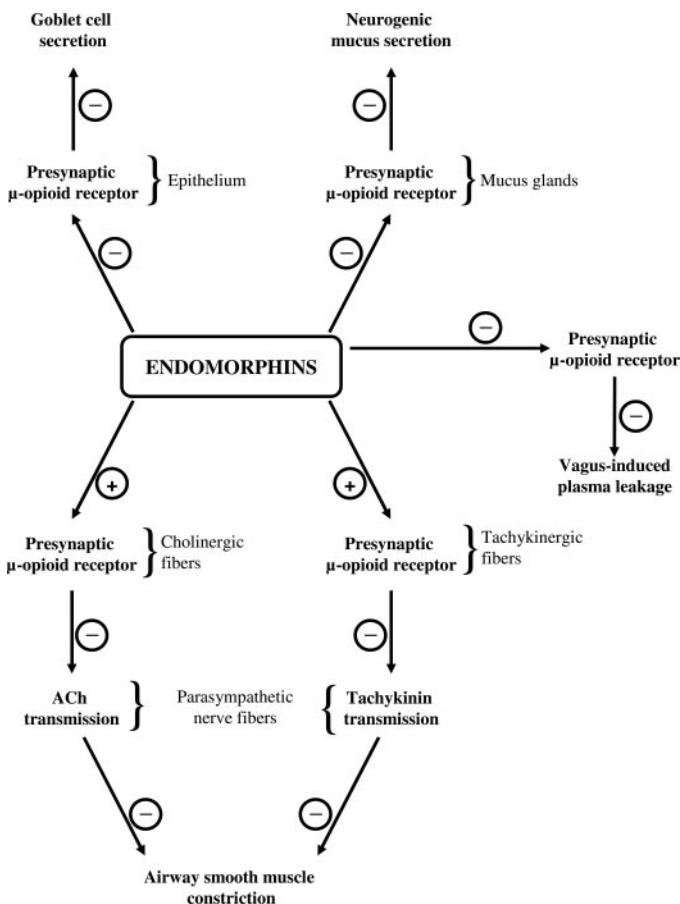
FIG. 9. Hypothetical effects of endomorphins on the respiratory system.

et al., 2000). In contrast, morphine monotonically decreased ventilation. It is likely that the depressant activity of endomorphins was mediated by the central opioid receptors, because it was prevented by systemic injection of the centrally and peripherally acting opioid antagonist, naloxone, but not the peripherally restricted opioid antagonist, methylnaloxone (Czapla et al., 2000). In contrast, the excitatory effects of endomorphins were probably nonopioid-mediated, as they were not antagonized by typical opioid receptor antagonists.

Czapla and Zadina (2005) examined whether i.v. administered endomorphin-1 and endomorphin-2 affect the hypercapnic ventilatory response in rats and whether this modulation is mediated by a central mechanism. Endomorphins, in doses far higher than these corresponding to their analgesic threshold, depressed ventilation by attenuating the ventilatory response to hypercapnia. However, the threshold doses for respiratory depression were considerably higher for endomorphin-1 and endomorphin-2 than for DAMGO or morphine. To test whether modulation of the hypercapnic ventilatory responses were centrally mediated, endomorphin-1, endomorphin-2, DAMGO, and morphine were systemically administered before and after the i.v. administration of naloxone and methylnaloxone. The ventilatory effects of all μ-opioid agonists were blocked by naloxone, but not by methylnaloxone, thereby indicating a central action of the agonists on respiratory function. These results suggested that endomorphin-1 and endomorphin-2 produce weaker depression of the ventilatory response to hypercapnia than DAMGO or morphine. Differences in both pharmacokinetic factors, such as metabolic stability or penetration rate into the brain through the blood-brain barrier, and pharmacodynamic factors, such as receptor selectivity, agonist efficacy, or distinct cellular signaling could contribute to the observed dissimilarity among the μ-opioid receptor agonists. It is unclear, however, whether the opioids modulate the ventilatory response to hypercapnia by depressing neural function in chemosensitive neurons, in neurons associated with ventilatory rhythm generation, or in both.

The involvement of the nonopioid systems in endomorphin-induced effects on the respiratory activity was also studied. Fischer and Undem (1999) demonstrated that endomorphins produced a concentration-dependent inhibition of the electrical field stimulation-induced tachykinin-mediated contractions of the guinea pig bronchus. Surprisingly, only endomorphin-1 effects could be blocked by naloxone (10 μM), whereas endomorphin-2 effects were not affected by any opioid receptor-specific antagonist. Patel et al. (1999) showed that endomorphin-1 and endomorphin-2 gave a concentration-dependent and naloxone-sensitive inhibition of cholinergic contractile responses in guinea pig trachea. Both peptides also inhibited the electrically evoked ACh release from cholinergic nerves innervating guinea pig

and human trachea. These results suggested that endomorphins can inhibit cholinergic, as well as nonadrenergic, noncholinergic (NANC) parasympathetic neurotransmission from postganglionic parasympathetic nerve fibers, which innervate airway smooth muscles, to the airways via the activation of classic opioid receptors (Belvisi et al., 1992). Different functional studies concluded that these effects are established via prejunctional μ-opioid receptors localized on both cholinergic and tachykinergic fiber types.

Recently, Asakawa et al. (2000) reported that endomorphins influenced oxidation processes. Intracerebroventricular injection of endomorphin-1 increased oxygen consumption in mice in a naloxone-reversible manner, indicating that this effect was mediated by the opioid receptors.

14. Effects on Gastrointestinal Tract. The effects of μ-opioid receptor ligands on the gastrointestinal tract are well established. Morphine inhibited gastrointestinal functions, transit and gastric emptying, in a naloxone-reversible manner (Asai, 1998; Asai et al., 1998). The μ-opioid receptor agonists were shown to influence small intestine motility both in vivo and in vitro (Puig et al., 1977; Nishiwaki et al., 1998; Allescher et al., 2000a), neurotransmitter release (Cosentino et al., 1997; Nishiwaki et al., 1998), and peristaltic reflex (Allescher et al., 2000a). Studies also revealed the fact that intestinal ascending contraction decreased via μ- and κ-receptors and latency increased via μ- and δ-opioid receptors (Olson et al., 1996; Allescher et al., 2000a,b). However, whereas opioid receptors present in the CNS have been thoroughly characterized, most groups failed to localize the μ-opioid receptor on the smooth muscle of the intestine, although different techniques, such as immunohistochemistry and receptor binding studies were used (Storr et al., 2002b). Grider and Makhlouf (1987) functionally characterized the μ-opioid receptor on the gut muscle layer, but because of the nerve/muscle preparation used, a neuronal effect could not have been excluded.

As expected, endomorphin-1 and endomorphin-2 also play important roles in the regulation of gastrointestinal functions. Tonini et al. (1998) analyzed the effects of endomorphin-1 and endomorphin-2, in a wide range of concentrations (3×10^{-12} M to 10^{-6} M), on ileum longitudinal muscle-myenteric plexus preparations from guinea pig ileum. Both peptides inhibited the amplitude of electrically induced twitch contractions in a concentration-dependent, CTOP-reversible manner, up to its abolition. Conversely, endomorphins failed to affect contractions to applied ACh on nonstimulated ileum longitudinal muscle-myenteric plexus preparations. These results demonstrated that endomorphins selectively activated μ-opioid receptors located on excitatory myenteric plexus neurons.

The effects of endomorphins on activity of smooth muscles in rat small intestine preparations were also characterized (Storr et al., 2002a). Both endomorphins inhibited the ascending contraction (i.e., attenuated the ascending excitatory reflex), increased the descending relaxation (stimulated the descending inhibitory reflex), and increased latency of onset of the contractile responses. Furthermore, endomorphin-1 and endomorphin-2 significantly reduced electrically induced smooth muscle twitch contractions. Inhibition of rat ileal smooth muscle activity was reversed by the μ-opioid receptor antagonist CTOP, confirming a specific μ-opioid receptor-mediated effect of endomorphins on the neural tissue in this preparation. Similar effects were observed for smooth and striated muscles of the rat esophagus (Storr et al., 2000a,b).

Endomorphins were shown to impair gastrointestinal transit by influencing various neural pathways. Yokotani and Osumi (1998) showed that endomorphin-1 and endomorphin-2 inhibited vagally evoked release of ACh from the isolated vascularly perfused rat stomach and suggested that the μ-opioid receptor is involved in this process. Cosentino et al. (1997) demonstrated that endomorphins modulated the adrenergic system by NA release in guinea pig colon. Storr et al. (2002a) suggested that endomorphins must act via μ-opioid receptors located on the presynaptic membrane of inhibitory NANC neurons and that they exert their effect on NANC relaxation by reducing release of the neurotransmitters involved (NO and vasoactive intestinal peptide). These effects could also be mediated by an inhibition of cholinergic nerve activity (Tonini et al., 1998; Yokotani and Osumi, 1998), which may indirectly result in an inhibition of NANC nerve activity.

B. Endomorphins, Neurotransmitters, and Neurohormones

In general, the exogenous and endogenous opioid receptor ligands elicit their biological effects by modifying the function of various neurotransmitter and neurohormone systems. Modulation of neurotransmitter release by opioids has been reviewed extensively (Illes, 1989; Jackisch, 1991; Mulder and Schoffelmeer, 1993). For example, it has been shown that opioids are able to influence the secretion of DA (Henderson et al., 1979; Iwamoto and Way, 1979; Vizi, 1979; Langer, 1981; De Vries et al., 1989; Mulder et al., 1989, 1991a,b; Chen et al., 2001; Ukai and Lin, 2002a; Bujdoso et al., 2003; Huang et al., 2004), NA (Starke, 1977; Westfall, 1977; Henderson et al., 1979; Iwamoto and Way, 1979; Vizi, 1979; Langer, 1981; Szekely and Ronai, 1982a,b; Hagan and Hughes, 1984; Chen et al., 2001; Al-Khrasani et al., 2003; Hung et al., 2003), 5-HT (Hagan and Hughes, 1984; Passarelli and Costa, 1989; Wichmann et al., 1989; Mulder and Schoffelmeer, 1993; Chen et al., 2001), and ACh (Domino, 1979; Jhamandas and Sutak, 1980). Moreover, opioids may also alter the release of various neurohormones (Szekely and Ronai, 1982b; Pfeiffer and

Herz, 1984; Millan and Herz, 1985; Tuomisto and Männistö, 1985; Howlett and Rees, 1986).

In the last part of this review, the relationship between endomorphins and neurotransmitter and neurohormone systems is briefly summarized.

1. Modulation of Dopamine Transmission. The μ-opioid receptors are found in high density in dopaminergic pathways in the Nac and corpus striatum (Khachaturian et al., 1985; Fallon and Leslie, 1986; Eghbali et al., 1987; Mansour et al., 1988) and seem to play an important role in dopaminergic transmission in both structures. Activation of μ-opioid receptors in the Nac is known to increase accumbal DA release or DA efflux in rats (Spanagel et al., 1992). Okutsu et al. (2006) studied the ability of endomorphins to alter the accumbal extracellular DA level by means of microdialysis in freely moving rats. Infusion of endomorphin-1 or endomorphin-2 into the Nac produced a dose-dependent increase of the accumbal DA level. Endomorphin-1-induced DA efflux was abolished by intraccumbal perfusion of CTOP, systemic administration of naloxone, and systemic administration of the μ_1-receptor antagonist naloxonazine. The μ-receptor antagonists failed to affect endomorphin-2-induced DA efflux, suggesting that the effects produced by endomorphin-2 are not mediated by μ-opioid receptors. This result is in good agreement with those of earlier reports, showing that the ability of endomorphin-2 to stimulate the release of DA in the Nac and to increase the level of DA metabolites, DOPAC and HVA, in the shell of the Nac, resulted from activation of the mesolimbic system and disinhibition of the local GABA neurons (Johnson and North, 1992; Huang et al., 2004).

In the striatum, opioids act predominantly at the presynaptic opioid receptors and produce different indirect effects on DA turnover, depending on the receptor type (Clouet and Ratner, 1970; Smith et al., 1972; Gauchy et al., 1973). Locally administered μ-opioid agonists were shown to increase DA efflux through postsynaptic μ-opioid receptors (Dourmap et al., 1992). This efflux was not accompanied, however, by modification of DA uptake or an increase in the level of two main DA metabolites, DOPAC and HVA (Das et al., 1994). Dourmap et al. (1997) showed that modulation of DA release by μ-opioid receptors required the integrity of cholinergic and, to a lesser level, of GABAergic neurons in the striatum. The μ-opioid agonists applied to the striatum might also act at presynaptic sites on nigrostriatal DA neurons, activating DA receptors to prevent DA release.

In the study of Yonehara and Clouet (1984), the levels of DA and its catabolites after intracisternal administration of morphiceptin in rat were measured. Morphiceptin produced a decrease in the levels of 3-methoxytyramine, suggesting an inhibition of DA release (Das et al., 1994). However, no such studies were performed for other μ-opioid receptor-selective ligands, including endomorphins.

2. Modulation of Noradrenaline Transmission. The LC consists of a compact group of NA-containing cell bodies close to the floor of the fourth ventricle at the upper border of pons (Nestler et al., 1994). LC innervates all levels of the neuraxis and can simultaneously influence functionally diverse neural targets (Waterhouse et al., 1983, 1993; Simpson et al., 1997). It is the primary source of NA in the forebrain and the sole source of NA in the cortex and hippocampus (Aston-Jones et al., 1985; Lewis et al., 1987; McCormick et al., 1991). LC is the primary origin of descending noradrenergic analgesic fibers (Proudfit, 1988; Jones, 1991; Lipp, 1991). Electrophysiological studies and microscopic autoradiography revealed a high μ-opioid receptor density in the LC (Mansour et al., 1986, 1995).

Yang et al. (1999) investigated the influence of natural and synthetic μ-opioid selective peptides (morphiceptin, hemorphin-4, Tyr-MIF-1, Tyr-W-MIF-1, endomorphins, Tyr-D-Arg-Phe-Sar, and Tyr-D-Arg-Phe-Lys-NH$_2$) on the activity of the LC using intracellular recording techniques. All peptides tested reduced the excitability of LC neurons in a concentration-dependent, D-Phe-c(Cys-Tyr-D-Trp-Arg-Thr-Pen)-Thr-NH$_2$-reversible manner. They inhibited spontaneous firing, induced the hyperpolarization of the membrane potential by opening inward-going rectification potassium channels, and reduced the neuronal input resistance. Endomorphin-1 and endomorphin-2, which were the most effective endogenous opioid peptides, were almost equipotent, and their electrophysiological effects on LC neurons showed similar amplitude and time course. Furthermore, the concentrations of endomorphins required to elicit hyperpolarization of the membrane potential were much lower than those for other opioids, including morphine, β-endorphin, and DAMGO. The authors of the study speculated that the presence of an aromatic group in position 4 and the amidation of the C terminus in endomorphins are crucial both for high μ-receptor binding affinity and a significant electrophysiological response of LC neurons.

Peoples et al. (2002) examined the ultrastructural associations of endomorphin-1 with the NA neurons of the LC and the peptidergic neurons of Barrington's nucleus in the brainstem dorsal pontine tegmentum. Both structures, targeted by endogenous opioids (Sutin and Jacobowitz, 1988; Van Bockstaele et al., 1996c) and enriched with μ-opioid receptors (Atweh and Kuhar, 1977; Van Bockstaele et al., 1996a,b), might be involved in the reduction of the excitability of the LC neurons caused by endomorphins (Peoples et al., 2002). It was demonstrated that endomorphin-1-immunoreactive axon terminals form synaptic specializations with tyrosine hydroxylase- and CRF-containing dendrites in peri-LC and Barrington's nucleus, respectively, in the dorsal pontine tegmentum of the rat brain. Endomorphin-1 could modulate the LC neurons directly in this structure by tonically inhibiting the neurons in the Barrington's nucleus.

The potential interactions between endomorphin-1 and NA transmission in the spinal cord have also been studied (Hao et al., 2000). Intrathecally administered endomorphin-1 in rats activated local NA terminals and stimulated the release of NA in the spinal cord. Thus, the peptide indirectly activated α_2-receptors and produced analgesia in rats, as measured in the tail-flick, tail pressure, and formalin tests.

3. Modulation of Serotonin Transmission. Serotoninergic transmission is important in various physiological processes, especially nociception (Driessen and Reimann, 1992; Pini et al., 1997), stress and anxiety (Petty et al., 1992; Kawahara et al., 1993), and food intake (Kaye, 1997; Mercer and Holder, 1997), in which the opioid system has also been implicated. The largest population of serotoninergic cell bodies is located within the ventral portion of the PAG, in the dorsal raphe nucleus (DRN), an area involved in the integration of the responses to stress (Basbaum and Fields, 1984; Roche et al., 2003). Pain and stressful stimuli activate opioidergic neurons in the PAG, which in turn modulate the activity of serotoninergic neurons with projections to the sites involved in emotional states (Ma and Han, 1992; Grahn et al., 1999).

The extracellular level of 5-HT in DRN can be used as an indicator of neuronal activity and serotonin release in the forebrain (Tao and Auerbach, 1995). Electrophysiological studies showed that μ-opioids have inhibitory effects on serotoninergic neuronal discharge in the DRN (Haigler, 1978; North, 1986), whereas the in vivo microdialysis with μ-opioids stimulated serotoninergic activity (Tao and Auerbach, 1995; Kalyuzhny et al., 1996). This inconsistency could be explained by the interaction of the opioidergic and serotoninergic systems in the DRN. The μ-opioid receptor-containing synaptic endings are present in the DRN (Wang and Nakai, 1994), but the opioid agonists do not excite 5-HT neurons directly (Tao and Auerbach, 1995; Jolas and Aghajanian, 1997). The μ-opioid-induced change in 5-HT efflux represents a balance between two competing effects, inhibition of endogenous GABAergic afferents and disinhibition of 5-HT neurons by enhancing the excitatory influence of Glu, which is attenuated by direct inhibition of glutamatergic afferents (Tao et al., 1996; Jolas and Aghajanian, 1997; Kalyuzhny and Wessendorf, 1997; Gervasoni et al., 2000). Although the net effect depends on the initial equilibrium between GABAergic (inhibitory) and glutamatergic (excitatory) influences on 5-HT neurons, the μ-opioids seem to favor the increase in extracellular 5-HT.

The effect of in vivo microdialysis with endomorphin-1 and endomorphin-2 on extracellular levels of 5-HT in the rat DRN has been investigated (Tao and Auerbach, 2002). Both peptides increased the efflux and the extracellular level of 5-HT in the DRN. Endomorphins produced a shorter-lasting and, in the case of endomorphin-2, a lower response than DAMGO, which could be due to a faster rate of degradation of those peptides. The response was blocked by a selective μ-opioid receptor antagonist, β-funaltrexamine.

The influence of endomorphins on 5-HT levels in other brain areas has also been studied. Endomorphins injected into the VTA reduced serotoninergic activity in the medial prefrontal cortex and ventral striatum (Chen et al., 2001), which might suggest that they stimulate μ-opioid receptors and might mediate the depletion of 5-HT in both brain regions. As shown by Huang et al. (2004), both endomorphins showed no effect on the serotonin metabolite, 5-hydroxyindoleacetic acid in the Nac.

In the neocortex, endomorphin-1 produced a naloxone-reversible inhibitory effect on K^+-stimulated 5-HT overflow (Sbrenna et al., 2000). This observation clearly indicated that in the neocortex μ-opioid receptors are located on serotoninergic nerve terminals and suggested a direct presynaptic modulation of serotoninergic terminals by μ-receptors in this brain area.

In the medial prefrontal cortex, DAMGO and endomorphin-1 produced a strong, CTOP-reversible suppression of the 5-HT_{2A}-induced excitatory postsynaptic currents in layer V pyramidal cells (Marek and Aghajanian, 1998). It was also demonstrated that the μ-opioid receptor agonists acted on neuronal elements presynaptically to the pyramidal cells. It was proposed that 5-HT_{2A} and the μ-receptor ligands interact physiologically on a subset of glutamatergic afferents to medial prefrontal cortical layer V pyramidal cells and the apparent neocortical source for both receptor types might be layer VI pyramidal cells (Vogt et al., 1995). A subcortical candidate for this unique population of afferents might be the thalamus or basolateral nucleus of the amygdala (Krettek and Price, 1977; Delfs et al., 1994; Mansour et al., 1994; Bacon et al., 1996). It was concluded that the physiological interaction between 5-HT_{2A} and μ-receptors in the CNS may be of substantial importance, given the heavy and widespread cortical localization of both receptor types.

4. Modulation of Acetylcholine Transmission. As discussed in detail above (sections VI.A.11., VI.A.13., and VI.A.14.), endomorphins may modulate cholinergic neurotransmission in peripheral tissues, mainly in the respiratory system and the gastrointestinal tract. They are believed to exert these inhibitory effects by acting through prejunctional μ-opioid receptors to inhibit release of contractile transmitters.

Evidence for the possible role of endomorphin-1 in the regulation of cholinergic neurotransmission in the inner ear has also been reported. Lioudyno et al. (2002) investigated the ability of endomorphin-1 to modulate ACh-evoked currents in isolated frog saccular hair cells and rat inner hair cells by means of the patch-clamp recording method. They observed that endomorphin-1 inhibited ACh-evoked currents and suggested that endo-

morphins may interact with nicotinic acetylcholine receptors.

5. Modulation of Neurohormone Release. Oxytocin (OT) and AVP are neurohormones synthesized by magnocellular neurosecretory cells in the PVN and supraoptic nucleus (SON) of the hypothalamus. The PVN and SON project to the posterior pituitary, where OT and AVP are secreted directly into the systemic circulation (Hatton, 1990). In vivo studies demonstrated that the opioid systems participate in regulation of the electrical activity of the OT and AVP neurons and the release of both hormones (Russell et al., 1995; Brown and Leng, 2000). The μ-opioid receptor agonists decreased the basal levels of both OT (Clarke et al., 1979; Frederickson et al., 1981; Clarke and Patrick, 1983; Hartman et al., 1987) and AVP (Van Wimersma Greidanus et al., 1979; Aziz et al., 1981). However, more recent studies showed that the μ-opioid receptor agonists inhibited only OT cells, whereas the activity of AVP neurons was diminished exclusively by κ-opioid receptor agonists (Pumford et al., 1993).

The only report on the association of endomorphins and the level of both neurohormones was published by Doi et al. (2001), showing that i.c.v. administered endomorphin-1 in rats inhibited both OT and AVP SON cells, but in a different manner. The OT cells were much more sensitive to endomorphin-1-induced inhibition than the AVP cells, which may suggest that they express μ-opioid receptors at a much higher level.

Several studies have demonstrated that i.c.v. (Liu et al., 2002), i.p. (Agren et al., 1997), and intracisternal administration of OT (Arletti et al., 1993), as well as direct injections of OT to the PAG (Zhang et al., 2000) and the nucleus raphe magnus (Robinson et al., 2002), induced antinociceptive effects in animals and humans (Arletti et al., 1993; Lundeberg et al., 1994; Louvel et al., 1996; Petersson et al., 1996; Agren et al., 1997; Caron et al., 1998; Kang and Park, 2000). The involvement of opioid receptors in OT-induced antinociception in the CNS has also been investigated (Wang et al., 2003; Zubrzycka et al., 2005). The OT-induced antinociceptive effects to both thermal and mechanical stimulation in rats were blocked significantly by i.c.v. administration of β-FNA, indicating that μ-opioid receptors are involved. Unfortunately, so far there are no data on the involvement of endogenous μ-opioid receptor ligands, endomorphins, in OT-induced antinociception.

Likewise, the relationship between endomorphins and AVP needs to be elucidated. Recent evidence suggested that the increased vasopressinergic neuronal activity in the amygdala or hypothalamus represents an important step in the neurobiology of stress-related behaviors. For example, acute stress increased extracellular levels of AVP in the rat amygdala or hypothalamus (Ebner et al., 2002; Wigger et al., 2004) and the activation of V1 receptors by AVP may underlie anxiogenic and depressive behaviors in the rat (Liebsch et al., 1996; Griebel et al.,

2002; Wigger et al., 2004). These data suggest that further studies with endomorphins are needed.

A potential role of μ-opioid receptors for regulatory processes in the stomach is supported by autoradiographic (Nishimura et al., 1984, 1986), immunohistochemical (Fickel et al., 1997), and physiological (McIntosh et al., 1990) investigations. Lippl et al. (2001) examined the effects of endomorphins on gastric neuroendocrine functions. Endomorphin-1 has been shown to inhibit somatostatin stimulation in the perfused rat stomach. Blockade of μ-opioid receptors with CTOP, a selective μ-opioid receptor antagonist, significantly, but not completely, antagonized the inhibitory effect of endomorphin-1. These results suggest that the inhibitory effect of endomorphin-1 on somatostatin activity is mediated in large part by μ-opioid receptors.

VII. Conclusions

Since their discovery in 1970s, the opioid peptides have become increasingly important in our understanding of brain functions. Endomorphin-1 and endomorphin-2, isolated from mammalian brain nearly a decade ago, are endogenous opioid peptides with high affinity and extreme selectivity for the μ-opioid receptors. Endomorphins and the μ-opioid receptors display widespread localization in the central and peripheral nervous systems and seem to participate in many distinct neuronal circuits, thereby exerting a multitude of diverse functions in a variety of animal species. As matter of fact, endomorphins have been implicated in a broad range of biological processes, including nociception, cardiovascular, respiratory, feeding, and digestive functions, responses related to stress, and complex functions such as reward, arousal, and vigilance, as well as autonomic, cognitive, endocrine, and limbic homeostasis. Whether these activities reflect direct or indirect responses to endomorphins remains a matter of speculation. To answer this fundamental question, the elucidation of endomorphin synthesis and degradation pathways, the recognition of exact mechanisms of action, and the identification of the principal pathways involved in their effects are obviously required. Other important issues, such as the complex relationship with neurotransmitters and neurohormones, also need to be clarified.

The beneficial effects of endomorphins in various pathological conditions such as pain, mood, and feeding disorders, related to reward and cardiorespiratory function, will undoubtedly motivate the development of new ligands. These ligands would preferably be endomorphinomimetics with high μ-opioid receptor affinity and good resistance to proteolytic degradation, which could potentially be used as analgesic, anxiolytic or antidepressant agents.

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