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Endomorphin peptides: pharmacological and functional implications of these opioid peptides in the brain of mammals. Part two

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SUMMARY

Endomorphin-1 (EM1) and Endomorphin-2 (EM2) represent the two endogenous C-terminal amide tetrapeptides shown to display a high binding affinity and selectivity for the μ-opioid receptor as reported previously (see previous paper, Part I). Endomorphins injected into the VTA were shown to enhance the development of behavioral sensitization responses to amphetamine (AMPH), besides of inducing an increase of locomotion (horizontal) activity in animals. These studies showed that EM2 was significantly more potent than EM1 in modulating the increased opioid-mediated ambulatory responses by altering the dopamine (DA) projecting system in the globus pallidus in tested animals. Several transmission systems (e.g., GABA) have been shown to participate in the endormorphin-induced locomotor responses. EM1 injected into the VTA produced potent rewarding effects in rodents, similar to the rewarding responses produced by distinct opiate compounds. The opioid rewarding responses induced by EM1-2 were shown to be mediated via the activation of both GABAergic and the dopamine (VTA-NAc-PFCx) transmission systems in the brain. Moreover, EM1-2 peptides injected into the VTA, but not in the NAc, produced similar related-rewarding responses induced by low doses of morphine. However, ICV administration of EM1 was shown to enhance a significant conditioned-place preference (CPP); whereas EM2 displayed a place aversion in tested animals.

With regard to stress-related behaviors and physiological responses in mammals, endomorphin peptides have been proposed to modulate the HPA axis function via activation of the NTS-projecting neural system impinging on hypothalamic neurons, and/or via activation of the PAG (ventrolateral area) mediating analgesic responses-induced by stress. EM1-2 peptides have been shown to induce mood-related behaviors. For instance, administration of EM1 induced an increased anxiolytic response in mice when tested in elevated plus maze paradigms, results that showed that the μ-opioid receptor modulates mood-related responses in animals and humans, as well. Interesting enough is the recent observation that EM1-2 peptides may induce antidepressant-like behaviors in animals models of stress and depression, whereby EM1-2 peptides have been shown to up-regulate in a dose-dependent manner the neuronal expression of the BDNF mRNA in rat limbic areas involved in stress and depressive-like behaviors. Thus, these studies led to the proposition that endomorphin peptides may play crucial roles in psychiatric disorders (e.g., depression, schizophrenia). Furthermore, over the past years, it has been shown that μ-opioid receptor agonists (e.g., morphine, DAMGO; morphine-6β-glucuronide) displayed potent orexigenic activities in the CNS of mammals, similar to that displayed by EM1-2 peptides, whose dose-dependent orexigenic activity appears to be mediated by the endogenous opioid peptide, Dynorphin A, acting on its cognate κ-opioid receptor at the hypothalamus.

Extensive studies revealed the activity of the EOS (e.g., β-endorphin) on the regulation of gonadal hormones and sexually-induced behaviors (e.g., lordosis) in female rats. β-endorphin or morphiceptin have been shown to facilitate lordosis behaviors in estrogen- and/or estrogen/progesterone primed rats, whereas EM1-2 peptides injected into third ventricle or into the diagonal band (DB) produced dose- and time-dependent, naloxone-reversible lordosis responses in female rats. These results posit that EM1-2 peptides produce their sexual behaviors and mating responses via modulating the cell release of LHRH and modulating GABA transmission system in the brain. Endomorphins have been shown to impair short- and long-term memory processing in mice when exposed to different learning paradigms. These opioid mediated effects appear to be regulated through the interaction of both cholinergic and dopaminergic transmissions in the brain. In addition, endomorphins have been shown to modulate cardiovascular and respiratory bioactivities, acting on several rostrocaudal areas of the CNS of mammals. Administration of EM1-2 peptides induced a significant reduction of heart rate and blood pressure in normotensive and hypertensive rats, via regulation of GABA and glutamate transmission systems. Although the exact endogenous mechanisms by which EM1-2 peptides produce their vasoactive responses are still unclear, several studies suggested that the peptide activity depends on the synthesis and release of nitric oxide (NO) from endothelial cells enhanced by

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activation of μ-opioid receptors. Studies on respiratory function showed that EM1-2 peptides attenuate and produce significant respiratory depression in tested animals. Finally, EM1-2 peptides have been shown to induce important inhibitory gastrointestinal effects via the activation of μ-opioid receptors localized in myenteric-plexus neurons that innervate smooth-muscle cells producing a dose-dependent- and CTOP-reversible inhibition of electrically-induced twitch ileum contractions, probably mediated through a reduced release response of several peptide and non-peptide transmitters.

Key words: Endomorphins, physiology, locomotor sensitization responses, opioid reward, stress, HPA axis, sex, feeding, cardiovascular, respiratory, anxiolytic, social defeat.

RESUMEN

La endomorfina-1 (EM1) y la endomorfina-2 (EM2) son dos péptidos bioactivos que poseen la más alta afinidad de unión selectiva por el receptor opioide μ en comparación con la unión de distintos ligandos agonistas a este subtipo de receptor opioide (véase resumen y texto del capítulo anterior, parte I). Estudios farmacológicos y conductuales han demostrado que la inyección de las EM1-2 en el *área ventrotegmental* (AVT) genera respuestas conductuales de sensibilización locomotora a la anfetamina (AMPH), además de incrementar la actividad locomotora de tipo horizontal en los roedores tratados. Estos estudios mostraron que la EM2 fue significativamente más potente que la EM1 en inducir las respuestas locomotoras detectadas, mediadas a través de la alteración de la actividad sináptica de dopamina (DA) y en el *globus pallidus* de los animales tratados. Asimismo, estudios fármaco-conductuales similares demostraron que otros sistemas de transmisión participan conjuntamente con el sistema dopaminérgico en la generación de los efectos locomotores inducidos por las EM1-2, como es el caso del sistema *gabaérgico* (GABA). Más aún, la inyección de EM1 en la región AVT del cerebro de roedores mostró generar respuestas potentes de recompensa placentera, similares a las reportadas por distintos alcaloides opiáceos de alto potencial adictivo, posterior a su administración sistémica. Más aún, la inyección de endomorfinas en la región AVT del cerebro del roedor, mas no en el núcleo *accumbens* (NAc), mostró generar respuestas de recompensa paralela a la generada posteriormente a la administración de dosis bajas de morfina.

En línea con los efectos farmacológicos inducidos por las EM1-2, estudios fármaco-conductuales demostraron que la administración ICV de la EM1 fue capaz de generar respuestas de preferencia de lugar en roedores tratados CPP, por sus siglas en inglés, *conditioned place preference*, en tanto que la administración de EM2 generó respuestas opuestas, esto es, respuestas de aversión al lugar. Estudios conductuales relacionados con el fenómeno de estrés mostraron que las EM1-2 son capaces de modular la actividad funcional del eje HHA (eje hipotálamo/hipófisis/glándula adrenal) a través de la activación del sistema de proyección neuronal del tracto solitario (NTS, por sus siglas en inglés), al hipotálamo y/o a través de la activación del área ventrolateral de la sustancia gris periacueductal (PAG, por sus siglas en inglés); componente importante del sistema opioide endógeno, que median respuestas analgésicas (antinociceptivas) inducidas por estímulos estresantes. Asimismo, la administración de endomorfinas (v.g., EM1) mostró generar incrementos de conductas de naturaleza ansiolítica en ratones expuestos a paradigmas experimentales de generación de conductas estresantes (v.g., laberinto elevado). Estos estudios sugieren que la generación de conductas de estrés-emocional inducidas por las endomorfinas es mediada a través de la activación del receptor opioide μ en neuronas del hipotálamo responsables de regular la secreción de factores liberadores de distintas hormonas hipofisiarias (v.g., CRH, LHRH). Más aún, resulta interesante que las endomorfinas sean capaces de inducir conductas antidepresivas o de tipo antidepresivos como se ha reportado recientemente en modelos animales de estrés y depresión. Estos estudios mostraron que las respuestas conductuales de reacción al estrés y las conductas antidepresivas mediadas por las EM1-2 están ligadas con la expresión neuronal del mensajero de RNA que codifica para el factor trófico (*BDNF*, por sus siglas en inglés, *brain derived neurotrophic factor*), en áreas del *sistema límbico*, y que es inducida en forma dosis-dependiente por las endomorfinas, posterior a su administración ICV. Por lo tanto, estos estudios han permitido proponer que las endomorfinas cumplen un papel relevante durante el curso o desarrollo de las enfermedades mentales (v.g., esquizofrenia y depresión). En extensión a estos estudios conductuales, estudios recientes han demostrado la actividad orexigénica de las endomorfinas en forma similar a lo previamente detectado con distintos ligandos agonistas del receptor opioide μ (v.g., morfina, DAMGO; morfina-6β-glucurónido). Si bien estos estudios mostraron que tanto las EM1-2 como diversos agonistas del receptor opioide μ exhiben potentes actividades orexigénicas en el SNC de roedores, la actividad de las EM1-2 parece depender de la actividad de la *dinorfina A* y su unión sobre su receptor opioide κ en neuronas hipotalámicas. Más aún, diversos estudios han mostrado que el sistema opioide endógeno (a través de la β-endorfina) regula conductas de naturaleza sexual y apareamiento (v.g., lordosis), además de modular la secreción y/o actividad de hormonas de origen gonadal (estrógenos, progesterona).

Estudios similares en roedores hembras mostraron que la microinyección de EM1-2 en áreas específicas del sistema límbico y/o la administración IT de ambos péptidos era capaz de generar respuestas sexuales de apareamiento, similares a las detectadas por la β-endorfina y morficeptina en la misma especie de animal, siendo bloqueados los efectos por la administración de naloxona. Estas respuestas conductuales inducidas por las EM1-2 mostraron estar ligadas a la liberación neuronal de LHRH, como de la activación y modulación del sistema de transmisión gabaérgico. En cuanto a las funciones de memoria y aprendizaje, diferentes estudios han demostrado que la administración ICV de EM1-2 en ratones expuestos a diferentes paradigmas de aprendizaje experimental, los péptidos opioides alteran significativamente los mecanismos de procesamiento y consolidación de memoria a corto y largo plazo en los animales tratados. Estos efectos parecen depender de la modulación del sistema opioide (v.g., el receptor opioide μ) sobre los sistemas de transmisión colinérgica y dopaminérgica en el cerebro de los mamíferos. Asímismo, diversos estudios han demostrado que tanto las EM1-2 como los alcaloides opiáceos y opioides endógenos modulan funciones cardiovasculares y respiratorias. En este contexto, diversos estudios mostraron que la administración de EM1-2 en ratas normotensas e hipertensas produce cambios fisiológicos significativos en la presión sanguínea y la frecuencia cardiaca. Si bien no están del todo esclarecidos los mecanismos por los cuales las endomorfinas producen sus respuestas cardiovasculares, diversos estudios sugieren que la actividad de estos péptidos está en función de la actividad e interacción de los sistemas de transmisión gabaérgico y glutamatérgico, respectivamente. Más aún, otros estudios sugieren que las respuestas fisiológicas de estos péptidos dependen de la actividad del óxido nitroso (NO, por sus siglas en inglés) liberado de los vasos sanguíneos, en respuesta de la activación del receptor opioide μ. Finalmente, diversos estudios han mostrado que las EM1-2 y la activación del receptor opioide μ producen efectos inhibitorios sobre la contracción del músculo liso del tracto gastrointestinal, generados

a través de una reducción sostenida en la liberación de neurotransmisores de terminales sinápticas del plexo mientérico, mismas que inervan el tejido muscular liso del tracto gastrointestinal.

Palabras clave: Endomorfinas, fisiología, sensibilización locomotora, estrés, recompensa a opiacios, eje HPA, sexo, alimentación, cardiovascular, respiratorio, ansiolítico, conducta social.

VII. OPIOID RECEPTOR AGONISTS AND BEHAVIORAL SENSITIZATION

Repeated injections of psychoactive drugs into animals or humans usually lead either to a decrement of behavioral responses (tolerance) or an increase (sensitization) of psychomotor effects.[1] *Behavioral sensitization* is a term often used to describe the neurochemical responsiveness and behavioral effects detected from repeated and intermittent administration of lower doses of a psychoactive drug.[2] This phenomenon creates a drug «preference» state, which allows a drug to be used frequently once the substance of abuse has «sensitized» the active sites in the brain. Thus, drug-sensitization plays a crucial role in the development and maintenance of drug addiction,[3] which may persist for long-term periods after drug withdrawal,[4] enhancing an overwhelming urge (craving) for increased drug-seeking and drug-taking behaviors associated with a loss of behavioral control during long-term periods of abstinence.[3,5] Interesting enough is that all drugs abused by humans have been shown to generate drug-rewarding effects and behavioral sensitization responses in animal models of drug addiction.[6] The neuroanatomical and neurochemical bases of drug sensitization led researchers to focus on the *mesocorticolimbic projecting dopamine (DA) pathway* (neuron and axon fibers that emerge from the VTA and project to both NAC and mPFCx).[7] Several interacting transmission systems besides of the dopamine (DA) system[8,9] impinging directly or indirectly on VTA neurons; have been shown to mediate several of the evoked-behavioral sensitization responses to drug of abuse in animals, which includes the inhibitory GABAergic system,[10] the glutamate (GLU) / aspartate (ASP) excitatory neurotransmission system,[11] and the endogenous μ and δ-dependent opioid receptor systems[12] (figure 1). Microdialysis experiments demonstrated that rats exposed to either systemic administration or direct injection of μ-opioid receptor agonists (e.g., DAMGO and morphine) into the VTA (but not in the NAc)[12,13] enhanced a significant increase of DA release in the NAc,[14-17] and conversely, administration of μ-opioid receptor antagonists (e.g., CTOP) attenuated the release of DA in the NAc.[12] Likewise, chronic administration of endomorphins in the VTA produced a significant effect on the development of locomotor sensitization responses to amphetamine (AMPH).[18] Endomorphin treatment significantly increased the tissue concentration of GLU and its metabolites in several limbic structures (e.g., NAc, mPFCx, CPu) in either EM1-2 plus AMPH-treated rats or AMPH-treated animals, used as controls. These results demonstrated that μ-opioid receptor agonists, including both EM1-2 peptides, induce behavioral sensitization responses in animals mediated via the activation of both GABA and GLU transmission systems in the VTA.[10,19,20]

VIII. ENDOMORPHINS MODULATING LOCOMOTOR BEHAVIORAL RESPONSES

Specific subcortical structures of the brain have been shown to play an important role in the control of movement.[21,22]

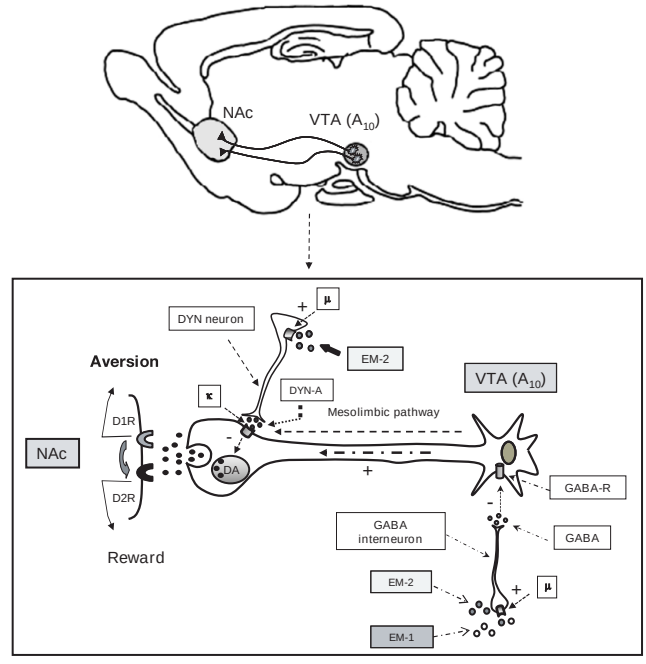

Figure 1. Schematic representation of pharmacological and motivational effects of endormorphin peptides on the reward system of the brain. As depicted in the figure **(upper panel)**; the brain reward system in rodents including humans; comprise of dopaminergic neural pathway that arise from the ventral tegmental area (VTA) and projects their axon fibers into forebrain limbic areas such as, the nucleus accumbens (NAc) and mPFCx (not shown here). This neural pathway mediates the rewarding responses to natural incentives and most drugs of abuse involved in drug addiction. μ-opioid receptors localized at the VTA region, on GABA neurons, have been shown to mediate several of the reinforcing and rewarding effects of different opioid agonists (e.g., morphine, heroin). Endomorphins injected into the VTA, have been shown to reproduce several of the motivational and psychomotor stimulating effects of opioid substances, after binding their cognate μ-opioid receptor localized on GABA cells or on specific *Dynorphin A*-secreting cells **(lower panel)** (see text for specific details) (text and figure were adapted from Fichna et al., 2007, and modified for the present review).

Within the basal ganglia, the globus pallidus contains a subpopulation of neurons that expresses high levels of μ-opioid receptor mRNA.[21,22] μ-opioid receptor agonists have been shown to increase locomotor sensitization responses that are influenced by a number of variables (e.g., ligand concentration, experimental paradigm, and timing of experiment performed). Most studies using psychomotor paradigms have shown that μ- and δ-opioid receptor agonists enhanced an increment of ambulatory responses, whereas κ-opioid receptor agonists produced opposite responses.[23,24] In this context, locomotor responses (e.g., horizontal and vertical locomotor) mediated by activation of the μ-opioid receptor by morphine-binding its cognate μ-opioid receptor[25-27] and grooming behavior mediated by activation of δ-opioid binding sites (localized in the VTA, NAc, and PAG)[26,28,29] have been shown to depend on the synthesis and release of DA from both nigrostriatal and mesolimbic dopaminergic neurons.[30] Interactions between DA and opioid systems in the brain have been extensively reported. These studies showed that opiates (e.g., morphine, heroin) increase behavioral sensitivity responses to DA agonists, enhancing an increase supersensitivity of DA receptors and the expression of stereotypic behaviors mediated by the activation of D1R/D2R-receptor by ligand agonists.[31] For instance, morphine potentiated an apomorphine-dependent climbing behavior in wild-type mice, as opposed to the mutant-μ-opioid receptor knockout mice.[32] Thus, these set of results demonstrated that μ-receptor ligands alter the DA projecting system by potentiating the climbing behavior responses in mice induced after administration of D1R/D2R-ligand agonists.[33]

Endomorphin peptides, like morphine, were found to increase locomotion (horizontal) activity[34,35] without affecting the vertical locomotor activity[19] in mice. These studies showed that low concentrations of EM2 (0.3 and 1.0 μg/animal, ICV administration) induce similar behavioral responses displayed by higher concentrations of EM1 peptide (10-30 μg/mouse, ICV administration). These results led researchers to posit that EM1-2 peptides not only activate different μ-opioid receptor subtypes in the basal ganglia,[36,37] but could modulate different opioid systems (i.e. enkephalinergic, dynorphinergic) that appear to be implicated in the expression of the sensitization responses mediated by endomorphins.[38,39] Similar studies demonstrated that morphine injected into the globus pallidus produced a robust increase in locomotor activity in mice,[40] whereas EM1 induced localized stereotyped behavioral responses (e.g., orofacial dyskinesia).[41] These opioid-mediated behavioral responses led authors to propose that the locomotor activity induced by morphine could be mediated via the activation of δ- and κ-opioid receptors, whereas EM1-inducing inhibitory activities would depend mostly on the activation of μ-opioid binding sites.[41] As shown for the interaction of GABA and several endogenous opioid peptides (e.g., enkephalins),[42] EM1 and GABA could mediate opposite behavioral responses in the control of movement at the globus pallidus. The resulting chemical unbalance induced in both neurotransmission systems could lead to the development of motor dysfunctions and the manifestation of localized dyskinesias.[19]

IX. ENDOMORPHINS REGULATING OPIATE REWARDING RESPONSES

Extensive studies have shown that the chronic administration of μ-opioid-receptor agonists (e.g., DAMGO, morphine, codeine, and sufentanyl)[43-45] produce potent drug- and/or stress-rewarding effects, associated to the development of drug withdrawal symptoms and physical dependence in animals.[46,47] Quite interesting to note is that opposite (non-rewarding) aversion responses in rodents appeared after administration of selective κ-opioid receptor agonists.[48]

Drug-rewarding effects have been shown to be mediated via the interaction between GABAergic neurons and the mesolimbic/VTA-dopaminergic transmission system (see extensive reviews in[49-51]) (figure 1). As described above, opioid receptor agonists inhibit GABAergic inputs to VTA/dopaminergic principal cells that project the NAc, inducing a disinhibitory effect, which in turn enhances a potent release of DA into this limbic structure.[10,52] Endomorphin peptides injected into the posterior area of the VTA induced a conditioned-place preference (CPP), displaying similar behavioral responses to those exhibited by morphine or DAMGO.[53] Moreover, EM1 injected into the VTA produced a potent rewarding effect in rodents exposed to the drug self-administration paradigm. However, injection of same peptide or DAMGO into the NAc produced poor and delayed rewarding effects compared to the VTA-detected responses.[53] These data suggested the absence or poor expression of μ-opioid-receptor sites in this mesolimbic area.[54]

ICV administration of endomorphin peptides shed inconsistent results on the induced rewarding responses. For instance, some authors reported that EM1 mediated a significant CPP, whereas EM2 displayed significant place aversion effects in mice.[55] Conversely, other authors reported that ICV administration of EM1-2 peptides (at low doses, 15 μg) induced significant antinociceptive responses in mice[56] producing no-effects on the CPP paradigm.[57] However, higher doses of EM1 (30 μg) produced barrel rotation of the body trunk, whereas EM2 evoked a significant place preference condition in tested mice.[57] Such discrepancies led authors to posit that the differential behavioral responses mediated after EM1-2 administration could be due to the activation of distinct opioid receptors (e.g., μ- and δ- opioid receptors); to the expression of a different pharmacogenetic background in animals; to the

asymmetric expression of opioid receptor sites in targeted brain areas and cells; and / or to the activation of different molecular mechanism that drive the rewarding effects and behavioral responses to opioid substances.[19]

Moreover, differences in the behavioral effects induced by endomorphin peptides could be due to the activation of μ-opioid receptors expressed in neurons localized at the brainstem PAG region, involved in the generation of aversive behaviors.[58] These effects have been associated to the disruption of the HPA axis, in addition to the deregulation of different mesolimbic transmission systems involved in rewarding functions, as shown in addicted humans exhibiting a history of long-term opiate abuse[59] (figures 1 and 2).

X. ENDOMORPHINS IMPLICATED IN STRESS-INDUCING ALTERED BEHAVIORS

Stressors have been implicated in the development of several psychiatric illnesses, where the HPA axis and endogenous opioid system have been shown to play a crucial role in stress responses. Although the precise role of endogenous opioid peptides and receptors to stress stimuli has not been fully elucidated, over the past years several works showed the existing interactions between stressors, the HPA axis and the endogenous opioid system, (see reference,[19] and references therein). These works showed a close relationship between the levels of the opioid ligands, corticosteroids, pituitary hormone levels, and immune-borne hormones (e.g., cytokines).[19] Activation of the HPA axis by external / internal stressful stimuli (e.g., stress, immune challenge) leads to the increase secretion of corticotrophin-releasing factor (CRF) and Arg-vasopressin (AVP) from the median eminence of the hypothalamus, enhancing the cell-release of ACTH from the anterior lobe of the pituitary.[60] Increased serum levels of ACTH enhance the release of glucocorticoids from the adrenal gland, which exert a negative feedback on pituitary adrenocorticotrophs and limbic regions of the mammalian CNS (e.g., amygdale), enhancing the homeostatic and neuroendocrine balance along the HPA axis.[61] Some authors have postulated that the endogenous opioid system, driven through β-endorphin (binding μ-opioid receptors) at the hypothalamus exerts a potent inhibitory activity on the HPA axis.[62,63] In this context, pharmacological studies showed that acute morphine administration, acting on μ-opioid receptors expressed along the HPA axis,[64] produced an important increase of ACTH and adrenal secretion of corticosterone[65] (figure 2).

Based on the aforementioned results, ICV administration of EM1 or EM2 peptides (10 μg) produced no stimulatory activity on the HPA axis and displayed no neuroendocrine effect on the ACTH and corticosterone secretion.[60] Moreover, EM1 failed to block the stimulatory effect of morphine on the ACTH-induced increased levels of corticosterone in plasma. Furthermore, chronic activation of the HPA axis by exposure of animals to chronic stress paradigms (e.g., chronic inflammatory stress of adjuvant-induced arthritis, the restraint stress model, and the immune-based lipopolysaccharide stress model) showed that plasma corticosterone, ACTH and β-endorphin were dramatically increased, whereas levels of endomorphin peptides showed no detectable changes compared to controls. These data suggested that EM1-2 peptides appear to display no significant roles on the neuroendocrine modulation of the HPA axis, mediating stress responses to challenging stimuli.[60]

Based on that μ-opioid receptors are expressed on pituitary cells and neuronal cells within the hypothalamus, and receptor ligand agonists (e.g., morphine) properly induce a potent activation of the HPA axis, it has been proposed

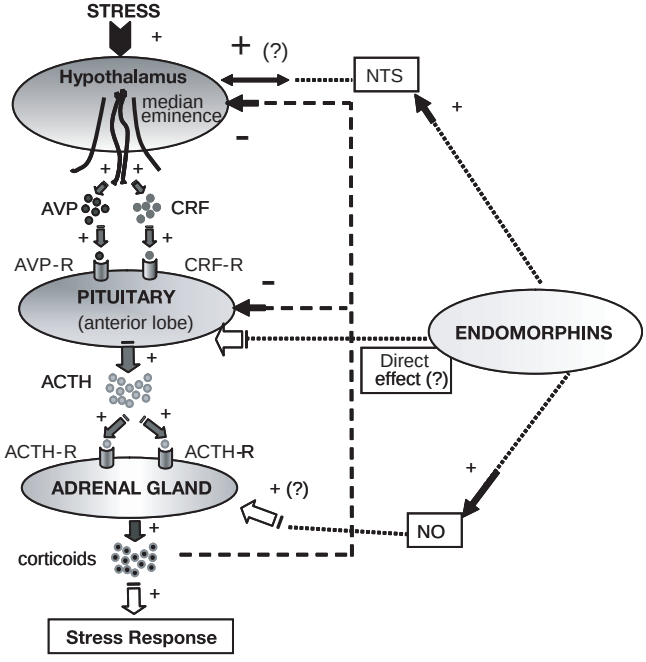

Figure 2. Schematic representation of the potential effects of endomorphin peptides regulating the activity of the HPA axis upon exposure to stressful challenges. As shown stressors (stress) impinging on the hypothalamus enhance the release of stress-related peptide hormones, such as, *Arg-vasopressin* (AVP) and *corticotrophin-releasing hormone/factor* (CRF) from their synthesizing neurons localized at the *median eminence*. Peptide hormones are released from presynaptic terminals into the *hypothalamo-hypophyseal portal system* (not shown here) and reach the anterior lobe of the hypophysis, where they enhance the secretion of substantial amounts of ACTH from ACTH-producing cells or *corticotropes*. ACTH will stimulate the adrenal gland, inducing the release of corticoids. In turn, corticoids will influence negatively on both hypothalamus and hypohysis, forming the neuroendocrine negative feedback loop in response to acute stressors. Several works led to the hypothesis that endomorphins may modulate and/or activate the HPA axis during stress responses (see text for more details) (text and figure were adapted from Fichna et al., 2007, and modified for the present review).

that the lack of endomorphin-induced neuroendocrine effects on the HPA axis could be due merely to the central metabolism or degradation of these peptides (see degradation of EM1-2 peptides in previous paper, part I) (figure 2).

Other plausible explanation offered relies on that μ-opioid receptor agonists display different stimulating properties of Gi/Go protein, as demonstrated in animal models of pain.[38,66] Thus, intracellular molecules may provide important insights into the differential cell-responses of endomorphin peptides modulating several neural systems involved in distinct physiological effects and responses.

For instance, different works showed recently that nitric oxide (a chemical messenger molecule involved in different physiological and pathological processes in mammals) released from cells mediates several physiological responses induced by opioid peptides (e.g., β-endorphin) or morphine[67-69] besides of the aforementioned endomorphin bioactivities (e.g., vasodilatory responses, modulation of HPA axis activity).[35,67] This situation led researchers to propose the term «endomorphin-NO-HPA axis»,[35,70] where endomorphin peptides acting either on hypothalamic neurons, may stimulate the HPA axis; or through the proposed NTS-endomorphin projecting neural system impinging on hypothalamic neuron cells (this neural component has been shown previously to activate directly the HPA axis in animals) (see previous studies in references[71,72]) (figure 2).

Other neural pathways emerging from the PAG have been shown to mediate stress-induced immobility and analgesia in adult rats and rat pups.[73] Pharmacological experiments showed that μ-opioid receptor antagonists (e.g., naltrexone, CTOP) acting on the ventrolateral PAG blocked several analgesic responses mediated by endogenous opioids, providing evidences that endomorphins could mediate PAG-inducing stress-related analgesic effects in rats, as shown for different μ-opioid receptor ligand agonists.[73] Thus, these neural and neuroendocrine driving mechanisms provide important insights that endomorphin peptides might be directly or indirectly involved in the activation of the HPA axis, enhancing the release of CRH from PVN / hypothalamic neurons.[74,75]

XI. ENDOMORPHINS INVOLVED IN MOOD-RELATED AND PSYCHIATRIC DISORDERS

Over the past decades extensive studies demonstrated that ICV (central)[76,77] or IP (peripheral)[78,79] administration of μ-opioid receptor agonists (e.g., morphine) produce anxiolytic responses, whereas μ-opioid receptor antagonists promote anxiogenic effects.[80] These responses were shown to be mediated through the interaction of the EOS and the GABAergic system (e.g., GABA and BZD);[81,82] the monoaminergic (e.g., 5-HT, BZD) and peptidergic systems.[19] Moreover, these pharmacological and behavioral studies demonstrated that the anxiolytic and anxiogenic activities of opiates substances are dose- and site-dependent after their local administration into the rat neural tissue. For instance, low doses of morphine injected into rat midbrain tectum induced anxiolytic–like responses, whereas the injection of higher doses displayed anxiogenic-like effects.[83] Converserly, morphine injected into the dorsal PAG[84] or lateral septum of the rat brain[85] has been shown to produce aversive responses. Previous reports describing the neuroanatomical co-localization between endomorphins and μ-opioid receptors in both limbic and brainstem regions and nuclei in the CNS of rodents,[86,87] led to postulate the hypothesis that EM1-2 could modulate mood-related behaviors (e.g., anxiety and stress-related behaviors) in animals and humans.[19] For instance, ICV administration of EM1 into mice induced an increased anxiolytic behavior responses in the elevated plus maze[88] supporting previous observations that administrations of μ-opioid receptor agonists in humans produce anxiolytic symptomology (e.g., drowsiness, warmth feelings, and sensation of well-being).[80]

Earlier findings showed the expression of high density μ-opioid receptors and high concentration of endogenous opioid ligands (e.g., β-endorphin) in limbic areas of animals exposed to stressful challenges.[89-92] These data led authors to postulate that the EOS and endomorphins play a crucial role in modulation in psychiatric disorders,[93] such as depression and schizophrenia,[94-98] in spite of the absence of a clear therapeutic benefit of opioid ligands to treat mental illnesses.[19] Molecular and behavioral studies showed that knockout mice lacking the μ-opioid receptor display altered emotional states consistent of depressive-like behaviors, similar to those studies that have extensively demonstrated the use of a wide variety of μ-opioid receptor agonists, as antidepressant agents (e.g., oxycodone and oxymorphone)[99] for treating depressive symptoms among many other mental illnesess.[100-102] For instance, morphine has been used as an antidepressant-like agent to relieve stress behaviors in experimental animals.[103] These data demonstrate the importance of the EOS in the etiology of mental disorders, besides of the controversial issue on the clinical use of μ-opioid receptor agonists as therapeutical agents to relieve psychiatric disorders.[19]

Based on the immunoreactive co-localization of EM1-2 peptides and μ-opioid receptors in both forebrain (e.g., septum, NAc, amygdala, thalamic nuclei) and brainstem regions (e.g., LC) in the CNS of mamamals, and shown previously with regard to their functional implication in the pathophysiology of depression;[86,104,105] several authors have postulated the importance of these amide tetra-peptides in the etiology of depressive disorders, based on their potential antidepressant-like effects observed in animal models of depression and stress.[19] The antidepressant-like effects detected for both EM1-2 peptides

(0.3–30 μg / animal, ICV) in mice were dose-dependent and short-lasting (enduring only 10-15 min after their brain administration).[19] The magnitude of the antidepressant responses displayed by both peptides was comparable to the several compounds shown to display potent antidepressant activities.[106,107] These studies provided strong evidences that these opioid peptides, like conventional antidepressants, may generate antidepressant-like responses (reducing behavioral-immobility in paradigms of stress and depression [e.g., forced swimming test, FST])[108] and which may be blocked by μ-opioid receptor antagonists (e.g., naloxone, β-funaltrexamine) but not with selective δ- or κ-opioid receptor antagonists (e.g., naltrindole, nor-binaltorphimine, respectively).[19] Additional studies regarding the implication of EM1-2 in the pathophysiology of depression showed that ICV administration of endomorphins (e.g.,20-50 μg / animal), into rats induces a dose-dependent up-regulation of BDNF mRNA expression in limbic areas of the rat brain (mPFCx, hippocampus, amygdala) that was blocked by specific μ-opioid receptor antagonists (e.g., naltrexone), but not with specific δ-opioid receptor antagonists (e.g., naltrindole).[109]

This neurotrophic factor (BDNF) has been shown to modulate primordial functions, such as neuronal survival, differentiation, and plasticity,[110] and shown recently to play an important role in the therapeutic actions of several antidepressants acting on different neurotransmission systems.[111-115]

XII. ENDOMORPHINS MEDIATING FOOD-INTAKE BEHAVIOR

The neural pathways and transmission systems that regulate food-intake behavior in mammals are complex.[19] Several regulatory peptides (i.e., NPY, GHRH, 26RFa peptide) have been shown to display orexigenic activities in the brain.[116-119] In a similar context, μ-opioid receptor ligand agonists (e.g., morphine, DAMGO), including the active morphine metabolite (e.g., morphine-6β-glucuronide), have been shown to display an orexigenic activity[120-123] regulating gustatory neural pathways arising from the NTS neurons[124,125] (a neural pathway projecting to hypothalamic areas and other limbic structures).[126] In a similar context, ICV administration of EM1 or EM2 (0.03–30 nmol) produced a dose-dependent food-intake behavior in non food-deprived mice for up to 4 h after peptide injection.[88] These EM1-2 mediated effects were attenuated by the specific μ-opioid receptor antagonist, β-funaltrexamine.[127,128] However, the endogenous κ-opioid receptor ligand peptide, Dynorphin A (DYN A), was shown to display a potent stimulatory food-intake bevavior compared to EM1-2 peptides. These results posit that the orexigenic activity induced by EM1-2 peptides appears to be mediated, via activation of κ-opioid receptors, where μ-opioid receptors appear to play a minor role in this opioid and non-opioid dependent physiological activity.[19]

XIII. ENDOMORPHINS MODULATING SEXUAL BEHAVIOR RESPONSES

Extensive studies demonstrated the direct and indirect effects of endogenous opioid peptides (e.g., β-endorphin) on gonadal hormones, regulating both sexually-induced behaviors (e.g., lordosis) and reproductive functions in female rats.[129-131] These effects have been shown to be mediated through the activation of μ-opioid receptors expressed along the limbo-hypothalamic neural circuits that mediate the release of gonadal hormones (LH, FSH) from the pituitary and the release of its releasing peptide hormone (LHRH) from the hypothalamus.[132] LH / FSH have been extensively shown to influence female rat sexual behavior,[133] besides of modulating the release and expression of sexual steroids during mating behavior.[134] Based on the neuroanatomical distribution and cell expression of μ-opioid receptors within specific hypothalamic and mesencephalic regions that coordinate and regulate female reproductive behavior (e.g., VMH, mPOA, MCG),[104,135] several authors showed that μ-opioid receptor ligand agonists produce dual effects on lordosis in hormonally-primed female rodents during mating.[135,136] In a similar context, ICV or local administration of low doses of β-endorphin into the MCG or mPOA produces a potent inhibition of lordosis in gonadectomized, steroid-primed female rats.[137-140] Converserly, similar route of administration of high doses of β-endorphin or morphiceptin facilitated lordosis in estrogen- or estrogen / progesterone primed rats.[137,138,141-143] Moreover, ICV administration of EM1-2 peptides into the third ventricle or bilateral infusion into the diagonal band (DB) (septum-horizontal limb of the diagonal band, MS-HDB, an area shown to project axon fibers to the mPOA of the hypothalamus)[144] produced dose- and time-dependent lordosis responses in female rats[135] which was attenuated with naloxone.[134] However, similar responses were not detected when peptides were injected into VMH, mPOA or MCG.[134] These results led authors to propose that EM1-2 peptides modulate[104,145] the release of active neuropeptides (e.g., LHRH) and non-peptide (Ach, GABA) transmitters within the MS-HDB, inducing their opioid-dependent behavioral effects.[19]

XIV. ENDOMORPHINS INVOLVED IN LEARNING AND MEMORY PROCESSING

The implication of the EOS among several other brain transmitters in learning and memory has been extensively

studied and reported elsewhere.[19] For instance, the EOS has been shown to play important roles in operant and classic conditioning and different cognitive tasks, including memory processing.[19] These studies showed that either μ-opioid receptor (e.g., DAMGO and Tyr-D-Arg-Phe-β-Ala) and δ-selective opioid receptor (e.g.,D-Pen_2,L-Pen_5-enkephalin and D-Ala_2-deltorphin II) agonists, respectively, induced an impairment of both short-term and long-term memory processing in mice exposed either to passive avoidance paradigms[146-148] or spatial memory tasks.[149] Conversely, μ-opioid receptor antagonists enhanced memory retention in animals exposed to different learning tasks.[150] Similarly, κ-opioid receptor agonists (e.g., dynorphin A_{1-13}) have been shown to attenuate aberrant learning and memory processing in rodents exposed to aversive and non-aversive memory tasks.[151]

With regard to endomorphin molecules, a single report from Ukai et al.[152] showed that endomorphins impaired short-term memory processing in mice exposed to spontaneous alternation performance task. Whereas both tetrapeptides induced an important inhibitory activity on long-term memory processing when tested in passive avoidance learning task in mice,[153,154] EM2 peptide was shown to mediate its memory attenuating effect[155] by inducing an opioid receptor dependent cytosolic and mitochondrial protein synthesis mechanism in the lobus paraolfactorius in chicks (a brain area structurally related to the caudate putamen in mammals).[156,157] These studies showed that EM2 reverted the amnesic effects induced by anisomycin administration into chicks, and blocked the inhibitory effect on protein synthesis induced by this drug in this related striatal-brain structure.[155] However, other authors proposed that this EM2-dependent inhibition of passive avoidance learning task resulted from a functional disconnection of the hippocampus, a brain area known to be crucially important for processing and conversion of short-term memories into long-term memories (see reference[158]). Besides of the aforementioned studies, several authors proposed that both cholinergic and dopaminergic transmitter systems could mediate or participate in the opioid peptide-induced long-term memory impairment, although their exact roles have not been clearly elucidated.[19] In this context, behavioral and pharmacological studies showed that spatial working memory requires at least the interaction between μ-opioid receptor ligand agonists (including both EM1/EM2 peptides) and the ACh transmission system.[159] Cellular studies demonstrated that endomorphin peptides decreased significantly the release of ACh from neuronal cells in brain areas associated with memory processing and storage[153,154,160] and that physostigmine (a cholinesterase inhibitor) reverted the endomorphin induced passive avoidance learning impaired response.[146,154]

Besides of the interaction between ACh and opioid system, several authors showed that D2-receptor antagonists were capable of attenuating the EM2-induced passive avoidance learning impairment in rodents.[153] These results suggested that the inhibitory effect of EM2 would be mediated from stimulation of heterosynaptic D2 receptors expressed in dopaminergic neurons innervating both the striatum and the NAc, during acquisition and consolidation of memory.[153] In addition, ICV administration of EM1-2 peptides was shown to increase BDNF mRNA expressions in the hippocampus and amygdala.[109] This trophic factor, acting via its neuronal NT-3 receptor subtype, has been shown to mediate several plastic events in the brain, such as development and establishment of long-term potentiation (LTP) in hippocampal neurons; morphologic changes in active synapses and neurons in brain regions involved in learning and memory processing (e.g., hippocampus and cortex).[161,162] Thus, based on the information described above, endomorphins acting via μ-opioid receptors could be implicated in learning and memory processing in several areas of the brain regulating BDNF activity on neuronal cells.[19]

XV. ENDOMORPHINS REGULATING CARDIOVASCULAR AND RESPIRATORY BIOACTIVITIES

Over the past years it has been shown that several regions along the rostracaudal axis (e.g., VLM, NTS, LH, PVN) of the rat brain, including the dorsal hippocampus and limbic system, regulate cardiovascular and respiratory bioactivities, areas shown also to express a high density of μ-opioid receptors in neurons within each functional brain region.[19]

1. Cardiovascular effects

Although several works have acknowledged the important role of the EOS mediating cardiovascular responses, the effects of distinct opioid receptor ligand agonists on blood pressure and heart rate have been unclear and confusing. For instance, local injections of μ, δ and κ-opioid receptor agonists into the specific areas of the rat brain (e.g., PVN, DH, RVLM) in normotensive and/or hypertensive animals, were shown to reduce heart rate and blood pressure,[163] whereas ICV administration of μ-opioid receptor agonists (e.g., morphine, β-endorphin, DAMGO) produced hypotension in different species.[93,164-166]

In a similar context, ICV or IV administration of EM1-2 produced a significant reduction of heart rate and blood pressure in normotensive and hypertensive rats[167-170] showing a reversible and dose-dependent biphasic change in systemic arterial pressure[165] upon administration of μ-opioid receptor antagonists (e.g., naloxone or β-funaltrexamine). These results suggest that endomorphin peptides produce their cardioavascular responses via μ-opioid binding sites.[164,168-170] However,

differences in the administration route of EM2 into rats (IV versus ICV) produced different graded responses,[171] which led authors to suggest that although peripheral μ-opioid receptors might play an important role in the opioid peptide-inducing hypotensive effects in animals,[172] the mechanisms by which endomorphin peptides mediate both peripheral and central cardiovascular activities are not completely elucidated.

This assumption is based on previous results that demonstrated, on the one hand, that bradycardia results from activation of the vagus nerve, and bilateral vagotomy (or atropine) abolished EM1 effect on heart rate in rats, suggesting thus, that EM1-2 mediate their vascular effects via activation of vagal afferents.[171] On the other hand, EM1-2 peptides which exert potent inhibitory activities on neurons,[173-175] their cellular activities are expected to increase blood pressure and heart rate due an increase response on neuronal firing on cardiovascular regulatory areas at both medulla and cervical spinal cord.[176-178] Recent studies showed that infusion of EM2 into the rat NTS (mNTS) attenuated reflex responses upon stimulation of the carotid sinus and aortic baroreceptors,[176-178] showing that depressor and bradycardic responses resulted from a peripheral inhibitory effect on the baroreflex response and from the excitatory activity of mNTS neurons. These authors proposed that EM2 mediated its cardiovascular responses after being injected into the mNTS (a brainstem area control under local inhibitory-GABAergic neurons[179,180] and glutamate [GLU] projecting neurons descending from insular cortex)[181,182] through direct activation of μ-opioid receptors expressed on mNTS-GABA neurons, producing an hyperpolarization response, decreased of GABA release and increase of GLU release from presynaptic terminals, enhancing an overall reduction of GABAergic activity on mNTS postsynaptic neurons (disinhibitory effect) leading thus, to a final increase of neuronal excitability of mNTS neurons.[19,176-178]

Moreover, EM2 acting on μ-opioid receptors expressed on glutamate baroreceptor afferent terminals in response to baroreceptor stimulation were shown to decrease GLU release, resulting in attenuation of the baroreflex activity.[19] Despite that the depressor and bradycardic responses induced by EM2 could be explained through the aforementioned cellular mechanisms,[19,183] the endogenous mechanisms by which EM1-2 induced vasoactive responses are still unclear and further research may clarify this issue. However, several authors proposed different mechanisms by which endomorphins may mediate their hypotensive responses. One proposed mechanism is through the intracellular stimulation of NO synthesis[164,184] and NO release from the vessels[185] after binding their cognate opioid receptor on endothelial cells. Other mechanisms proposed is through the hyperpolarization of vascular smooth muscle cells and/or the cell-release of vasodilator prostanglandins or via an endomorphin inhibition of presynaptic release of NA from nerve endings distributed along vessel walls.[172] Whichever the mechanisms involved, none of these have been completely confirmed, which requires further research to clarify the exact endogenous opioid-dependent mechanism.

2. Respiratory effects

Several studies demonstrated that μ-opioid receptor agonists induce a potent respiratory depression.[186] Opioid-receptor agonists, such as morphine (see reference[19] and references therein), heroin,[187-189] fentanyl,[190,191] buprenorphine,[192-194] and DAMGO[171,195] have been shown to induce a respiratory depression by decreasing the sensitivity of brainstem nuclei regulating-respiratory activity to carbon dioxide, followed by a decrement of respiratory rate (see reviews in[46,196,197]).

Although immunochemical studies showed that both EM1-2-LI[198] and μ-opioid receptors[199,200] appear to be co-localized at specific neuroanatomical areas and nuclei of the brainstem of mammals (e.g., NTS and PBN)[104] shown to play a crucial role in the respiratory control,[19] very few studies have focused on the effects of endomorphin peptides on respiratory activity. For instance, IV administration of supra-analgesic doses of EM1 and EM2 peptides in rats produced biphasic responses, characterized by rapid initial ventilatory depression (inhibitory effect that lasts for 4-6 s) followed by an increase ventilation activity (excitatory effect that lasts for 10-12 min).[201] In contrast to the monotonically-induced decreased ventilation activity by morphine, EM1-2 appear to mediate their depressant respiratory activity via activation of central μ-opioid receptors, based on that methylnaloxone (a peripheral restricted μ-opioid receptor antagonist) was unable to block peripherically the opioid effects, whereas naloxone prevented the respiratory-depressed activity induced by endomorphin peptides.[201] Interestingly, EM1-2 peptides administered (I.V.) at doses higher above their corresponding analgesic threshold or higher above their respiratory depression-threshold dose (as shown for DAMGO or morphine) showed to attenuate hypercapnic ventilatory responses in rats, producing a respiratory depression in tested animals.[201] Although EM1-2 peptides induced a weaker depression activity on ventilatory responses compared to the hypercapnia effects induced by either DAMGO or morphine,[201] the reduced effects mediated by EM1-2 peptides have been extensively discussed based on several pharmacokinetic and pharmacodynamic parameters and factors discussed elsewhere.[19,201] In this context, the increased ventilation activity induced by EM1-2 peptides appeared to be mediated through a central non-opioid mechanism, based on that conventional μ-opioid receptor antagonists were unable to block the increased respiratory response.[201]

Interaction of endomorphins and other neuro-transmission systems regulating respiration activity[202,203]

showed that both EM1 and EM2 produced dose-dependent inhibition of tachykinin-mediated contractions of the guinea pig bronchus, with the exception of EM1 activity which was blocked by naloxone, whereas that of EM2 was not antagonized by any μ-opioid receptor antagonist.[202]

In the same context, Patel et al.[203] showed that EM1-2 peptides produced a dose-dependent inhibition and naloxone-reversible antagonism activity on cholinergic induced-contractile responses of the guinea pig trachea. These studies showed that EM1-2 peptides, acting on μ-opioid receptors distributed in rodent airways,[204] interact with both cholinergic and tachykinergic fiber types.[202,203] Thus, EM1-2 peptides were shown to induce a potent inhibition on the electrically-evoked release of ACh from cholinergic nerves innervating guinea pig trachea,[203] including the release of NA from nonadrenergic postganglionic nerve fibers innervating the airway-smooth muscle cells.[203] Recent studies showed that ICV administration of EM1 in mice induced an increased oxygen consumption that was blocked by naloxone, suggesting a μ-opioid receptor peptide dependent effect.[205]

XVI. ENDOMORPHINS INVOLVED IN GASTROINTESTINAL ACTIVITY

Opioid agonists regulating gastrointestinal activity have been extensively reported. IHC techniques and binding studies, using neuromuscular preparations, showed that μ-opioid receptors may be localized in smooth muscle cells and neurons, as well[206] where μ-opioid receptor agonists (e.g.,morphine) have been shown to exert, in a naloxone-reversible fashion, their inhibitory effects on GI activity and/or motility,[207-209] besides of modulating the evoked release of neurotransmitter release from nerve terminals (e.g., ACh, NA)[209-211] and influencing peristaltic reflex.[207]

In a similar context, EM1-2 peptides were shown to modulate different GI activities. For instance, application of a concentration range of EM1-2 peptides (10^{-12} M to 10^{-6} M) on a specific guinea pig ileum preparation (longitudinal muscle-myenteric plexus preparations from ileum) produced a dose-dependent- and CTOP-reversible inhibition of the amplitude of electrically-induced twitch ileum contractions.[212] However, EM1-2 peptides failed to inhibit muscle contractions induced by ACh stimulation. Moreover, both peptides displayed a potent inhibitory effect on the ascending excitatory reflex and increased stimulation of the descending inhibitory reflex, inducing an increase latency on the onset of ileum muscle-contractile responses.[213] Similar inhibitory responses were detected at the smooth and striated muscles of the rat esophagus.[214]

Overall, these results led authors to suggest that EM1-2 induce their inhibitory GI effects, via activation of μ-opioid receptors localized either in presynaptic terminals of non-adrenergic/non-cholinergic inhibitory neurons and/or in myenteric-plexus neurons that innervate smooth-muscle cells.[212,214] Activation of μ-opioid receptors may lead to a reduced release response of different neurotransmitters (e.g., NO, VIP), including ACh and NA from local and myenteric neurons, respectively.[210-213]

XVII. ENDOGENOUS OPIOIDS AND SOCIAL BEHAVIORS

A clear example of stress-inducing increased HPA activity occurs in foraging and defensive behaviors in animals.[215] For example, after social defeat, subordinate animals display physiological, neuroendocrine, neurochemical and behavioral changes induced by the endogenous stress-driving mechanisms in socially-interacting species.[216] These behavioral and functional changes have been suggested to be highly connected to the development of fear, anxiety, depression, and panic disorders,[217,218] including the development of drug-seeking and drug-taking behaviors in both animals and humans.[219] Moreover, few studies have shown that μ-opioid receptor results to be up-regulated in restricted regions of the rat brain (e.g., VTA) after social defeat.[220] ICV administration of EM1 into Syrian hamsters failed to inhibit the consolidation of conditioned defeat (without stimulating locomotor activity or inducing sedation),[221] whereas morphine impaired the consolidation of newly acquired memories in rats and mice.[222-225] These authors suggested that such reduced or failed behavioral responses mediated by EM1 could be due to cellular responses and pharmacological activities mediated through the binding of the peptide to its cognate receptor (see detailed explanations in[19] and references therein). For instance, morphine, DAMGO, besides of other potent endomorphin peptide analogs (e.g., Tyr-D-Arg-Phe-β-Ala),[88,226,227] have been shown to inhibit memory retrieval, increase anxiolytic responses and produce fear-conditioning responses in animals.[77,78,228,229] Such responses appear to depend on the activation of NA neurons and NA neural system.[230]

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RESPUESTAS DE LA SECCION AVANCES EN LA PSIQUIATRIA

Autoevaluación

1. C
2. A
3. A
4. A
5. C
6. B
7. B
8. A
9. A
10. D
11. C
12. D