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Neurotransmitter role of endogenous morphine in CNS

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Summary

Endogenous morphine is present in the mammalian brain as determined by gas chromatography/mass spectrometry. The criteria essential for satisfying the definition of morphine as a neurotransmitter are examined. The detection of endogenous morphine-like compounds inside brain neurons by immunocytochemistry and the Ca^{+} dependent release of endogenous morphine from rat brain slices provide evidence for its transmitter status. Indirect evidence that endogenous morphine modulates thermonociception and weakens memory through mu opioid receptors again supports a neurotransmitter role for this chemical messenger. Evidence has been found for its endogenous synthesis in animal tissues as well. These findings indicate that endogenous morphine might function as neuromodulator/neurotransmitter agent in the CNS.

key words: **endogenous morphine • neurotransmitter • opiate receptor • mu3 opiate receptor subtype • nitric oxide • amygdala • hippocampus • memory**

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Background

A morphine-like material in animal tissues was originally demonstrated by immunological recognition [1]. Subsequently, endogenous morphine-like compounds have been identified in mouse and calf brain [2,3]. The molecular structure of the HPLC purified compound was confirmed as morphine by liquid and gas chromatographic retention times and mass spectrometry in various tissues such as bovine brain, rat and mouse brain, hypothalamus and adrenal glands, mammalian lung, invertebrate and human tissues, human cerebrospinal fluid and human plasma [4–16]. Codeine and morphine also have been identified in human cerebrospinal fluid [14] and non human primate brain [17]. Morphine precursors, reticuline [18], thebaine [19] and codeine [20–22] are some of the main intermediates of morphine biosynthesis in the poppy plant and have been found in mammals.

The pathway for morphine biosynthesis has been established in the opium poppy and animal tissue. In this regard, radiolabeled reticuline was transformed into salutaridine by rat liver microsomes *in vitro* and a similar conversion has been observed *in vivo* [23]. The conversion of salutaridine, thebaine and codeine into morphine has been demonstrated in several rat tissues, including the brain, providing evidence for the biosynthetic pathway of endogenous morphine (eM) in mammals [5,24]. Incubation of human neural cancer cell lines with L-tyrosine leads to the formation of morphine as well [25]. Addition of reticuline to intact invertebrate ganglia *in vivo* leads to the formation of morphine [26]. Ascaris suum, a mammalian parasite produces morphine in mammalian hosts and *in vitro,* a phenomenon that may dull the host discomfort so that the parasite can escape host surveillance [27].

Immunocytochemical Localization of Endogenous Morphine in Mammalian Brain

Morphine-like immunoreactivity has been demonstrated in cell bodies, fibers and terminals of neurons in different brain areas of the rat [28], mouse [2] and man [29] by indirect immunofluorescence and unlabeled antibody enzyme procedure. The antisera revealed numerous nerve cell bodies with variable morphology, dendrites and nerve fibers throughout the cerebral cortex. In the caudate putamen numerous medium size immunoreactive perikaria were visible. Nerve cell body and fibers were stained in the hyppocampal formation; dot-like immunoreactivity was visible around granule cells of dentate gyrus and dorsal hippocampus; immunoreactive nerve cell bodies were also revealed throughout the brainstem. In the cerebellum numerous granule cells in the granular layer and small neurons and fibers in the molecular layer are immunoreactive. In human brain tissue, morphine-like immunoreactivity was identified in cerebral and cerebellar cortex. Morphine positive terminals were observed in rat hyppocampal formation sometimes forming synaptic contact with the soma of unlabeled cells [29]. In different regions of the brainstem positive morphine-like nerve fibers apposed to μ opiate receptor (μOR) immunoreactive cell bodies were observed (Figure 1).

Morphine Release

Neurotransmitters and neuromodulators are released from neurons by depolarization in a Ca^{++} dependent manner. Neuron depolarization by high potassium concentrations caused the release of endogenous morphine from eel chromaffin cells [20]. The release of endogenous morphine from rat brain slices was observed *in vitro* in the presence of high potassium concentration; this effect disappeared when Ca^{++} was omitted from the superfusion medium [7]. Furthermore, exogenously applied morphine can affect the release of other neurotransmitters, i.e., dopamine, in nervous tissues suggesting a similar role for endogenous morphine [30].

Morphine Uptake

The slow i.c.v. infusion of [^{3}H]morphine demonstrated the capacity of specific brain neurons to store the alkaloid [28]. Otherwise synaptosomes from mid-brain, cerebral cortex and cerebellum failed to take up and store any trace of the tritiated alkaloid [28]. However, this finding also suggests that an alternate degradation process may be operating. The major pathway for the detoxication of exogenous morphine is conversion of morphine to morphine 6 glucuronide and/or morphine 3 glucuronide. The presence of morphine 6-dehydrogenase in mammalian liver [31] suggests the metabolic pathway of morphine to morphinone as a route in endogenous morphine metabolism. Thus, endogenous morphine conversion may represent the way to terminate its action.

Role of Endogenous Morphine in The Modulation of Thermal Nociception

The depletion of endogenous morphine in mouse brain was obtained by immunoneutralization of this molecule from brain extracellular spaces. An affinity purified anti-morphine IgG was administered to mice, which then underwent the hot plate test. As shown by the licking latency in the hot plate test, the nociceptive threshold in response to thermal stimulus was significantly lowered by anti-morphine IgG with respect to controls. We attributed this effect to a decrease in endogenous morphine levels present in mouse brain extracellular spaces [8]. Endogenous morphine depletion attenuated the antinociceptive effect of the mu selective agonist DAMGO in the hot plate test whereas enkephalin or DPDE antinociceptive effect was unmodified [8]. Therefore, endogenous morphine appears to be involved in the modulation of thermonociception in mice via the μOR receptor [8].

Role of Endogenous Morphine in Weakening Memory Processes Under Stress Condition

Classically, exogenous morphine administration impairs memory processes [32] and a highly significant regression coefficient between analgesia and memory impairment was found after opiate administration [33]. Twelve hour food deprivation (fasting) induced a stress condition, which does not interfere with motor coordination, spontaneous motility and exploratory activity. An anti-morphine IgG was administered to mice in order to induce morphine depletion in extracellular spaces. Fed and stressed animals were both administered with anti-morphine IgG and submitted to passive avoidance test to study the effect of endogenous morphine depletion on working memory [34]. Fed animals were used as a control. Acquisition and consolida-

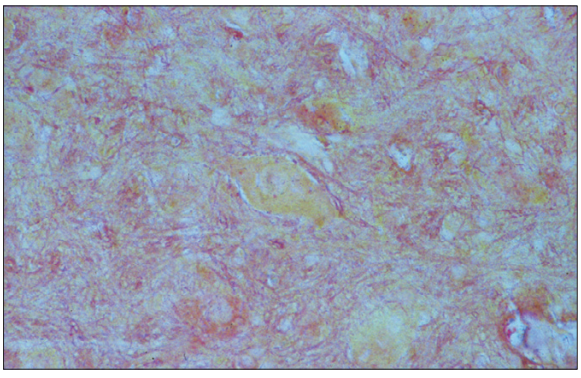

Figure 1. Bright field of coronal section of mouse brainstem double stained with anti-μOR (brown) and anti-morphine (violet) IgG. Morphine-like immunoreactive nerve fibers are closely apposed to a μOR positive neurons.

tion of memory was significantly impaired in stressed animals with respect to control. This was demonstrated by a decrease in entry latency into the dark room in the retention session of the passive avoidance test. This effect was reversed to the basal value after endogenous morphine depletion. Therefore, endogenous morphine may be involved in weakening memory processes under stress conditions [34]. This may provide a strategy for limiting future hesitancy when action is called for.

Mu Receptor Interaction: Postsynaptic Response System

Recently the μ_3OR has been cloned in human neural (cell line), immune and vascular tissues [35]. This μ_3OR splice variant is morphine selective and opioid peptide insensitive and is coupled to nitric oxide release [36]. In rat limbic tissues (hippocampus and amygdala) morphine is present as demonstrated by biochemical, including mass spectrometry, and immunocytochemical techniques [28,37,38]. Additionally, adding exogenous morphine to these tissues results in constitutive nitric oxide release, which does not occur with opioid peptides, indirectly demonstrating the μ_3OR subtype [36]. Taken together, a highly specific postsynaptic receptor exists in these tissues for morphine.

Criteria for defining a chemical messenger as a neurotransmitter exists. These criteria are the following: 1) The chemical must be produced and found within a neuron; 2) When a neuron is depolarized, it must release the chemical in a Ca^{++} dependent manner; 3) When released, the chemical messenger must act on a post-synaptic receptor and cause a biological effect; 4) After exerting its action, the chemical messenger must be inactivated via a reuptake mechanism or by an enzyme that terminates its action; and lastly, 5) If the chemical messenger is applied on the post-synaptic membrane, it should have the same effect as when it is released by a neuron. Taken together, the earlier noted studies demonstrate that morphine functions as a neurotransmitter.

Conclusions

Reticuline [18], thebaine [19] and codeine [12,20,38] are some of the main intermediates of morphine biosynthesis in the poppy plant and these compounds have been found in mammals and invertebrates, especially in their neural tissues. Radiolabeled reticuline was transformed into salutaridine by rat liver microsomes *in vitro* and a similar conversion has been observed *in vivo* [23]. The conversion of salutaridine, thebaine and codeine into morphine has been demonstrated in several rat tissues, including the brain, providing evidence for the biosynthetic pathway of endogenous morphine in mammals [5,24]. Reticuline exposure to invertebrate ganglia leads to morphine formation as well [26]. Presently morphine appears to be endogenously synthesized by brain microsomes even if microsomes are intracellular membrane structures belonging to different brain cell type. In a neural cancer cell line L-tyrosine exposure leads to morphine formation [25]. Morphine immunoreactivity has been demonstrated inside the cell body, fibers and terminals of neurons in different brain areas of the rat [28], mouse [2], humans [29] and invertebrate neural tissues [39]. We also have demonstrated that endogenous morphine can be released in a chemically detectable form from rat brain slices in a Ca^{2+} and K^{+} dependent manner, fulfilling a criterion for endogenous morphine as a neurotransmitter [12]. When endogenous morphine was depleted from mice brain extracellular spaces, acute thermonociception was increased and working memory weakness, a classic effect of exogenous morphine administration, was impaired [8,34]. Both these effects appeared to be mediated through μOR. We failed to observe an active uptake of [^{3}H]morphine by rat brain synaptosomes [28]. However, its enzymatic conversion to different metabolites may satisfy this criterion. A novel μ_3OR exists, which is coupled to nitric oxide release and only responds to morphine. This occurs in both human, mammalian and invertebrate tissues. Endogenous morphine and the μ receptor are associated throughout the phylogenesis of the nerve system of mammal and lower animals, suggesting that this molecule is a physiological ligand of the μ receptor.

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