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Melatonin exerts its analgesic actions not by binding to opioid receptor subtypes but by increasing the release of β-endorphin an endogenous opioid

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Abstract

The occurrence of systematic diurnal variations in pain thresholds has been demonstrated in human. Salivary melatonin levels change following acute pain when other factors that could explain the change have been removed or controlled. Melatonin-induced analgesia is blocked by naloxone or pinealectomy. By using selective radioligands [3H]-DAMGO, [3H]-DPDPE, [3-U69593, and 3H]-nociceptin, we have shown that the bovine pinealocytes contain delta and mu, but not kappa or ORL1 opioid receptor subtypes. In the present study, by using melatonin receptor agonists (6-chloromelatonin or 2-iodo-*N*-butanoyl-5-methoxytryptamine) or melatonin receptor antagonist (2-phenylmelatonin), we have shown that these agents do not compete with opioid receptor subtypes. However, we observed a time-dependent release of β-endorphin an endogenous opioid peptide, by melatonin from mouse pituitary cells in culture. Hence, it is suggested that melatonin exerts its analgesic actions not by binding to opioid receptor subtypes but by binding to its own receptors and increasing the release of β-endorphin.

Keywords: Pineal gland; Melatonin; Opioid receptor subtypes; Enkephalin; β-Endorphin; Naloxone

1. Introduction

The term “pain” applies both to an unpleasant experience linked to a physical damage and to the feeling of emotional suffering accompanying anxiety and depression. Pain is applied to a variety of subjective phenomena ranging from the perception of noxious stimuli to excruciating pain associated with terminal cancer, thalamic syndrome, or trigeminal neuralgia. Therefore, pain is considered to be a multifactorial phenomenon in which biochemical, humoral, neurophysiological, and psychological factors are integrated.

Primary afferent fibers transmit sensory information and have central processes that terminate primarily in the dorsal horn of the spinal cord and brain stem. Activation of primary afferent nociceptors results in the release of several neurotransmitters and neuromodulators including substance P, calcitonin gene-related peptide, neurokinin A, and glutamate [26]. There are many receptor subtypes that have been implicated in transmission of nociceptive information in the dorsal horn, which include alpha-2 adrenoreceptors, tachykinins (substance P, neurokinin A), opioid (mu and delta), and glutamate (AMPA and NMDA) receptors. The production of nitric oxide may take place secondary to activation of NMDA receptor and influx of Ca^{2+} [31].

It has long been recognized that microglia migrate to differentiate and proliferate at sites of brain injury and inflammation, and activation of mu opioid receptors inhibits microglial cell chemotaxis [8]. It is interesting that interleukin-2 involved in inflammatory processes exerts analgesic actions [46]. Functional relation exists between mu opioid receptors

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and alpha-2 adrenergic receptors, and clonidine, an alpha-2 adrenergic agonist, is analgesic in nature. Opioid receptor immunoreactivity is found in substance P containing neurons, and delta opioid receptors modulate the release of substance P. Mu opioid receptors are localized in GABAergic neurons, and flumazenil, a benzodiazepine receptor antagonist, enhances the analgesic action of morphine [16]. Furthermore, opioid receptor modulate NMDA receptor-mediated actions [30].

Present treatments for acute and/or chronic pain rely on non-steroidal anti-inflammatory agents and norcotic analgesics. However, there are various persistent painful conditions such as neurodegenerative diseases that respond poorly to existing analgesics and hence await novel therapeutic avenues. Physicians have noted since antiquity that their patients complained of less pain and required fewer analgesics at night times [11]. In human, the circulating levels of melatonin, a pineal substance with analgesic and hypnotic properties, exhibit a pronounced circadian rhythm with serum levels being high at night and very low during day times [2,48]. Moreover, melatonin exhibits maximal analgesic effects at night, pinealectomy abolishes the analgesic effects of melatonin, and opioid receptor antagonists disrupt the day–night rhythm of nociception [13].

Previous studies have suggested that opioidergic peptides influence the synthesis of melatonin. For example, the subcutaneous injection of des-tyrosine-γ-endorphin increased melatonin levels [17], and morphine stimulated the release of melatonin from the rat pineal gland [14,15]. Subsequently, the presence of opioid peptides derived from three precursors, namely, pro-opiomelanocortin, pro-enkephalin, and pro-dynorphin have been detected in mammalian pineal gland by radioimmunoassay [10,40,43,44]. Moreover, by utilizing immunohistochemical techniques, the presence of opioidergic nerve fibers have been demonstrated in the pineal gland of guinea pig [32], human [35], cow [33], European hamster [12] and tree shrew [37]. In addition, the immunoreactive intrapineal neuronal-like cells [32,35], as well as pinealocytes [12] containing enkephalin, were also observed in the pineal gland of guinea pig, human and European hamster.

In recent years, Govitrapong et al. [19], and Aloyo [1], by using [^{3}H]diprenorphine, have reported the existence of opioidergic receptors in the bovine pineal gland. Moreover, Bzdega et al. [7] have found that, in the mouse brain, the delta opioid receptor is expressed in low levels but its transcripts are found in large amounts, particularly in the anterior pituitary and pineal gland. Several studies indicated that melatonin exerts analgesic effects [18,49]. The analgesic effects of melatonin were attenuated by naloxone and benzodiazepines (BZP) antagonists suggesting its relationship with opioid and BZP receptors [18]. Moreover, melatonin treatment not only reversed the morphine-induced tolerance and dependence but also augmented the morphine-induced analgesia [23,39]. However, the exact mechanisms how melatonin induces analgesia is still poorly understood.

In the present study, we intended to learn in detail the pharmacodynamics of melatonin-induced analgesia by characterizing the opioid receptor subtypes and defining the effects of melatonin receptor agonists and antagonists on the opioid receptor binding. Furthermore, we intended to learn whether or not melatonin altered the release of endogenous opioids.

2. Materials and methods

2.1. Animals

Bovine pineal glands were dissected from freshly obtained brains of 6–12-month-old cows (Bos Taurus) at a local slaughterhouse between 10 and 12 h and kept in ice-cold DMEM containing 10% fetal calf serum (pH 7.4).

2.2. Isolation of bovine pinealocytes

Bovine pinealocytes were isolated by mechanical disruption. The glands were dissected free of connective tissue and blood vessels. They were minced for 15 min, suspended in DMEM and then triturated with a Pasteur pipette for 15 min. All steps were carried out on ice. The minced tissues were resuspended in DMEM and precipitated on ice for 7 min, following which the suspensions were filtered through a nylon mesh (pore size 41 μm, diameter 13 cm). Then, the suspensions were centrifuged at $4000 \times g$ at 4 °C for 5 min. The pinealocytes thus obtained were used for membrane preparation.

2.3. Preparation of bovine pinealocyte membranes

The pinealocyte pellets were resuspended in 20 volumes (original wet weight/volume) of ice-cold 50 mM Tris–HCl buffer (pH 7.4), homogenized for 10 s with a Polytron homogenizer (Ultra Turax 25, setting at 13,500 rpm), and then centrifuged (Sorvall RC26 Plus, Sorvall, Wilmington, DE) at $48{,}000 \times g$ for 20 min at 4 °C. The pellets were then resuspended (20 volumes) in ice-cold Tris–HCl (pH 7.4) and homogenized. The resulting membrane suspensions were used for binding assays.

2.4. Radioligands and chemicals

[^{3}]-[D-Pen^2,D-Pen^5] enkephalin (DPDPE) (specific activity 45.0 Ci/mmol) and [D-Ala^2,*N*-methyl-phe^4,glycol^5 (tyrosyl-3,5-^{3}H)]DAMGO (specific activity 54.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). [^{3}H]-(5α, 7α, 8β)-(−)*N*-methyl-*N*[7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8yl]-phenyl-benene-acetamide (U69593) (specific activity 65.0 Ci/mmol) and [^{3}H]-nociceptin (specific activity 155.0 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). Methionine-enkephalin was purchased from Peninsula Laboratories Inc. (Belmont, CA). Leucine-enkephalin, DPDPE ([enkephalin, [D-Pen^2, D-Pen^5]], DADLE (enkephalin, [D-Ala^2,D-Leu^5]),