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Effect of melatonin injection into the periaqueductal gray on antinociception and tonic immobility in male rats

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ABSTRACT

Melatonin (MLT) is a neurohormone with significant involvement in several biological functions, of which antinociception and tonic immobility (TI) may be the key neurobehavioral components to survive in adverse conditions such as a predator attack. TI-induced antinociception can be elicited, facilitated, or increased through opioid and γ-aminobutyric acid (GABA) among other chemical mediators at several levels of the central nervous system, mainly in the periaqueductal gray (PAG). The aim of this study was to assess the effect of the microinjection of MLT into the main PAG regions that are related to different integrated defensive responses, namely dorsal (D) and ventrolateral (VL), on both antinociception through the tail-flick (TF) test and TI duration as single behavioral response and on combined behavioral responses (TF/TI). We found that the microinjection of MLT into the main PAG areas produced antinociception but did not affect the TI duration. The microinjection of MLT into the D-PAG decreased TF latency during TI in the combined trial (TF/TI), which implies that TI-induced antinociception was blocked. The microinjection of MLT into the VL-PAG maintained the antinociceptive capability of the TI without addition or increase in the antinociceptive effects, implying a permissive effect by MLT on the TI-induced antinociception. MLT administration into the D-PAG decreased the TI duration on the TF/TI, whereas MLT administration into the VL-PAG had the opposite effect of significantly increasing TI duration with the TF/TI trial.

1. Introduction

Melatonin (MLT; *N*-acetyl-5-methoxytriptamine) is a neurohormone synthesized and released mainly from the pineal gland, with both circadian and seasonal rhythms of secretion being higher at nighttime and in winter (scotophasic peaks of synthesis), and diverse physiological functions including regulation of circadian rhythms, sleep, enhancement of the immunological system, free radical scavenging and antioxidant effects, antinociception, and mood regulation (Srinivasan et al., 2010). MLT exhibits high- affinity binding to its receptors at concentrations < 500 pM (Sugden et al., 2004). A selective MT_1 and MT_2 localization on neuronal cell bodies and dendrites in numerous regions of the rat telencephalon, diencephalon, and mesencephalon was demonstrated (Lacoste et al., 2015). Somadendrites endowed with MT_2 receptors were mostly observed in the CA3 field of the hippocampus, reticular thalamic nucleus, supraoptic nucleus, inferior colliculus, substantia nigra pars reticulata, and ventrolateral periaqueductal gray (VL-PAG; Lacoste et al., 2015). The analgesic effects of MLT result from the activation of MT_1 and mainly MT_2 MLT membrane receptors (López-Canul et al., 2015a, 2015b; Yu et al., 2000c), which leads to reduced cyclic AMP formation (Ambriz-Tututi and Granados-Soto, 2007; Ambriz-Tututi et al., 2009; Reppert, 1997; Srinivasan et al., 2010). The PAG is a major component of the descending pain inhibitory system (Basbaum and Fields, 1979, 1984; Roychowdhury and Fields, 1996).

Tonic immobility (TI) is an antipredator or defensive behavior characterized by a reversible state of motor inhibition that is elicited by manipulation and restriction of movement of an animal. In nature, TI, also known as "immobility response", "immobility reflex", "animal hypnosis", "death feigning", and "behavioral arrest" (Carli et al., 1976; Klemm, 2001; Sandoval-Herrera et al., 2011), can be practically elicited in every animal species by a wide variety of stimuli (e.g., visual, auditory, olfactory, and tactile) (Klemm, 2001; Miranda-Páez et al., 2016). TI can be induced by injecting MLT in several animal species that become more susceptible to TI at night (Tsoukolas, 2012). Both antinociception and immobility are the main components of defensive reaction involving the PAG as one of the major centers. The layers above and lateral to the mesencephalic aqueduct located in the intermediate (middle) and caudal PAG coordinate active defensive reactions, such as reactive immobility or freezing, including non-opioid hypoalgesia (not

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attenuated or blocked by naloxone); instead, the layers of neurons mainly located in the caudal, VL-PAG integrate an opposite behavioral reaction consisting of quiescence, behavioral arrest or TI, and opioid analgesia (Depaulis et al., 1994; Miranda-Páez et al., 2016; Morgan, 1991; Morgan et al., 1998).

Several studies in mice, hamsters, rats, and humans show that there is a circadian rhythm involved in pain perception (Srinivasan et al., 2010). During darkness or nighttime, when MLT plasma levels are higher, animals are less responsive to nociception and more sensitive to morphine in the tail-flick (TF) test (Rosenfeld and Rice, 1979).

MLT-induced antinociception has been explained by a variety of mechanisms (Barrett et al., 1999; Wilhelmsen et al., 2011) as follows: through GABA receptors (Dhanaraj et al., 2004; Golombek et al., 1991, 1996), β-endorphin (Wang et al., 2006; Yu et al., 2000b), μ-opioid receptors (Barrett et al., 1999; Dai et al., 2007; Li et al., 2005; Wang et al., 2006), and the NO-arginine pathway (Ulogol et al., 2006). Furthermore, MLT can activate opioid receptors indirectly (Pang et al., 2001; Wang et al., 2006), open potassium channels and modulate $GABA_A$ receptor function (Ambriz-Tututi et al., 2009; Dhanaraj et al., 2004), and inhibit the expression of 5-lipoxygenase and cyclooxygenase (Ambriz-Tututi et al., 2009; Steinhilber et al., 1995).

Certain situations that are potentially dangerous for the animal may carry implicit antinociception integrated within the defensive behavioral response, and probably the most studied of these is TI-induced analgesia, with the main center of origin in the midbrain PAG (Depaulis et al., 1994; Miranda-Páez et al., 2014, 2016; Morgan, 1991).

With this background, we studied the effect of MLT microinjected into both the main regions of PAG (dorsal (D) and VL) on both antinociception and TI in terms of (a) isolated or single behavior and (b) combined behavior in a single trial (TI-induced antinociception) through the TF test.

2. Materials and methods

2.1. Animals and housing

Thirty-six male Wistar rats weighing 250–300 g were obtained from our animal care facilities (Harlan Laboratories were the original source). The experimental protocol for the study was according to the procedures established by The Guide for the Care and Use of Laboratory Animals of the Mexican Council for Animal Care (NOM-062-ZOO-1999) and the National Institutes of Health (USA) for the care and use of laboratory animals (NIH Publications No. 8023). Every effort was made to alleviate any distress that might be experienced by the animals during this set of experiments. We used the minimum number of animals required to attain the goals of the study. Rats were isolated in individual cages 1 week before stereotaxic surgery under a normal dark/light cycle (lights on at 07:00 AM) with controlled temperature (20–22 °C) and humidity (45–55%) and free access to food and water. The rats were returned to the individual cages for 1 week after surgery (recovery period). All behavioral tests were conducted between 10:00 AM and 04:00 PM.

2.2. Stereotaxic surgery

The animals were anesthetized with ketamine (80 mg/kg, Pisa®) and xylazine (15 mg/kg, Pisa®), both intraperitoneally (ip). A one-sided guide cannula was implanted from the skull to the D-PAG (AP: −7.3 mm, ML: 0.5 mm and DV: 4.0 mm) or at the VL-PAG (AP: −7.5 mm, ML: 0.5 mm and DV: 7.0 mm with a 15° angle) using a stereotaxic frame (mod: 502650, World Precision Instruments®, Sarasota, FL, USA) and coordinates from the atlas of Paxinos and Watson (2014). The guide cannula was held in place to the skull with two screws, dental acrylic, and a stylet inserted into the guide.

One week after the stereotaxic surgery, the stylet was removed from the guide cannula and MLT at different concentrations was microinjected into the respective PAG region through the cannula (31 gauge × 10 mm), that extended 1 mm beyond the tip of the guide cannula. The injection cannula was connected to a 1-μL syringe (Hamilton Co., Reno, NV, USA) with a PE-20 tubing filled with sterile water. Microinjections through the cannula were made for 60 s, and for another 60 s, the injection cannula was left undisturbed to avoid backflow of the drug. In addition, any struggling movement of the rat was gently restricted and only calm movements were allowed. The stylet was returned to the guide cannula after all the behavioral tests.

2.3. Drugs and microinjections

MLT (Sigma-Aldrich®, St Louis, MO, USA) at 0.5, 1, 2.5, and 5 (μg/0.5 μL) concentrations were dissolved in 2-Hydroxypropyl-β-cyclodextrin solution, 45% (w/v) solution in water (CDX; Sigma-Aldrich®), and CDX 45% with a pH of 7.4, was used as a vehicle. MLT microinjection (0.0 = CDX, 0.5, 1, 2.5, and 5) was randomly assigned at 48–h intervals in each rat, with a maximum of three microinjections per animal. Each animal received only one dose of MLT or CDX as the vehicle on the day of testing.

2.4. Behavioral testing

Rats (n = 18 each for D-PAG and VL-PAG) were tested using the TF, TI, and a combined TF/TI tests 2 min after the respective microinjection. All tests were conducted in the same animal in a random order with a 3-min intertrial interval. Each animal then received three microinjections and four behavioral assessments, resulting in 12 observations for each animal. After each test, the rats were left in a plastic cage in the same laboratory for 3 min to reduce any further influence of the environment. The surfaces of the table and TF apparatus were cleaned with a 10% ethyl alcohol solution. All experiments were recorded for further revision. During our testing, we did not find any link between our results and the order in which the behavioral tests were performed or the way in which the rats were experimentally handled.

2.5. TF test

TF test was performed by a TF test meter (Columbus Instruments®, Columbus, OH, USA) after restricting the movements of rats by an air permeable plastic container and waiting until the animal was tranquil.

2.6. Tonic immobility

TI was elicited by clamping the neck with two 5-cm rubber-tipped alligator clips, one on the dorsal and the other on the ventral part of the neck. Each clip exerted 1300 g/cm^2 of force on approximately 1 cm^2 of neck skin. The animal was then inverted onto a supine-lateral position and gently maintained in this posture until it stopped struggling (if applicable) and remained immobile. The duration of TI was measured from the time the experimenter's hand was removed until the animal recovered the prone position (contact righting reflex) for a maximum of 180 s (Miranda-Páez et al., 2014, 2016; Sandoval-Herrera et al., 2011).

2.7. Combined TF test and TI

We assessed TI-elicited antinociception using the combined TF/TI test. Briefly, TI was induced over the surface of the TF test meter apparatus, and the tail was placed over the light beam. This procedure left the animal immobile without using a plastic container and simultaneously allowed for the evaluation of the TF latency (Miranda-Páez et al., 2016).

2.8. Histology

After all the behavioral experiments, the animals were overdosed with a lethal injection of sodium pentobarbital (90 mg/kg, ip).