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Research report

Behavioural actions of two new 1-N substituted analogues of melatonin

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HIGHLIGHTS

- In two animal models of anxiety, two 1-N melatonin analogues show better effects than the original molecule.
- Two new 1-N analogues produce sedative effects that last several hours.
- MT and its 1-N analogues reduce the seizures induced by pentylenetetrazole.

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ABSTRACT

Melatonin has been mainly used for alleviating some disorders related with insomnia and circadian rhythmicity. The use of this hormone has been limited, among others, due to its short half-life and instability. This study reports some behavioural actions of two new melatonin analogues that incorporate a phenyl or a benzoyl group at the nitrogen atom of the melatonin molecule. Although diazepam was about 10 times more potent than either of the melatonin analogues, results show that in general these last display better anxiolytic, anticonvulsant and sedative actions than the original molecule.

1. Introduction

Melatonin (MT) is an indolic endogenous hormone synthesized and released into the pineal gland of all vertebrates [1–3]. This hormone has been involved in a wide variety of pathological and physiological events including, those related with disturbances in circadian rhythms, i.e. seasonal depression [4,5], the jet–lag phenomena [6], and the sleep–wake cycle [7]. Furthermore, MT possesses hypnotic, antidepressive, anticonvulsant, analgesic and relaxant properties [8–15]. In spite of this evidence, there are major drawbacks in its therapeutic value due its short half-life [16], strong light-induced variations, instability [17], low potency and selectivity to MT receptors. In order to reduce these deficiencies, new MT analogues have been synthesized that have a longer half-life and better binding affinity toward MT receptors [1,18–20]. Two of these new MT analogues are M6B (N-{2-[1-(4-cyanobenzoyl)-5-methoxy-1H-indole-3-yl]-ethyl}-acetamide) and M7C (N-{2-[1-(4-chlorophenyl)-5-methoxy-1H-indole-3-yl]-ethyl}-acetamide). These two compounds (Fig. 1) are characterized by the incorporation of either a phenyl or a benzoyl group at nitrogen atom, which delocalizes the electronic distribution of the indole ring, possibly altering its biological activity.

It is known that electron donors such as methoxy or acetamide as well as electron acceptors and carbonyl added at 5-C increase the affinity of MT to its receptors. This effect is also achieved when adding bulky or aromatic groups at 2-C [1]. Nevertheless, biological activity of this hormone seems to be mediated by radicals present at 1-N [20]. Theoretically a cyanobenzoyl or chlorophenyl group at indole ring would improve the biological activity of MT molecule.

The aim of the present report is to explore, by means of several animal models, the behavioural properties induced by the two 1-N MT analogues M6B and M7C. Although we detected that some of these models are more sensitive than others [21,22], most of the paradigms used here have been broadly validated, under a range of physiological and pharmacological conditions, as useful tools for

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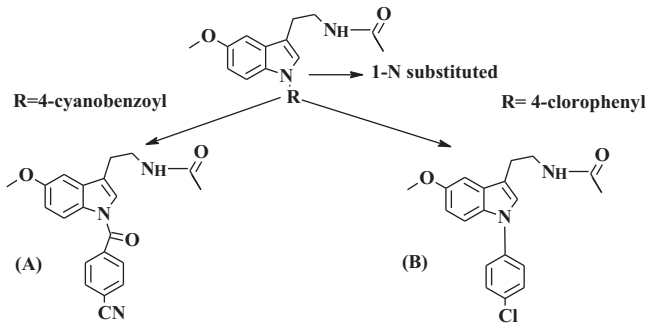

Fig. 1. Chemical structure of the 1-N substituted melatonin analogues where (A) M6B: N-{2-[1-(4-cyanobenzoyl)-5-methoxy-1H-indole-3-yl]-ethyl}-acetamide and (B) M7C: N-{2-[1-(4-chlorophenyl)-5-methoxy-1H-indole-3-yl]-ethyl}-acetamide.

discerning new anxiolytic, antiseizure, sedative and miorelaxant drugs [23–31].

2. Materials and methods

2.1. Animals

Male CD1 mice (25 + 5.0 g) were purchased from Harlan Laboratories (Harlan Mexico S.A de C.V) and submitted for two weeks to 12:12 h inverted dark–light cycle (9.00–21:00 dark period). All animals had free access to food and water. Care and handling of the animals were in agreement with internationally accepted procedures and approved by our own Institutional Committee following the recommendation indicated in the Mexican Normative 062-ZOO-1999: Technical Specifications for the Production, Care, and Use of Laboratory Animals [32].

2.2. Treatments

MT and pentylenetetrazole (PTZ) were purchased from Sigma (St. Louis, MO), polyethyleneglycol 25% from Merck, Diazepam (DZP) from Hoffman La-Roche, Basel, Switzerland), Water was Mili Q grade. M7C and M6B compounds were synthesized by Lira-Rocha A. at Chemistry Faculty (UNAM) [20]. For all doses and routes of administration, drugs and vehicles were administered at 10 ml/kg. At exception of PTZ (subcutaneously injected), all drugs were injected i.p. Dosage schedules were selected taken into account previous reports [29,33–35]. With exception of the intact group, all experimental groups were administrated 30 min before testing.

2.3. Burying behaviour test

Experimental anxiety was evaluated by using the burying behaviour test, which has been validated as a useful model for testing anxiety compounds [23,24]. This model consists of an acrylic cage (27 cm × 16 cm × 23 cm) containing a prod (7 cm long) emerging from one wall 2 cm above the bedding material (fine sawdust). Through the pod the animal receives an electric shock of 0.3 mA which is supplied by a current constant shocker (La Fayette Instruments, model 5806). During the test the animal is placed in the cage and its behaviour recorder over a 10 min period. In this paradigm, the following parameters were registered: (1) burying behaviour latency (the time in seconds from first shock to the burying behaviour display) and (2) the cumulative burying behaviour (the time in seconds that the mice spend burying the proud during the test). In this paradigm, burying behaviour latency inversely reflects the animal's reactivity, while the cumulative burying behaviour directly denotes its anxiety states [23,24].

2.4. Exploratory behaviour test

The exploratory behaviour test is a broadly used procedure to study experimental anxiety. Briefly, these tests consist of an acrylic cage (45 cm × 27 cm × 27 cm) divided into a small, darkened compartment (1/3 of total size) and a large and highly illuminated compartment. Both the compartments are connected by a little opening (13 cm × 15 cm) located at the floor level. Here, each mouse is introduced into the bright area and the number of transitions throughout the opening as well as the time spent in illuminated compartment is registered for 10 min. In this test, it is considered that both an increase in the number of crossings together with an increase in time spent in bright compartment, reflects low levels of anxiety [25,26]. After each session the test cage is carefully cleaned with a moist cloth.

2.5. Rotarod test

This test is used to detect a lack of motor control and therefore an impaired balance in rodents [29], and consists of an automated accelerating rotarod (Ugo Basile North America Inc. model 47600). In this apparatus mice are placed on the rod until they learn to remain there for at least 2 min. In the current study, two hours after this experience mice were selected for their ability to maintain themselves on the rod (constant speed = 16 rpm), injected, and again placed on the device. Parameters evaluated were; the total number of falls and the time spent on the rod during the 10-min test. An increase in the first parameter together with a short time on rod, are related to deficiencies of motor coordination due to muscle relaxation [30].

2.6. Horizontal wire test

Muscle tone and motor coordination can also be evaluated by means of the horizontal wire test. This consists of a horizontal steel wire (20 cm high, 1 mm diameter, 15 cm long) in which mice are allowed to grasp for 2 min. During the test, two parameters are registered: the total number of falls accumulated and the time spent by the mouse on the wire. Similar to the rotarod test, an increase in the number of falls together with a decrease in the time spent on the wire are interpreted as a lack of motor coordination [31].

2.7. Anticonvulsive activity

For this test, animals were injected subcutaneously with the chemoconvulsant PTZ (90 mg/kg) 30 min after treatment with M7C, M6B and MT and then placed in individual cages. Latency to the first seizure after PTZ injection was registered during the next 30 min [31].

2.8. Sedative activity

For this test, animals were injected and immediately placed in an acrylic cage (60 cm × 40 cm × 40 cm) whose floor was covered with sawdust. Two parameters were registered: (a) sedation latency, which is the period of time from the injection of compound until the animal lost its regular ambulation, and (b) sedation; which is the period of time from the loss of regular ambulation to the recovery of the same.

2.9. Spontaneous motor activity

This test was implemented only after anxiety tests and was carried out for controlling false results due to alterations in motor abilities of rats. Thus, all animals were tested immediately after either the burying behaviour or exploratory behaviour test in an automatic activity counter. This consists of an acrylic cage measuring 51.1 cm × 9.5 cm × 69.2 cm with two arrays of 15 infrared emitting beams placed perpendicular to each other. Beams are spaced 2.5 cm apart in such a way that the interruption of each beam generates an electric impulse, which is presented as a count (Opto-Varimex; Columbus Instruments, OH, USA). Ambulation and vertical activity (rearing) are registered over a 5-min test period [36].

Behavioural evaluations were carried out with independent groups ($n = 6$ per dose) between 10:00 and 14:00 h. Two anxiety tests were considered in the current study; cumulative burying behaviour and the exploratory behaviour test, while for evaluating coordination both rotarod and horizontal wire test were used. Anticonvulsant, hypnotic and sedative properties of the mentioned 1-N analogues were registered by a single observer, who was blind to treatment conditions.

2.10. Statistics

In order to homogenize the statistical analysis, since not all the data passed the normality test, results were statistically compared by using Kruskal–Wallis one-way analysis of variance, followed by Dunnett's test post hoc comparisons. A value of $p < 0.05$ was considered statistically significant. ED_{50} values for both MT and its analogues were calculated by means of log-probit analysis (SPSS 16.0 version).

3. Results

With exception of MT, which at low doses produces anxiogenic actions in the burying behaviour paradigm (Fig. 2; second set of bars; upper panel), results derived from the two analogues show clear anxiolytic actions in both animal models. In the first trial, all compounds tested decreased burying behaviour (Fig. 2 upper panel). However, this action was accompanied by equivalent increases in burying behaviour latencies. Interestingly, this effect was more pronounced with DZP than with melatonergic compounds, where only high doses lengthened latencies (Fig. 2 lower panel). Statistical analysis for latencies was: DZP, $H = 44.05$, $p < 0.05$ MT, $H = 36.74$, $p < 0.05$; M7C, $H = 36.76$, $p < 0.05$; M6B, $H = 27.226$, $p < 0.05$). Statistical analysis for cumulative burying behaviour was: DZP, $H = 44.56$, $p < 0.05$; MT, $H = 36.30$, $p < 0.05$; M7C, $H = 38.62$, $p < 0.05$; M6B, $H = 36.48$, $p < 0.05$). Regarding M7C, these results