

EXPERIMENTAL RESEARCH ON MICE REGARDING THE IMPLICATION OF MELATONIN IN PAIN MANAGEMENT

ANDREEA LETIȚIA NIȚULESCU-ARSENE¹, NICULINA MITREA¹,
AURELIA CRISTEA², CRISTINA MANUELA DRĂGOI¹

*University of Medicine and Pharmacy „Carol Davila”, Bucharest,
Faculty of Pharmacy, Traian Vuia no. 6*

¹*Department of Biochemistry*

²*Department of Pharmacology and Clinical Pharmacy*

Abstract

Melatonin (N-acetyl-5-methoxytryptamine) is the main hormone biosynthesized by the pineal gland. This biomolecule coordinates and synchronizes the expression of the most important physiologic sleep-awake effects and biological rhythms. Numerous studies in the scientific literature also describe its multi-regulatory roles in endocrine, immune and nervous systems. Recently, melatonin has been suggested to play a regulatory role in organisms' pain sensitivity. Our previous studies investigated the antinociceptive activity of melatonin when we performed an evaluation regarding the dose-dependent dynamics of the analgesic effect of melatonin. We found that the biomolecule exerts an important analgesic effect for the dose of 50mg melatonin/kgbw.

The present study investigated the antinociceptive pharmacologic effects of melatonin (50mg /kgbw, i.p.) in co-administration with ondansetron (4mg/kgbw, i.p.), petidine (4mg/kgbw, i.p.) and tramadol (3.5mg/kgbw, i.p.).

Experiments were performed *in vivo*, using Albino Swiss male mice. Pain sensitivity was assessed using the hot-plate test (60°C).

The present study also outlines an amplification of the analgesic effect when petidine is co-administered with melatonin and an antagonism between ondansetron and the pineal biomolecule.

Rezumat

Melatonina este principalul hormon sintetizat la nivelul glandei pineale. Această biomoleculă este responsabilă de coordonarea și sincronizarea expresiei efectelor fiziologice ale alternanței somn-veghe și a ritmului biologic. În literatura de specialitate sunt descrise o serie de studii ce evidențiază rolurile multiple ale melatoninei la nivelul sistemelor endocrin, imun, nervos. Efectul său reglator asupra sensibilității dureroase a fost de curând atribuit acestei molecule. Studiile experimentale anterioare au demonstrat dinamica doză-dependentă a efectului analgezic al melatoninei. Concluziile experimentale au arătat că această biomoleculă are un efect analgezic important la o doză de 50 mg melatonină/kgc.

Plecând de la aceste premise, ne-am propus ca pe parcursul acestui studiu experimental să evaluăm efectul antinociceptiv al melatoninei (50 mg melatonină/kgc, i.p.), în co-administrare cu ondansetron (4mg/kgc, i.p.), petidină (4mg/kgc, i.p.) și tramadol (3.5mg/kgc, i.p.).

Studiile s-au desfășurat *in vivo*, pe șoareci masculi Albino Swiss. Sensibilitatea la durere a fost evaluată cu ajutorul testului „hot-plate” (testul stimulului termic, la 60°C).

Studiul experimental evidențiază amplificarea efectului analgezic în cazul co-administrării petidinei cu melatonină și antagonismul existent între ondansetron și biomolecula pineală.

Keywords: *melatonin, analgesic effect*

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is the main hormone biosynthesized by the pineal gland. Numerous studies in the scientific literature describe its multi-regulatory roles in endocrine, immune and nervous systems.

Recent studies have revealed a variety of mediators, with different chemical structures, which are located in the pineal cells and modulate the biochemistry of melatonin [1, 7].

The complex biological effects of melatonin are mediated by three specific receptors (ML1, ML2, ML3). Experimental studies have demonstrated the important role of these receptors in the balance of the sleep-awake process, the body temperature, the cortisol secretion, the reproductive, immune-stimulatory and antioxidant functions [3].

Recently, melatonin has been suggested to play a regulatory role in organisms' pain sensitivity, due to the presence of opioid fibers in the pineal gland, demonstrated by the experimental studies on animals missing the pineal gland [5]. Our previous studies investigated the antinociceptive activity of melatonin when we performed an evaluation regarding the dose-dependent dynamics of the analgesic effect of melatonin (25, 50, 100mg/kgbw, i.p.). We found that the biomolecule exerts an important analgesic effect for the dose of 50mg melatonin/kgbw [6].

The present study investigated the antinociceptive pharmacologic effects of melatonin (50mg /kgbw, i.p.) in co-administration with ondansetron (4mg/kgbw, i.p.), petidine (4mg/kgbw, i.p.) and tramadol (3.5mg/kgbw, i.p.).

From the pharmacological point of view, the molecule of ondansetron is a serotonin 5-HT₃ receptor antagonist, mainly used as an antiemetic to treat nausea and vomiting following chemotherapy. Pétidine is an agonist of μ and κ opioid receptors, clinically used for its morphine-like analgesic effects. Tramadol is an analgesic reported in literature as an agonist of μ opioid receptors, as well as on the noradrenergic and serotonergic systems. It is clinically used for treating moderate to severe pain.

Materials and methods

The study was performed on Albino Swiss mice, having the approximate weight of 25-30 grams. The animals were brought from the 'Carol Davila' University of Medicine and Pharmacy Biobase, and located in the Biochemistry Laboratory of the Faculty of Pharmacy Bucharest.

They were divided into five groups of 10 mice each, considering the intraperitoneal administered substances:

- group 1 (CONTROL): animals that received saline solution (NaCl 0.9%) (0.1ml/10g bw, i.p.);
- group 2 (named M): animals that received 50 mg/kgbw i.p. melatonin;
- group 3 (named M+O): animals that received (simultaneously) 50 mg/kgbw i.p. melatonin and ondansetron (4mg/kgbw);
- group 4 (named M+P): animals that received (simultaneously) 50 mg/kgbw i.p. melatonin and petidine (4mg/kgbw);
- group 5 (named M+T): animals that received (simultaneously) 50 mg/kgbw i.p. melatonin and tramadol (3.5mg/kgbw).

The mice were housed in a room and maintained at 25 ± 2 °C and 45-55% relative humidity, with an alternating 12h light-dark cycle. They had free access to food and water until the morning of the experiment. All animals used in this study were maintained in facilities fully accredited and the experiments described here were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002.

Substances

In this study, there was used melatonin of high purity purchased from SIGMA,USA. Ondansetron, petidine and tramadol were a king gift from the Department of Toxicology of the Faculty of Pharmacy, Bucharest.

Evaluation of pain sensitivity of mice

The pain sensitivity was assessed using the Ugo Basile *hot-plate* test, at 60°. The parameter used for evaluating the pain sensitivity of mice is the jumping-time from the hot plate.

Results and discussion

The analgesic activity of melatonin was evaluated comparing the initial (basal) and final (after melatonin administration) jumping-time (Tj) of mice.

The basal pain sensitivity, for every group of mice, was expressed by the mean values of the jumping-time. After administration of the substances tested, the pain sensitivity (mean values of Tj) has changed as shown in Table I:

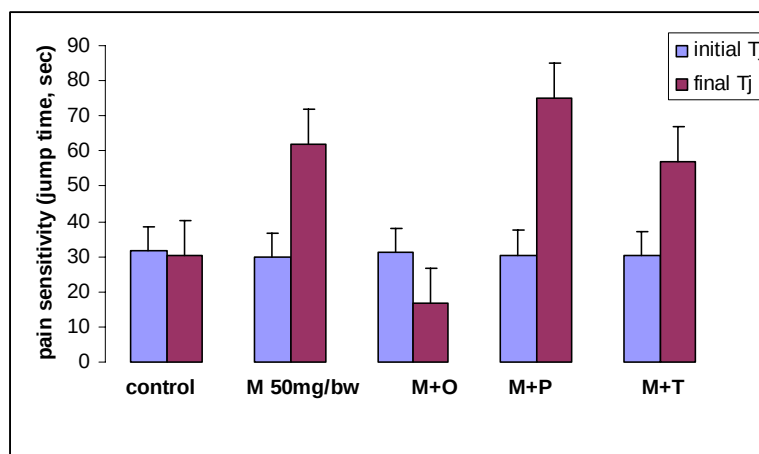
Table I

The analgesic effect expressed as mean values of the jumping time (Tj). Melatonin (50mg/kgbw) and its co-administration with ondansetron (4mg/kgbw), petidine (4mg/kgbw) and tramadol (3.5mg/kgbw).

Group of mice	basal, initial Tj (sec)	final Tj (sec)
control	31.45±10.05	30.21±10.23
M 50mg/kgbw	29.78±9.12	62±18.03
M 50mg/kgbw +O 4mg/kgbw	31.17±8.54	16.86±4.11
M 50mg/kgbw +P 4mg/kgbw	30.34±8.15	75±12.53
M 50mg/kgbw +T 3.5mg/kgbw	30.17±9.47	57±12.64

The statistic evaluation of the experimental data of the study was performed using the student “t” test and Anova of the STATISTICA software.

In figure 1, there is a graphic presentation of the dynamics of endogenous analgesia before and after the administration of the drugs tested.

**Figure 1**

Pain sensitivity before the experiment (initial jumping time, initial Tj) and after administration of 50mg/kgbw melatonin (M 50mg/kgbw) in association with ondansetron 4mg/kgbw (M+O), petidine 4mg/kgbw (M+P) and tramadol 3.5mg/kgbw (M+T)

Our results confirm the previous experimental study, regarding the analgesic effect of melatonin. As it can be observed, when administered in a dose of 50 mg/kgbw, the pineal hormone produces an increase of the jumping time, which reflects a diminution of animals' pain sensitivity.

Comparing with the group of mice that received only melatonin (50mg/kgbw), the animals that received petidine (4mg/kgbw) co-

administrated with melatonin (50mg/kgbw) developed higher values of the jumping time (which reflects the reduction of the pain sensitivity).

The same pattern was noticed and when the pineal hormone was co-administered with tramadol (3.5mg/kgbw).

Interestingly, the association of ondansetron (4mg/kgbw) with melatonin (50mg/kgbw) produced an increased response to the algogenic stimulus (mice exhibited low values of the jumping time). It seems that melatonin and ondansetron have antagonistic-like effects.

Figure 2 depicts, comparatively, the dynamics of the analgesic effect of the substances administered for the studied groups of mice.

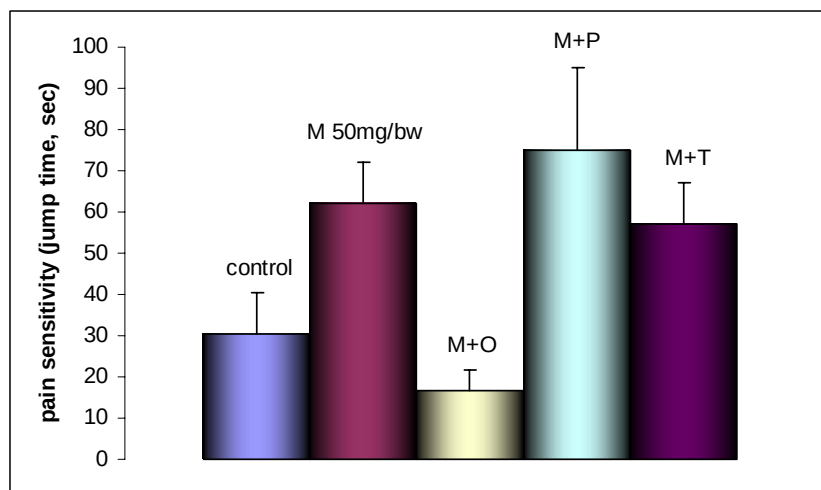


Figure 2

The dynamics of the analgesic effect of the substances administered for the studied groups of mice

Conclusions

As previously demonstrated, melatonin, in a dose of 50mg/kgbw modulates pain and decreases organisms' algogenic reactions.

Furthermore, the association of the pineal hormone with petidine is a positive one. Our data suggested an increase of the analgesic effect when the two substances are co-administered.

Interestingly, associating ondansetron with melatonin produced an increased response to the algogenic stimulus. It seems that melatonin and ondansetron have antagonistic-like effects. This phenomenon might be explained through the structural similitude of the two compounds, both ondansetron and melatonin possessing an indolic ring. This aspect remains to be elucidated.

References

1. Bothorel B, Barassin S, Saboureau M, Perreau S, Vivieb- Roels B, Malan A, and Pevet P – In the rat, exogenous melatonin increases the amplitude of pineal melatonin secretion by a direct action on the circadian clock, *Eur J Neurosci*, 2002, 16,1090-1098
2. Diaz E, Garidou ML, Dardente H, Salingre A, Pevet P, and Simonneaux V – Expression and regulation of mRNA in the Syrian hamster pineal gland, *Brain Res Mol Brain Res*, 2003, 122, 163-169
3. Ganguly S, Coon SL, and Klein DC –Control of melatonin synthesis in the Mammalian pineal gland: the critical role of serotonin acetylation, *Cell Tissue Res*, 2002, 309, 127-137
4. Garidou ML, Diaz E, Calgari C, Pevet P, and Simonneaux V –Transcription factors may frame *Aa-nat* gene expression and melatonin synthesis at night in the Syrian hamster pineal gland, *Endocrinology*, 2003, 144, 2461-2472
5. Garidou ML, Vivien Roels B, Pevet P, Miguez J, and Simonneaux V – Mechanism regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland, *Am J Physiol Regul Integr Comp physiol*, 2003, 284,R1043-R1052
6. Nitulescu-Arsene A.L., Mitrea N., Cristea A., Drăgoi C.M. – Experimental research on mice regarding the analgesic effect of melatonin, *Farmacia*, 2006, 6, 94-100
7. Valerie Simonneaux, Christophe Ribelayga – Generation of the Melatonin Endocrine Message in Mammals:A Review of the Complex Regulation of Melatonin Synthesis by Norepinephrine, Peptides, and Other Pineal Transmitters, *Pharmacol Rev*, 2003, 55, 325-395.

Manuscript received: 4.11.2008