Commentary

Melatonin actions in the heart: more than a hormone

Darío Acuña-Castroviejo[1,2*], Maria T. Noguera-Navarro[1], Russel J. Reiter[3], Germaine Escames [1,2]

[1]Centro de Investigación Biomédica, Departamento de Fisiología, Facultad de Medicina, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Granada, Spain.
[2]CIBERfes, Ibs.Granada, Unidad de Gestión Clínica de Laboratorios Clínicos, Complejo Hospitalario de Granada, Granada, Spain.
[3]Department of Cell Systems and Anatomy, UT Health, San Antonio, Texas, USA
*correspondence: dacuna@ugr.es, Tel: +34 958241000 ext. 20169

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Summary

Due to the broad distribution of extrapineal melatonin in multiple organs and tissues, we analyzed the presence and subcellular distribution of the indoleamine in the heart of rats. Groups of sham-operated and pinealectomized rats were sacrificed at different times along the day, and the melatonin content in myocardial cell membranes, cytosol, nuclei and mitochondria, were measured. Other groups of control animals were treated with different doses of melatonin to monitor its intracellular distribution. The results show that melatonin levels in the cell membrane, cytosol, nucleus, and mitochondria vary along the day, without showing a circadian rhythm. Pinealectomized animals trend to show higher values than sham-operated rats. Exogenous administration of melatonin yields its accumulation in a dose-dependent manner in all subcellular compartments analyzed, with maximal concentrations found in cell membranes at doses of 200 mg/kg bw melatonin. Interestingly, at dose of 40 mg/kg b.w, maximal concentration of melatonin was reached in the nucleus and mitochondrion. The results confirm previous data in other rat tissues including liver and brain, and support that melatonin is not uniformly distributed in the cell, whereas high doses of melatonin may be required for therapeutic purposes.

Keywords: melatonin; heart; subcellular distribution; pinealectomy

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Melatonin is frequently referred to as hormone or even more restrictedly, as a neurohormone. This is because it behaves as a hormone, i.e., it is produced by an organ in the brain, the pineal gland and secreted into the blood as well as into cerebrospinal fluid (CSF). It sometimes acts on the targeting organs via specific receptors. This classical description of the endocrine secretory product has changed, and this definition of hormone is no longer supportable. In fact, it is known that some hormones including aldosterone and IGF-I are produced by many different tissues including the heart (1-4). Many hormones act via a variety of mechanisms, including membrane, nuclear, and mitochondrial receptors, e.g., steroid hormones. Even hormone metabolites, which

were considered to lack endocrine effects, display metabolic actions; this is the case of diiodothyronine, a metabolite of thyroxine which targets the mitochondria of heart (5).Thus, the concept for a hormone as a molecule produced in an endocrine organ, released into the blood, and acting on a specific targeting organ possessing selective membrane, cytosolic, or nuclear receptors, should be revised. A hormone may be produced in different organs, may act through multiple mechanisms, and its metabolites may also have endocrine effects.

With regard to melatonin, many features distinguish it even from this new concept of a hormone. Melatonin is produced in most, if not all organs and tissues of the body, being synthesized via the same enzymatic machinery as in the pineal gland (6). It acts through specific membrane receptors, of which two, MT1 and MT2, have been identified in humans (7); these receptors mediate chronobiotic properties of this indoleamine. The genomic actions of melatonin prompted the identification of binding sites belonging to the ROR/RZR family of nuclear transcription factors (8). Melatonin also binds to calcium-related proteins, including calmodulin and calreticulin, in both cases with high affinity and a K_d in the low nanomolar range. Thus, they fulfill pharmacologic features of a receptor (9, 10). Melatonin also regulates mitochondrial homeostasis by several mechanisms including nuclear and mitochondrial DNA transcriptional activity (11, 12). Mitochondria have been proven to possess melatonin receptors and they synthetize melatonin also (13).

Perhaps the most intriguing feature of melatonin is the differences in the way it is handled by the pineal and extrapineal sources. Pineal melatonin is not stored in the gland, where it is released into the blood and CSF soon after it is produced, this occurs with the typical nocturnal peak. This rhythm conveys a chronobiotic signal that synchronizes multiple functions of the body to a 24 hour day/night cycle (14). The maximal concentration of melatonin in blood at night rarely exceeds 1 nM, although it is in much higher levels in the CSF (6).

The situation of melatonin at extrapineal sites is very different. The presence and expression of the genes coding for arylalkylamine N-acetyltransferase (AANAT) and acetylserotonin methyl transferase (ASMT), the enzymes that control melatonin synthesis, have been identified in most tissues including the heart (15). These enzymes synthesize melatonin in these organs where it functions in the same manner as pineal-derived melatonin (6). The physiochemical features of melatonin, which allow it to cross all biological membranes, suggest that some of intracellular melatonin could derive from the blood. To address this and other questions regarding extrapineal melatonin, plasma and subcellular distribution of melatonin after its peripheral administration, were assessed in liver and brain of rats, as published elsewhere (6).

A number of studies have focused on the myocardial function of melatonin during various diseases including aging, sepsis, muscle dystrophies, ischemia/reperfusion, and even heart transplantation; in all of these situations melatonin administration reportedly has beneficial effects (16-19).

In view of these findings, we considered it of interest to analyze the subcellular distribution of melatonin in cardiomyocytes to identify its intracellular targets. To test whether pineal gland affects subcellular distribution of melatonin, sham-pinealectomized (SPx) and pinealectomized (Px) rats were sacrificed at 08:00, 12:00, 16:00, 20:00, 24:00, 02:00, 04:00, and 06:00 h under a 12:12 light/dark cycle. To study the dose-dependent effects of exogenous melatonin administration on its subcellular distribution, additional groups of control rats were i.p. injected with 0, 10, 40, 100, or 200 mg/kg bw melatonin at 08:00 h, and sacrificed 4 h later, when maximal intracellular melatonin was detected (6). The experiments were performed in accordance with the Ethical Committee of the Granada University (CEEA 462-2013); the Spanish Protection Guide for Animal

Experimentation (R.D. 53/2013), and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS # 123). Heart melatonin levels were analyzed for the effects of pinealectomy, the light:dark cycle, and the exogenous melatonin administration in subcellular organelles. The results showed a daily variation in the subcellular levels of melatonin, tending to increase after Px (Fig. 1). Cosinor analysis reported the absence of circadian rhythm in these oscillations.

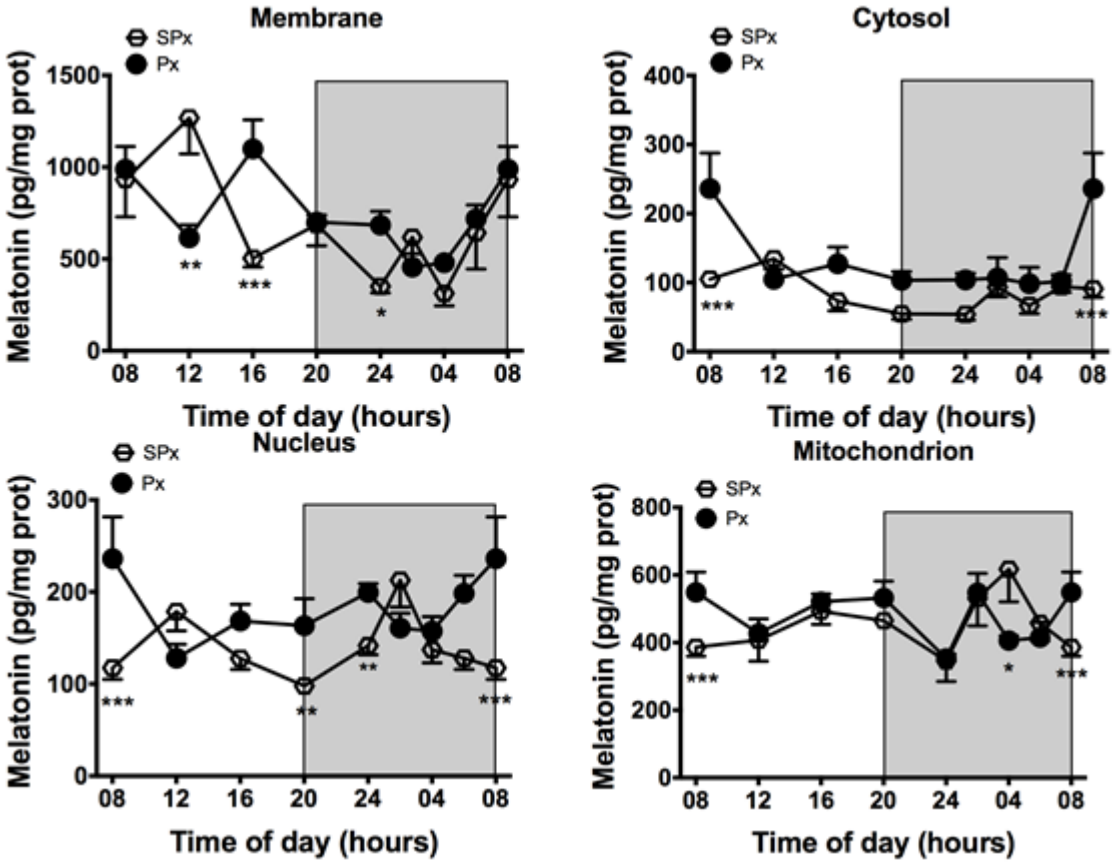

Figure1. Daily fluctuations of melatonin in subcellular compartments of rat myocardial tissue in sham (SPx) and real pinealectomized (Px) animals.

*Rats were maintained in a 12:12 hr cycle and sacrificed at the indicated hours. Shaded boxes indicate the dark period. *P < 0.05, **:P < 0.01, and ***:P < 0.001 vs SPx.*

Exogenously melatonin accumulated in the cell membranes in a dose-dependent manner, with an estimated one tenth of this melatonin entering the cells. Moreover, nuclear and mitochondrial levels became saturated with melatonin at the dose of 40 mg/kg, with the higher doses not further elevating the concentration of melatonin in these compartments (Fig. 2). Blocking the MT1 and MT2 membrane receptors with luzindole had minimal effect on intracellular melatonin levels (data not shown). Apart from cell membranes, mitochondria had the higher levels of melatonin in the cardiomyocytes.

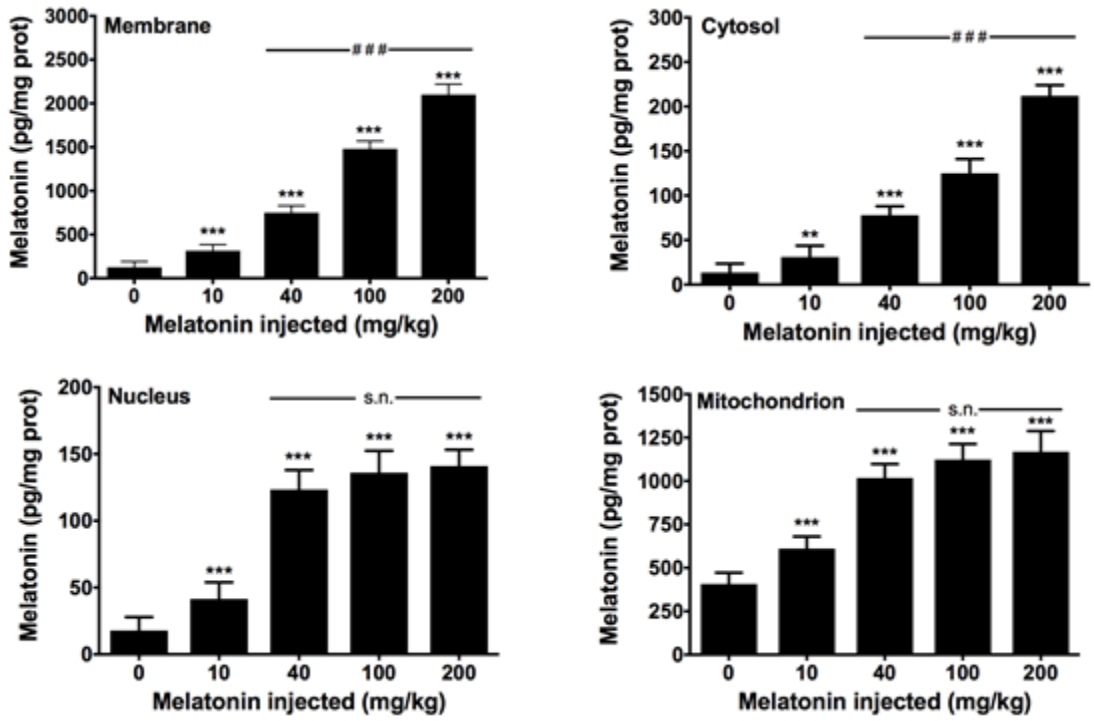

Figure 2. Effects of different doses of melatonin administration on its subcellular distribution in rat myocardial tissue.

*Rats received i.p. injections of 10, 40, 100, and 200 mg/kg bw melatonin or vehicle (0) at 10:00 h, and they were sacrificed four hours later. **:P < 0.05, and ***:P < 0.001 vs. 0.*

These data further support the results of previous studies in liver and brain of rats under the same experimental protocol (6), suggesting a local production of melatonin by the heart, which changes along the day but it is not under photoperiodic control. Of note, the content of melatonin in mitochondria along the 24 hours is much more elevated in heart than that elsewhere reported in liver or brain (6), suggesting a relationship between mitochondrial activity and melatonin content. These data also suggest that the variations of melatonin levels in different organelles over a 24-hour period may be due to utilization (e.g. as a radical scavenger), coupled with slight fluctuations in synthesis, rather than to variations in only synthesis. The fact that Px animals showed higher levels of melatonin than that of SPx rats in some hours along the 24 period is difficult to explain at this time, but it may reflect some degree of modulation of extrapineal melatonin by the pineal one.

The saturation of melatonin content in nuclei and mitochondria, the major targets of melatonin in the cell, at doses of 40 mg/kg bw suggest that doses ranging from 5 mg/kg bw to 40 mg/kg bw should represent the therapeutic range of melatonin in this species. To obtain a therapeutical range of doses from animals to humans, it is used the human equivalent dose (6, 20). Based on this calculation, the human doses ranged from 1.6 to 6.5 mg/kg bw, i.e., 112 to 455 mg for an adult human of 70 kg bw. Given that several diseases cause a reduction in the intracellular melatonin content, these data help to provide the first therapeutic approximation of melatonin in heart diseases. The absence of adverse effects of melatonin as reported in the literature indicate melatonin may have a high safety profile in the treatment of cardiac pathologies in humans.

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CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest to this work. Submitting author declares that all co-authors have read the manuscript and they confirm agreement with its final version.

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