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## **CARDIOPROTECTIVE USE OF MELATONIN CAUSES GENDER-SPECIFIC CHANGES OF VEGETATIVE CARDIAC CONTROL IN A SETTING OF CONSTANT ILLUMINATION**

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### **Abstract**

The aim of the work was to assess gender-specific differences in vegetative cardiac control with development of myocardial necrosis in a setting of constant illumination and use of melatonin. An experiment in mature laboratory breed albino rats of either sex simulated abnormal lighting regime. The simulation included keeping the animals for 10 days under constant illumination of 500 lux. On Day 11, myocardial necrosis (MN) was modeled by administering adrenaline (0.5 mg/kg intramuscularly); a follow-up electrocardiography was performed at 1 hour and 24 hours later to assess for heart rhythm variability. A half of animals was given melatonin 1 hour prior to MN modeling (5 mg/kg intraperitoneally). The animals kept under day/night balance were used as controls. The results have shown constant illumination to cause gender-specific changes in effects of vegetative nervous system on formation of heart rhythm in rats; the activity of the parasympathetic component increased in

females and decreased in males. In females, the development of adrenaline-induced myocardial necrosis in a setting of constant illumination was accompanied by changes in cardiac interval (cardiointervalography) findings. The development of these changes with time was identical to that in day/night balance, with a more pronounced activity of the parasympathetic component and a synergetic enhancement of the sympathetic component. When exposed to constant illumination, males had a response different from that in day/night balance; their response to constant illumination was characterized by reduced activity of the parasympathetic component and by predominance of the sympathetic component. Cardioprotective use of melatonin was shown to increase parasympathetic tone compared to day/night balance; in males, this increase was evident at initiation of necrosis and in females, the increase was seen at the peak of necrotic foci formation in the myocardium.

**Key words: myocardial necrosis; constant illumination; vegetative cardiac control; melatonin; gender**

**Introduction.** Globally, cardiovascular disease remains a predominant group of diseases [1, 2]. This defines the importance of research studies exploring the unresolved issues of pathogenesis of cardiovascular disease. Stress is an important risk factor of coronary artery disease, including myocardial infarction. A combination of natural adaptive responses underlying the essence of stress may have detrimental consequences when exposure to stress is long-term. These include the hazardous cardioneclerosis-forming effects of adrenaline, the principal stress hormone, as demonstrated by experimental models of catecholamine necrosis and in a clinical setting [3, 4, 5]. Currently, there are many scientific reports showing that important factors of acute or chronic stress include abnormalities of circadian rhythms, which, under normal conditions, are fundamental for the functioning and adaptation of virtually all forms of life. They define the health status, the capacity for professional activity, the mood and cardiac activity [6, 7, 8]. However, modern lifestyle factors, including active migration, frequent changes of time zones and specific features of professional activity cause excessive photonic stimulation of visual sensory system, which is well beyond physiological limits. All these become the basis for cardiovascular problems, both new and exacerbations of existing ones, including myocardial infarction [9, 10]. Given the above, studies of melatonin effects are attracting significant attention, melatonin being the principal substance regulating circadian rhythms. Melatonin has effects in virtually all organs in the body. However, the cardiologic interest is focused on assessment of cardioprotective effects of this hormone.

Melatonin may reduce ischemic reperfusion injury in the myocardium, both due to its antioxidant properties and due to its effects on vegetative control. Established effects of melatonin include stimulation of vagal effects and inhibition of sympathetic activity. This ensures anti-anginal, anti-arrhythmic, antihypertensive and anti-stressor effects of this hormone in subjects under stress [11, 12, 13, 14, 15]. However, gender-specific aspects of cardioprotective efficacy of melatonin in a setting of impaired circadian rhythms are not completely understood. The aim of the study was to establish gender-specific aspects of melatonin effects on vegetative heart rhythm control in a rat model of adrenaline-induced myocardial necrosis in a setting of constant illumination.

### **Materials and methods**

The experiments were performed in albino rats of either sex (males and females) weighing 220-270 g, which were kept in a vivarium and on a standard vivarium diet. The animals were divided into 3 groups. The animals of Group 1 (controls) were kept under day/night balance. Day/night balance included the following alternating cycles: “day” at 500 lux for 12 hours and “night” at 0.5-1 lux for 12 hours. The animals in Group 2 were constantly kept at 500 lux for 10 days. The animals in Group 3: the lighting parameters were identical to those in Group 2, but 1 hour before simulated adrenaline-induced myocardial necrosis (MN) the animals in this group were given intraperitoneal melatonin (MEL, at 5 mg/kg). MN was modeled with intramuscular adrenaline (at 0.5 mg/kg). The principal tests were performed at 1 hour and at 24 hours after adrenaline injection. ECGs in 2 standard leads were recorded in all animals using a “Cardiolab” computerized ECG system (Kharkiv, Ukraine). The investigators obtained the durations of 1000 consecutive cardiac R-R intervals accurate to 0.001 second. The following parameters were determined: Mo (sec) is the most frequently registered duration of the R-R interval; AMo (%) is the percentage of R-R intervals corresponding to the Mo value, and  $\Delta X$  (sec) is the difference between the longest and the shortest R-R duration. The statistical analysis of results was performed using a parametric method of variation statistics, when normality was demonstrated for the data rows compared, with  $n=6$  for each of the rows. The following parameters were determined: the arithmetic mean (M), standard deviation ( $\bar{\sigma}$ ) and Student's t-test (t). The difference between the arithmetic means was considered significant at t value not less than 2.228 ( $p \leq 0.05$ ). The calculations were performed with Microsoft Excel and BioStat, version 6 (by AnalystSoft Inc., USA). All experiments were conducted in accordance with the ethical principles of animal experimentation, approved at the First National Congress of Bioethics (Kyiv, 2000)

and in harmony with the premises of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes.

### **Results and discussion**

All data present in table 1. The most important parameter of rhythmic cardiac activity is the heart rate (HR). Heart rate analysis has shown that HR values changed differently in males and in females exposed to constant illumination. Males responded to constant illumination with a 7% increase in HR, while females responded with a 36% reduction in HR. Such changes suggested that male and female rats employed different mechanisms of adjustment to abnormal lighting regime, with both genders' mechanisms involving the vegetative nervous system (VNS). The activities of the two components of the VNS (the sympathetic and parasympathetic) were assessed by changes in basic parameters of variational cardiointervalography. In males, there was a 7% reduction in Mo values, while AMo values increased by 17%, which was reflective of activation of the sympathetic component of the VNS. A simultaneous 35% reduction in  $\Delta X$  suggested reduced parasympathetic influence on heart rhythm. Such changes can be interpreted as manifestations of stress due to constant illumination, impaired sleep patterns and impaired melatonin synthesis. Under similar conditions, females had an expected increase in Mo (36%) and an expected reduction in AMo (30%) in a setting of reduced HR, which was reflective of weaker influence of sympathetic component of the VNS on heart rhythm. That said, the 82 % increase in  $\Delta X$  suggested a simultaneous increase in the activity of the parasympathetic component of the VNS and high degree of lability in a stress due to constant illumination.

The development of MN in this setting also demonstrated various mechanisms through which VNS influenced the hearts of male and female rats. At hour 1 of MN (the period of hyperadrenalinemia and initiation of necrotic foci), males had lower heart rates (by 18%) than in Group 1, higher Mo values (by 18%) and lower AMo values (by 82%), which suggested a lesser influence of sympathetic component of the VNS on heart rhythm at this stage of the disease process. The simultaneous 63% higher (compared to Group 1)  $\Delta X$  values reflected a higher parasympathetic tone in spite of post-injection hyperadrenalinemia. Such changes may be attributable either to “enhanced antagonism”, i.e. increased parasympathetic activity in a setting of high sympathetic tone [16], or to the phenomenon of functional desensitization of receptors in a setting of catecholamine excess [17], which can be another marker of stress.

Table 1 - HR, Mo, AMo,  $\Delta X$  findings in rats with adrenaline-induced myocardial necrosis ( $M \pm \bar{o}$ )

Parameter	Gender	Control (n = 6)	MN 1 hr. (n = 6)	MN 24 hrs. (n = 6)
HR				
Group 1	♂	472 $\pm$ 21 #	531 $\pm$ 17 *	491 $\pm$ 29
Day/night balance	♀	438 $\pm$ 19 #	496 $\pm$ 27 *	481 $\pm$ 12 *
Group 2	♂	503 $\pm$ 16 ^#	450 $\pm$ 27*^#	506 $\pm$ 31 #
Constant illumination	♀	323 $\pm$ 7 ^#	392 $\pm$ 22*^#	442 $\pm$ 26 *^#
Group 3	♂	474 $\pm$ 13#	454 $\pm$ 17^	363 $\pm$ 9*^#
Constant illumination+ MEL	♀	450 $\pm$ 10#	458 $\pm$ 24	449 $\pm$ 10^#
Mo				
Group 1	♂	0,127 $\pm$ 0,006#	0,113 $\pm$ 0,002*#	0,122 $\pm$ 0,007
Day/night balance	♀	0,137 $\pm$ 0,006 #	0,121 $\pm$ 0,007*#	0,125 $\pm$ 0,003*
Group 2	♂	0,119 $\pm$ 0,004^#	0,133 $\pm$ 0,008*^#	0,119 $\pm$ 0,008#
Constant illumination	♀	0,186 $\pm$ 0,004^#	0,153 $\pm$ 0,009*^#	0,136 $\pm$ 0,008*^#
Group 3	♂	0,127 $\pm$ 0,004#	0,132 $\pm$ 0,005^	0,165 $\pm$ 0,004*^#
Constant illumination+ MEL	♀	0,133 $\pm$ 0,003#	0,132 $\pm$ 0,007	0,134 $\pm$ 0,003^#
AMo				
Group 1	♂	31,7 $\pm$ 3,8	59,2 $\pm$ 5,8*#	43,7 $\pm$ 7,5*
Day/night balance	♀	33,8 $\pm$ 4,5	45,2 $\pm$ 5,0*#	41,2 $\pm$ 3,1*
Group 2	♂	37,1 $\pm$ 1,7^#	32,6 $\pm$ 2,6*^	47,0 $\pm$ 9,8*
Constant illumination	♀	26,0 $\pm$ 2,8^#	29,8 $\pm$ 2,0 ^	40,4 $\pm$ 5,0*
Group 3	♂	50,5 $\pm$ 4,7^#	31,0 $\pm$ 3,0*^#	24,2 $\pm$ 3,6*^#
Constant illumination + MEL	♀	31,5 $\pm$ 1,5#	37,5 $\pm$ 3,7*^#	37,0 $\pm$ 2,9*#
$\Delta X$				
Group 1	♂	0,65 $\pm$ 0,22	0,35 $\pm$ 0,08*	0,58 $\pm$ 0,04#
Day/night balance	♀	0,55 $\pm$ 0,14	0,33 $\pm$ 0,05*	0,40 $\pm$ 0,06*#
Group 2	♂	0,48 $\pm$ 0,10#	0,57 $\pm$ 0,16^	0,47 $\pm$ 0,12#
Constant illumination	♀	1,00 $\pm$ 0,18^#	0,73 $\pm$ 0,12*^	0,65 $\pm$ 0,10*^#
Group 3	♂	0,45 $\pm$ 0,08#	0,79 $\pm$ 0,09*^#	0,83 $\pm$ 0,10*^#
Constant illumination+ MEL	♀	0,73 $\pm$ 0,08^#	0,60 $\pm$ 0,06*^#	0,67 $\pm$ 0,12^#
Note: * = a significant ( $p \leq 0.05$ ) difference from controls; ^ = a significant difference from Group 1 animals; # = relative to animals of the opposite sex				

The review of test parameters at hour 24 of MN development (i. e at peak of necrosis formation) has shown that male HRs were not different from the respective values in Group 1, while female HRs remained 9% lower. Mo, AMo and  $\Delta X$  values in males of Group 2 were identical to those in Group 1. In females at hour 24 of MN, Mo values were 9% higher compared to Group 1, AMo values were not different and heart rhythm variability ( $\Delta X$ ) remained 63% higher. This was suggestive of preserved high tone of the parasympathetic VNS component in heart rhythm control and reflected a more active involvement of cholinergic stress-limiting mechanisms in cardiac adjustment to adrenaline damage.

Cardioprotective use of melatonin contributed to the fact that HR, Mo and  $\Delta X$  in control males were identical to the respective values in Group 1, while AMo was 59% higher. In the meantime, HR, Mo and AMo in females were identical to the respective values in Group 1, while  $\Delta X$  was 33% higher. This suggested that melatonin, when administered as a single dose to animals exposed to 10 days of constant illumination, was significantly effective in VNS, but such efficacy was dependent on the sex of animals. In Group 3 males, melatonin increased the cardiac rhythm effects of sympathetic VNS; in Group 3 females, melatonin increased the cardiac rhythm effects of parasympathetic VNS. At hour 1 of MN development in males, HR was 17% lower compared to Group 1; Mo was 17% higher, AMo was 91% lower, while  $\Delta X$  was 2.3 times higher. When the same comparison was performed in females, both HR and Mo values were found to be identical to those in Group 1, AMo was 21% lower and the difference for  $\Delta X$  was 1.8-fold. These differences have demonstrated different VNS effects of melatonin in a setting of disease in animals of different sex. In part, it was obvious that inhibition of sympathetic cardiac effects and enhancement of parasympathetic cardiac effects were both more prominent in males. At hour 24 of MN development, HR was 35% lower compared to Group 1; Mo was 35% higher, AMo was 81% lower, while  $\Delta X$  was 43% higher. When the same comparison was performed in females, HR was 7% lower, Mo was 7% higher, AMo was identical to AMo in Group 1 and  $\Delta X$  was 68% higher. The above analysis suggests that the use of melatonin in a MN model in animals exposed to constant illumination substantially changed regulatory activities of sympathetic and parasympathetic components of the VNS with the exact nature of such changes being gender-dependent. In particular, both at the stage of initiation of necrosis (characterized by hyperadrenalinemia), and during maximal necrosis formation, the changes in activities of either VNS components were more substantial in male animals. It is worth noting that females had less pronounced changes with time in the parameters reflective of activity of sympathetic component of the VNS (namely, Mo and AMo). However, the activity of parasympathetic VNS was maximal during the peak of myocardial necrosis (i. e., 24 hours after administration of adrenaline). This difference shows different pathogenetic roles of sympathetic and parasympathetic components in cardiac functioning in a setting of MN, including the situation of constant illumination. Previous research has demonstrated that administration of melatonin in our model had a more substantial cardioprotective influence on female myocardium [18]. This fact demonstrates that females are characterized by better efficacy and harmony concerning the entirety of responses in both VNS components.

## Conclusions

Constant illumination causes gender-specific changes in effects of vegetative nervous system on formation of heart rhythm in rats; the activity of the parasympathetic component is increased in females and decreased in males. In females, the development of adrenaline-induced myocardial necrosis in a setting of constant illumination was accompanied by changes in cardiac interval findings. The development of these changes with time was identical to that in a setting of day/night balance, with a more pronounced activity of the parasympathetic component and a synergetic enhancement of the sympathetic component. When exposed to constant illumination, males had a response different from that in day/night balance; their response to constant illumination demonstrated reduced involvement of the parasympathetic component and predominance of the sympathetic component. Cardioprotective use of melatonin increased parasympathetic tone compared to day/night balance; in males, this increase was evident at initiation of necrosis and in females, the increase was seen at the peak of necrotic foci formation in the myocardium.

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