BIOLOGICA NYSSANA
12 (2) December 2021: 167-176
DOI: 10.5281/zenodo.5759829

# Melatonin affects gene expression of noradrenaline transporter and enzymes in the hearts of stressed rats

*Original Article*

Bojana Stefanović
Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, the National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia
*stefanovic_bojana@vin.bg.ac.rs (corresponding author)*

Nataša Spasojević
Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, the National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia

Predrag Jovanović
CEDARS-SINAI, Centre for Neural Science and Medicine, 8700 Beverly Blvd., Los Angeles, CA 90048, USA

Harisa Ferizović
Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, the National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia

Milica Janković
Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, the National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia

Perica Vasiljević
Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Nis, Višegradska 33, 18000 Niš, Serbia

Slađana Dronjak
Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, the National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia

*Abstract:*

The role of stress is important in the etiology of depression which subsequently affects cardiovascular regulation. There has been a lot of evidence of the beneficial effects of melatonin in various cardiovascular pathologies. We examined the effect of chronic melatonin treatment on noradrenaline content, synthesis, transport and degradation in the left atria and ventricle of rats exposed to chronic mild unpredictable stress (CUMS). Our results show that CUMS decreased expression of mRNA TH and NET in the left atria and COMT in the left ventricles, whereas increased MAO-A enzymes in the left ventricles. Melatonin treatment induced a significant increase in gene expression of NET in the atria and a decrease in MAO-A in the ventricle. The observed beneficial effects of enhanced uptake and reduced degradation during melatonin treatment were most probably a compensatory mechanism which protects cardiomyocytes from the deleterious effects of noradrenaline overstimulation.

*Key words:*
melatonin, noradrenaline, heart, stress, transporter, enzymes

*Apstrakt:*

**Efekat melatonina na ekspresiju gena za noradrenalinski transporter i enzime degradacije u srcu hronično stresiranih pacova**

Uloga stresa je važna u etiologiji depresije koja posledično može uticati na kardiovaskularnu regulaciju. Postoji mnogo dokaza o blagotvornom dejstvu melatonina u različitim kardiovaskularnim oboljenjima. Iz tih razloga smo ispitivali uticaj hroničnog tretmana melatoninom na količinu noradrenalina, njegovu sintezu, transport i degradaciju u levoj pretkomori i komori pacova izlaganih hroničnom blagom nepredvidivom stresu (CUMS). Naši rezultati pokazuju da je CUMS smanjio količinu iRNK za TH i NET u levoj pretkomori i COMT u levoj komori, dok je povećao gensku ekspresiju MAO-A enzima u levoj komori. Tretman melatoninom je značajno povećao gensku ekspresiju NET u pretkomorama i smanjio količinu enzima MAO-A u komori. Uočeni korisni efekti melatonina na ponovno preuzimanje i smanjenu degradaciju noradrenalina predstavljaju kompenzacione mehanizme koje štite kardiomiocite od štetnih efekata njegove prekomerne stimulacije.

*Ključne reči:*
melatonin, noradrenalin, srce, stres, transporter, enzimi

*Received:* April 28, 2021
*Revised:* September 01, 2021
*Accepted:* November 01, 2021

## Introduction

Stress is an underlying trigger that leads to the development of depression as well as cardiovascular disease. Depression is found in 20–30% of cardiovascular patients (Whooley, 2006). Depression is associated with the autonomic nervous system dysfunction which could subsequently lead to cardiovascular disregulation (Carney et al., 2005). Several studies have demonstrated an exaggerated cardiac noradrenaline response in major depression (Veith et al., 1994; Yehuda et al., 1998; Mausbach et al., 2005). The methods inducing depression-like states in experimental animals are generally accepted as valuable tools for the studies of affective disorders. Among these methods, chronic unpredictable mild stress (CUMS) model of depression has been frequently employed (Willner et al. 1992). Grippo et al. (2006) reported that CUMS model of depression is characterized by

anhedonia and elevated sympathetic cardiac tone. Functional noradrenergic transmission consists of a balance between noradrenaline synthesis, secretion and reuptake. Noradrenergic activity is dependent on the synthesis of noradrenaline as determined by the rate limiting enzyme tyrosine hydroxylase (TH). Noradrenaline released from cardiac sympathetic nerve terminals is removed from the neuroeffector junction by the neuronal noradrenaline transporter (NET) and metabolized to dihydroxyphenylglycol (DHPG) via monoamine oxidase-A (MAO-A) (Esler et al., 1990). Jovanović et al. (2019) reported that exposure of the animals to social isolation for 12 weeks induced hypertrophy of cardiomyocytes in the wall of the left ventricle and a significant increase in noradrenaline levels in the left atria and the ventricle.

Melatonin (N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone, which is synthesized primarily by the pineal gland (Hardeland et al., 2006). Melatonin easily crosses all morpho-physiological barriers, and it can enter all cells of the body, affecting the function of a variety of tissues (Tan et al. 1999). Additionally, by virtue of its ability to detoxify free radicals and related oxygen derivatives, melatonin affects the molecular physiology of cells and contributes to improved cellular and organismal physiology (Reiter et al., 2010). The experimental data obtained from rodent studies suggest that melatonin has beneficial effects in the treatment of cardiovascular diseases (Reiter et al., 2010). Melatonin is also a well-established antioxidant molecule with many beneficial effects in various cardiovascular pathologies (Sun, et al., 2016). In humans, melatonin production not only diminishes with age (Sack et al., 1986) but is also significantly lower in many age-related diseases, including cardiovascular disease (Sakotnik et al., 1999; Altun et al., 2002; Dominguez-Rodriguez et al., 2002; Yaprak et al., 2003). The link between the heart and melatonin and the effect of endogenous melatonin on cardiovascular function is well established (Dominguez-Rodriguez et al., 2010). Melatonin interacts with the heart and blood vessels indirectly via the nervous system (Sewerynek, 2002; Pechanova et al., 2014), and directly, through its receptor-dependent and independent activities as a signaling molecule and a free radical scavenger (Paulis et al., 2012; Galano & Reiter, 2018). There is also an increasing amount of evidence regarding the beneficial effects of melatonin via exogenous administration (Tarocco et al., 2019). Recently, we have reported that melatonin treatment enhanced noradrenaline content and protein expression of TH, DBH, PNMT, and NET in the adrenal medulla of rats exposed to CUMS (Stefanovic et al., 2019).

Although many studies have indicated its cardioprotective actions, the underlying mechanistic links remain unclear. Hence, it is important to examine the regulation of more specific variables, such as the gene expression of noradrenaline biosynthetic and degrading enzymes and transporter in the left atria and ventricles of rats exposed to CUMS model of depression.

## Materials and Methods

### *Animals and treatment*

The experiments were performed on adult (two-month-old) male Wistar rats. The weight of males at the beginning of the experiment was 250-310 g. All animals were housed in a temperature-controlled room (20±2.0 °C) and synchronised to a 12-hour light/dark regime. Four rats were kept in a standard plastic cages and given ad libitum access to standard laboratory food (commercial rat pellets) and tap water. Care was taken to minimise the pain and discomfort of the animals in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). All procedures with animals were approved by the Ethical Committee for the Use of Laboratory Animals of the Vinča Institute and the Ministry of Agriculture and Environmental Protection, Authority for Veterinary, permission no. 323-07-04657/2015-05/02.

In this experiment, we used 24 animals, which were allocated to control (unstressed) and chronic unpredictable mildly stressed groups. These groups were further divided into two subgroups each and were receiving daily injection of vehicle (5% ethanol) or melatonin in dose of 10 mg $kg^{-1}$ body weight by intraperitoneally (i.p.) route one hour before the dark phase (Bassani et al., 2014). This time was chosen to avoid disruption of circadian rhythms that occurs if melatonin is administered throughout the day. Melatonin (Q-1300, Bachem, Switzerland) was dissolved in NaCl (0.9%) containing 5% ethanol. The dose of 10 mg $kg^{-1}$ is a supraphysiological dose chosen with the aim of assessing the drug's therapeutic effect and not for replacing or restorative purposes. The dosage of 10 mg/kg/day was regularly adjusted according to the body weight for the entire period of treatment. Exposure to CUMS and the vehicle, i.e. drug administration started on the same day and continued for 4 weeks.

The stressors were presented for 1 week and repeated during the following 4 weeks. The same stress method was not continuously applied to ensure the rats would be unable to anticipate the next type of stress that would be applied. Control animals were left undisturbed in their home cages with the exception of general handling (i.e. regular

cage cleaning and body weight measuring).

### *Chronic unpredictable mild stress*

The CUMS procedure was carried out according to the methods described by Grippo et al. (2002), and it was designed to maximise the unpredictable nature of the stressors. The CUMS groups of animals were exposed to the following stressors in random order: forced running (15 mins., 10 m/min); soiled cage (500 ml 22 °C water spilled into bedding for 5 h); 45° cage tilt along the vertical axis for 7 h; 17 h food deprivation, 5 h cold room (4 °C) and water deprivation (water bottles were removed from cage during this time), 5 h grouped housing (8 rats per cage) and individual housing (48 h). Control animals were left undisturbed in the home cage with the exception of general handling (i.e. regular cage cleaning and body weight measuring), which was matched to that of the CUMS group. Immediately after the CUMS procedure, all animals were sacrificed, between 10:00–11:00 a.m., by rapid decapitation with a guillotine (Harvard-Apparatus, USA). Left atria and ventricles were extracted quickly on ice, frozen in liquid nitrogen and stored at -70 ℃ until sample preparation for further molecular analysis.

### *High-performance liquid chromatography assay*

Ethylene glycol tetraacetic acid (EGTA) and DL-Norepinephrine hydrochloride (NA) were purchased from Sigma–Aldrich. Ammonium formate was supplied by Fisher Scientific (Loughborough, UK); formic acid (49–51%) by Fluka analytical (Switzerland); methanol by J.T. Baker (Deventer, The Netherlands), perchloric acid (70%) by Sigma–Aldrich and magnesium chloride ($MgCl_2 \times 5H_2O$) by Sigma–Aldrich. Purified water was obtained via a BlueClearRO600P reverse osmosis water cleaning system with integrated BlueSoft07-MB mixed bed salt remover (Euro-Clear Ltd., Hungary).

The stock standard solution of NA [1 mg/mL] was prepared in methanol and kept at -20 °C. Standard solutions in concentrations of 1, 2, 4, 8, 16, 32, 40 and 50 µg/mL were prepared by dilution of the stock standard solution in DEPROT (2% EGTA; 0,1N $HClO_4$; 0.2% $MgCl_2$). The ammonium formate buffer (100 mM, pH 3.6) was used as one of the mobile phase components. Tissue samples were homogenised in DEPROT (1 mg:10 µL) using an Ultra-turrax homogeniser (T10 basic), sonicated (Branson Sonic Power Company, Danbury, Connecticut; 3×10 s) and centrifuged (Sorvall SuperT21; 30 mins, 18.000 rpm, +4 °C). Supernatants were transferred in separate tubes and placed in the autosampler of the HPLC system. Data were obtained using a Thermo Scientific (DionexUltiMate 3000) HPLC system consisting of degasser unit, binary pump (HPG-3200SD), autosampler (WPS-3000 SplitLoop), column oven (Col. Comp. TCC-3000) and RS electrochemical detector (ECD-3000RS) equipped with the 6041RS ultra Amperometric Analytical Cell and glassy carbon working electrode. A Hibar 125-4 LiCrospher100 RP-18 (5 µm) HPLC column (Merck Millipore, Darmstadt, Germany) was used. Instrument control and data acquisition was carried out by the Chromeleon7 Chromatography Data System (Thermo Scientific). The following chromatographic conditions were obtained after the method optimisation. The mobile phase consisting of the ammonium formate buffer (100 mM, pH 3.6) as an A solution and methanol as a B solution was pumped at a flow rate of 500 µL/mins with the following step gradient. The run started with a mobile phase consisting of 98% A and 2% B solution. At the 9$^{th}$ minute of the run the part of B solution started to rise, reaching the 50% in the 15$^{th}$ mins and 80% in the 18$^{th}$ mins. Starting from the 20$^{th}$ mins until the end of the run (30$^{th}$ mins) the column was re-equilibrated with the initial mixture of mobile phase solutions (2% of A and 98% of B solution). The applied potential for electrochemical measurements was +850 mV and the separation temperature was set at 25 °C. The 50 µL of samples and standard solutions were applied to the system.

### *RNA isolation and real-time RT-PCR*

The total RNA from left atria and ventricles was extracted using TRIzol® Reagent (Invitrogen, CA, USA). Total RNA was isolated using chloroform-isopropanol extraction and quantification and RNA quality was carried out using spectrophotometer (NanoDrop 1000 Spectrophotometer, Thermo Scientific). Tissue samples were homogenised in 1 ml TRIzol® reagent per 100 mg of tissue, using electrical homogeniser (IKA-WERKE, GmbH & Co, Germany). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Bead (GE Healthcare Life Sciences, PA USA) and pd(N)6 primer according to the manufacturer's protocol. Real-Time RT-PCR assay was done exactly as previously described by Gavrilović et al. (2008). PCR reaction was performed in the ABI Prism 7000 Sequence Detection System at 50 °C for 2 mins., 95 °C for 10 mins., followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. TaqMan PCR reaction was carried out using Assay-on-Demand Gene Expression Products (Thermo Fischer Scientific, MA USA) for TH (ID: Rn00562500_m1), for NET (ID: Rn00580267_m1), for COMT (ID:Rn01404927_g1) and for MAO-A (ID:Rn01430950_m1). A reference endogenous control was included in each analysis to

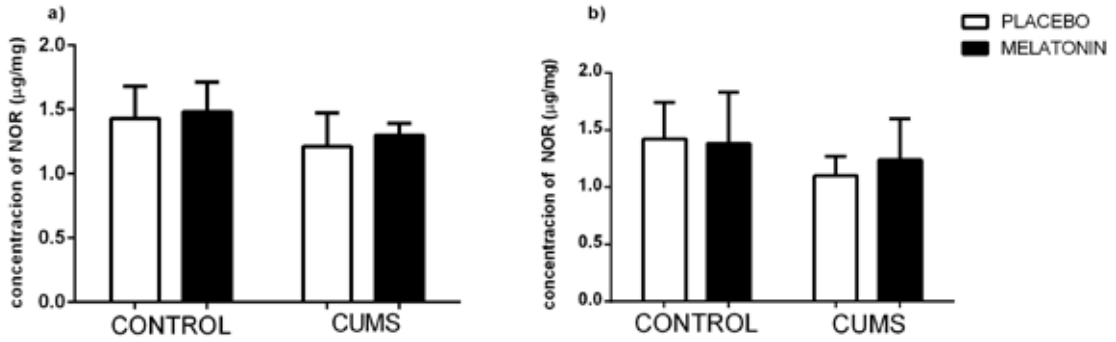


**Fig. 1.** The effect of chronic melatonin treatment on noradrenaline content in the left atria (**a**) and ventricle (**b**) of controls and rats exposed to CUMS. The results are presented as mean ± S.E.M. for a sample of 6 rats

correct the differences in the inter-assay amplification efficiency and all transcripts were normalised to cyclophilin A (ID:Rn00690933) expression. The obtained results were analyzed by RQ Study Add ON software for 7000 v 1.1 SDS instrument (ABI Prism Sequence Detection System) with a confidence level of 95% ($p<0.05$). The relative expression of the target gene was normalized to cyclophyline A and expressed in relation to the calibrator, i.e. the control sample.

### *Western blot analysis*

Left atria and ventricles were homogenised in RIPA Lysis Buffer System (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, sc-24948). After centrifugation (12 000 r.p.m., 20 mins at 4 °C), the supernatant was taken and protein concentration was determined by the method of Lowry et al. (1951). Samples were incubated for 5 mins at 100 °C in the appropriate amount of denaturing buffer according to Laemmli (1970). 30 µg of protein extract from tissue samples separated by 12% SDS-poly-acrylamide gel electrophoresis were transferred to a supported PVDF membrane (Immobilon-P membrane, Merck Millipore, Massachusetts, USA). The membranes were blocked in 5% non-fat dry milk in Tris-Buffered Saline-Tween 20 (TBST) for 1 h. All following washes (three times for 15 mins.) and antibody incubation (overnight at 4 °C for primary antibody and 1 h at ambient temperature for secondary antibody) were also performed in TBST at ambient temperature on a shaker. For measuring TH, NET, COMT and MAO-A, a rabbit polyclonal anti-TH primary antibody (ab51191, dilution 1:1000, Abcam, Cambridge, UK), a rabbit polyclonal anti-NET primary antibody (ab41559, dilution 1:1000, Abcam, Cambridge, UK), a rabbit polyclonal anti-COMT primary antibody (ab126618, dilution 1:5000, Abcam, Cambridge, UK) and a rabbit polyclonal anti-MAO-A primary antibody (ab126751, dilution1:1000, Abcam, Cambridge, UK), was used, respectively. Washed membrane was further incubated in the horseradish peroxidase-conjugated secondary anti-rabbit antibody for luminol-based detection (ab6721, dilution 1:5000, Abcam, Cambridge, UK). The secondary antibody was then visualized by Immobilon Western Chemiluminescent HPR Substrate (Merck Millipore, Massachusetts, USA) and exposed to X-ray film for Western Blot Detection. Image J analysis PC software (NIH, Bethesda, MD) was used for quantification densitometry of protein bands on X-ray film. Amounts of all analysed proteins were normalised to β-actin levels.

### *Statistical analysis*

The results are reported as means ± S.E.M. The significance of the differences in the catecholamine concentration, gene expression and protein levels of the examined TH, NET, COMT and MAO-A in the left atria and ventricles of the four groups of rats was estimated by a two-way ANOVA test. The Tukey post-hoc test was used to evaluate the differences between the groups. Statistical significance was set at $p<0.05$.

## Results

### *The Concentration of noradrenaline in the left atria and ventricles*

The results (**Fig. 1)** indicate that chronic exposure of rats to unpredictable stressors, as well as melatonin treatment, did not alter the noradrenaline concentration in the left atria and ventricles.

### *The gene expression of TH enzyme in the left atria and ventricles*

Two-Way ANOVA showed that chronic stress

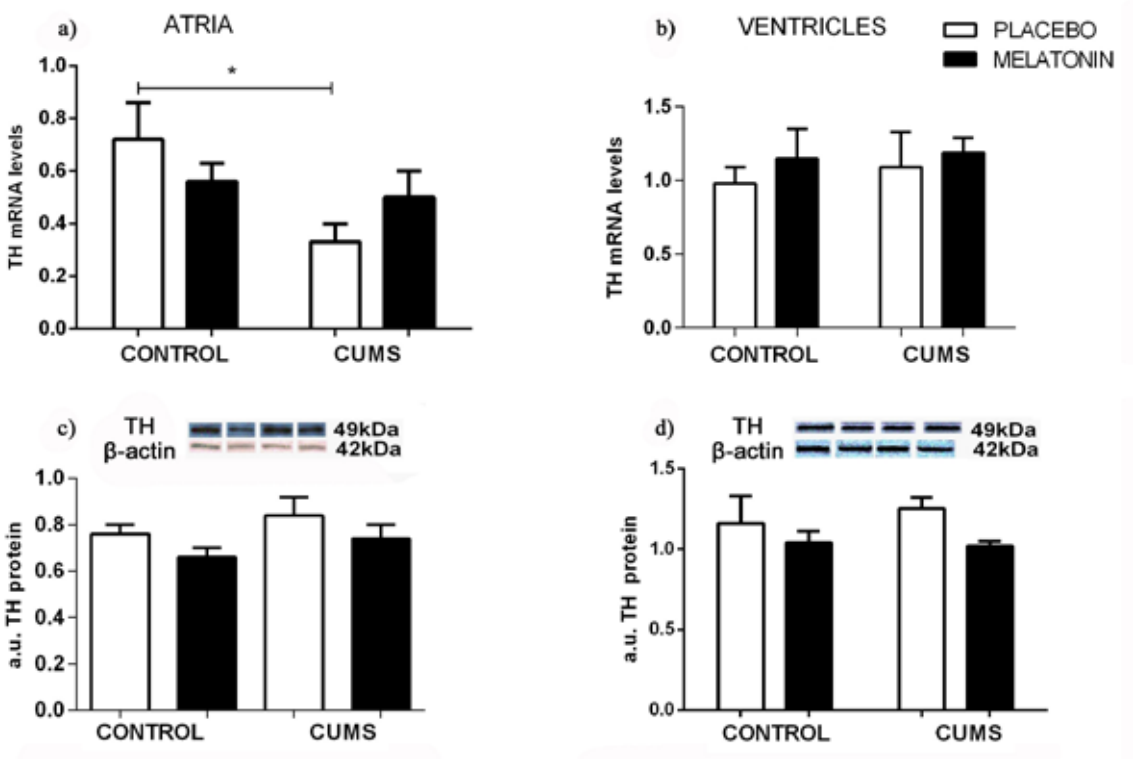


**Fig. 2.** The effect of chronic melatonin treatment on TH mRNA (**a**, **b**) and protein level (**c**, **d**) in the left atria and ventricle of controls and rats exposed to CUMS. The results are presented as mean ± S.E.M. for a sample of 6 rats. Statistical significance: *p<0.05 control vs. CUMS (Tukey test)

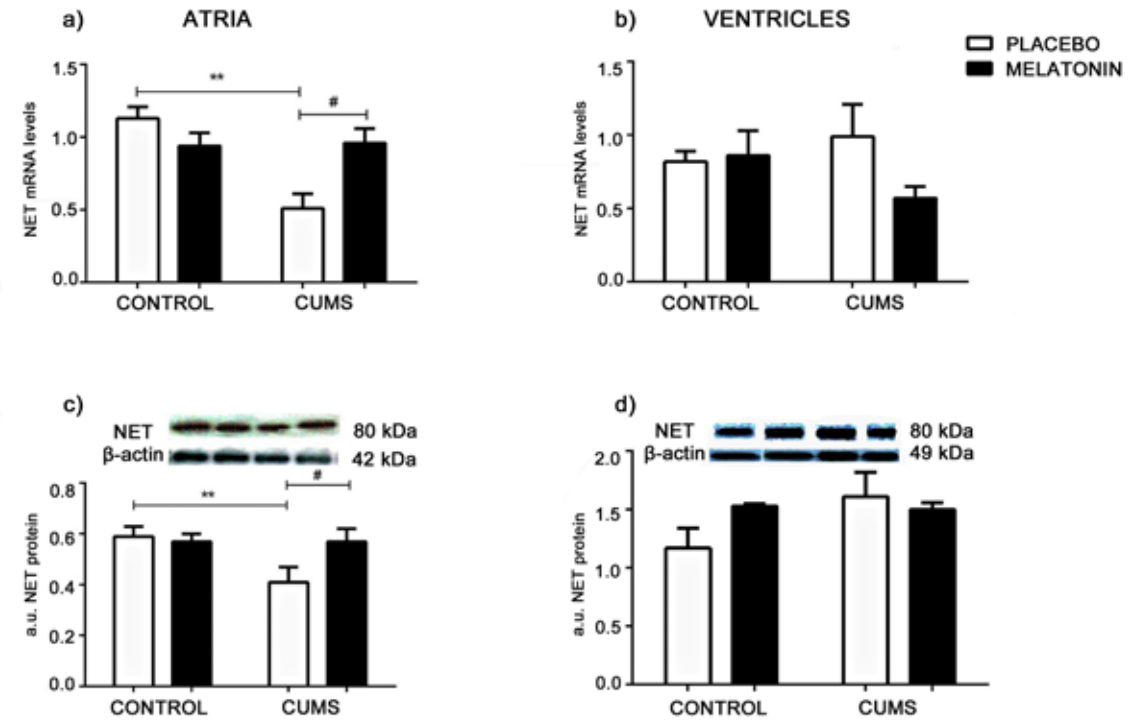


**Fig. 3.** The effect of chronic melatonin treatment on NET mRNA (**a**, **b**) and protein level (**c**, **d**) in the left atria and ventricle of controls and rats exposed to CUMS. The results are presented as mean ± S.E.M. for a sample of 6 rats. Statistical significance: **p<0.01 control vs. CUMS; #p<0.05 placebo vs. melatonin (Tukey test)

affects TH mRNA levels ($F(1.23) = 4.72$, $p<0.05$) in the left atria (**Fig. 2a**). Exposure to CUMS significantly reduced mRNA levels for TH (by 55%, $p<0.05$), but did not alter the protein levels of this enzyme in the left atria. There was not a significant effect of melatonin treatment on the gene expression of TH enzyme in the left atria.

CUMS did not change the expression of the TH gene in the left ventricles. The analysis of the results showed no significant effect of melatonin treatment on the TH mRNA and protein levels in the left ventricles.

### *The gene expression of NET transporter in the left atria and ventricles*

The analysis of the results showed in **Fig. 3** indicated the significant effect of both stress and melatonin treatment on the NET mRNA levels ($F(1.23) = 16.77$, $p<0.001$), as well as the protein content of this transporter ($F(1.23) = 4.63$, $p<0.05$) in the left atria. Chronic stress led to a decrease in both the mRNA level (by 55%, $p<0.01$), as well as the protein level of this transporter (by 30%, $p<0.01$). On the other hand, melatonin treatment induced an increase in NET gene expression ($p<0.05$) in animals exposed to CUMS, returning mRNA levels to control values.

The obtained results in **Fig. 3** showed that neither CUMS nor melatonin changed NET gene expression in the left ventricles.

### *The gene expression of MAO-A enzyme in the left atria and ventricles*

Changes as a result of the interaction between chronic stress and melatonin treatment on MAO-A mRNA ($F(1.23) = 48.13$, $p <0.001$) and protein levels ($F(1.23) = 69.28$, $p<0.001$) in the left ventricles are presented in **Fig. 4**. CUMS increased MAO-A gene expression in the left ventricles ($p<0.01$), while melatonin decreased mRNA ($p<0.01$) and protein levels ($p<0.05$) of MAO-A enzyme in the left ventricles of stressed rats.

### *The gene expression of COMT enzyme in the left atria and ventricles*

The results showed in **Fig. 5** indicate that chronic exposure to unpredictable stressors, as well as melatonin treatment, did not significantly alter COMT gene expression in the left atria of examined animals.

The analysis of the results showed that CUMS

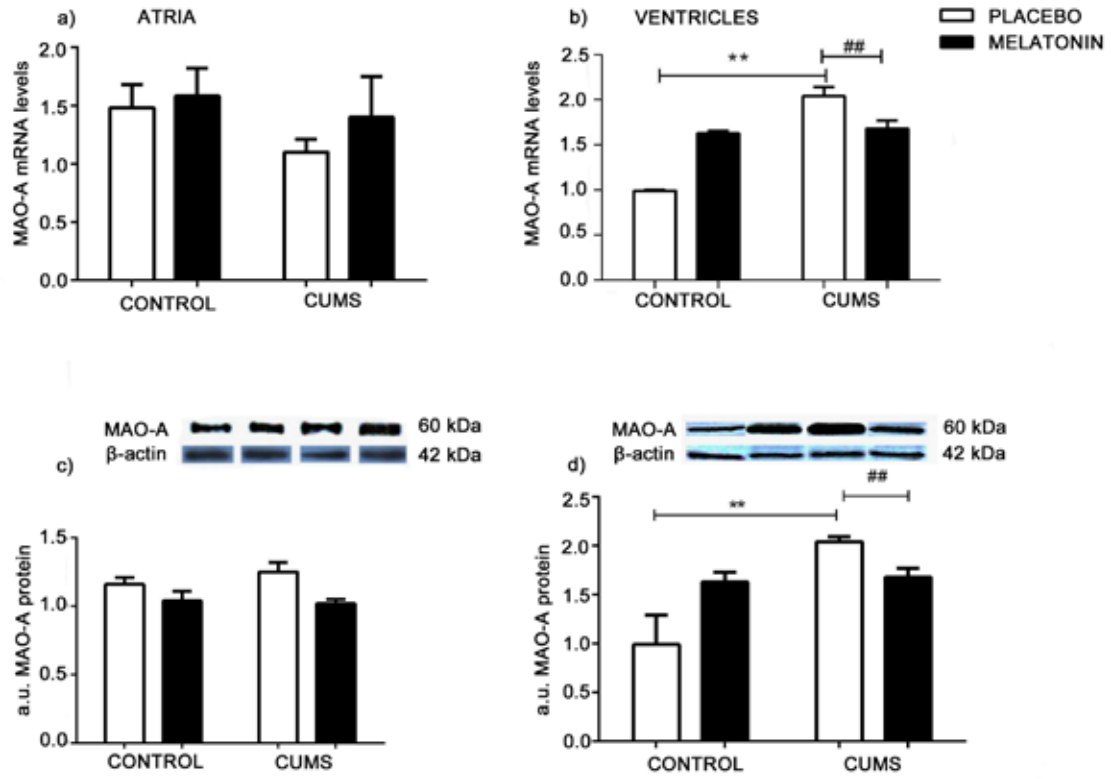


**Fig. 4.** The effect of chronic melatonin treatment on MAO-A mRNA (**a**, **b**) and protein level (**c**, **d**) in the left atria and ventricle of controls and rats exposed to CUMS. The results are presented as mean ± S.E.M. for a sample of 6 rats. Statistical significance: **p<0.01 control vs. CUMS; ##p<0.01 placebo vs. melatonin (Tukey test)

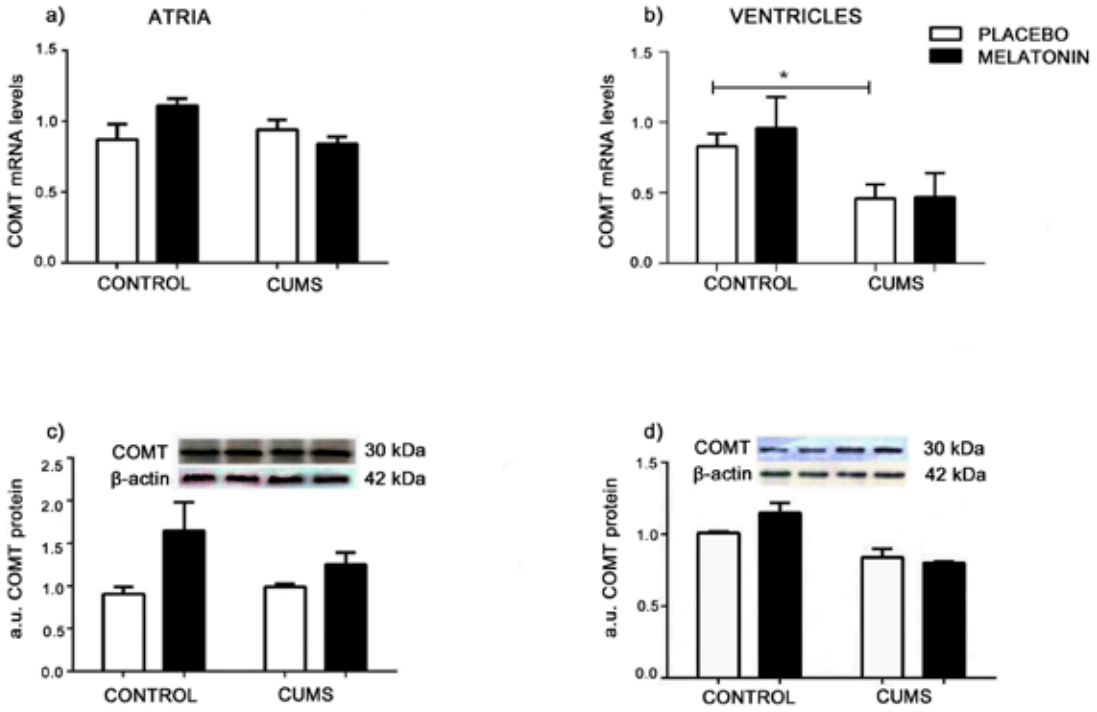


**Fig. 5.** The effect of chronic melatonin treatment on COMT mRNA (**a**, **b**) and protein level (**c**, **d**) in the left atria and ventricle of controls and rats exposed to CUMS. The results are presented as mean ± S.E.M. for a sample of 6 rats. Statistical significance: *p<0.05 control vs. CUMS (Tukey test)

reduced COMT mRNA levels (by 45%, p<0.05), while melatonin failed to change the mRNA levels of COMT in the left ventricles. There was also no significant change in COMT protein levels in the left ventricles under the stress protocol and melatonin treatment.

## Discussion

The changes in autonomic regulation of the heart, such as the activation of the sympathetic nervous system, the reduction in vagal tone and the increase in heart rate (HR) is often observed in depressed patients. The CUMS model of stress increases the sympathetic tone (Grippo et al., 2002), suggesting that autonomic changes may mediate changes in the heart rate of rats exposed to CUMS. Noradrenaline contribution to an increase in cardiac output is well established. Our results showed that CUMS as well as melatonin treatment does not change the concentration of noradrenaline in the left atria and ventricles (**Fig. 1**). Ganguly et al. (1997) also reported that the level of noradrenaline remained unchanged in rats with myocardial infarction. Induction of gene expression of the noradrenaline-synthesizing enzyme has a very important role in responding to stress and in the regulation of the cardiovascular system. Our results show that CUMS decreased the expression of mRNA TH in the left atria whereas gene expression of TH remained unchanged in the ventricle (**Fig. 2**). These findings are in agreement with Gavrilović et al. (2009) who have demonstrated that long-term social isolation stress also produced a decrease in TH mRNA level in left atria. The cardiac neuronal NET in sympathetic neurons is responsible for the uptake of released noradrenaline from the neuroeffector junction (Esler et al., 1990). There are studies that indicated reduced uptake in the diseased heart (Habecker et al., 2006). We showed a decrease in the gene expression of the NET transporter in the left atria, while the expression of this transporter is unchanged in the left ventricle under stress protocol (**Fig. 3**). In the study DOCA-salt hypertension, NET mRNA was reduced in the left atria (Wehrwein et al., 2013). Decreased sympathoneural uptake of noradrenaline has been associated with cardiovascular disease while faulty NET activity is a contributing factor to the increase in cardiac pathology (Shanks et al., 2013). Catechol O-methyltransferase (COMT) and MAO-A contributes to the removal of noradrenaline in the myocardium and it is believed to exert degradative action at high noradrenaline levels. In our experimental model there was no change in the

level of mRNA and the COMT protein in the atria but the level of mRNA for the same enzyme decreased in the left ventricles (**Fig. 5**). These effects of CUMS were not observed at the levels of COMT protein in the left ventricle (**Fig. 5**). Changes caused by stress in COMT degrading enzyme certainly depend on the type and duration of the stressor and numerous other factors. Kuroko et al. (2005) have shown that COMT contributes to the inactivation of high levels of noradrenaline during myocardial ischemia and it has been assumed that this degrading enzyme is more active in more severe heart damages. Namely, higher levels of catecholamine are necessary for the activation of COMT. In the study of Kaludercic et al. (2014), MAO activity has been pointed out as an another mechanism potentially implicated in heart failure. In peripheral tissues, MAO is involved in oxidative catabolism of amines, which generates aldehyde, which is in its turn metabolized to the corresponding acid by aldehyde dehydrogenase, and also ammonia and $H_2O_2$, products that are toxic in high concentration (Kaludercic et al., 2011). In our experimental CUMS model, the gene expression of MAO-A enzymes in the left ventricles has been increased (**Fig. 4**). MAO-A contributes to heart failure progression via enhanced noradrenaline catabolism and oxidative stress. Based on our results we could suggest that enhanced gene expression of MAO-A might results in augmented ROS generation, contributing to left ventricular dysfunction in hearts rats subjected to CUMS.

Among endogenous substances with great success against cardiac illness certainly is melatonin. That is also a hormone with marked antioxidant properties. Studies reports that circulating levels of melatonin and his synthesis are reduced in patients with different cardiovascular diseases (Dominguez-Rodriguez et al., 2012). Effects of melatonin on noradrenaline biosynthesis, uptake and degradation in the heart of depression model rats are still poorly understood. The melatonin treatment in our experiment induced a significant increase in gene expression of NET in the atria (**Fig. 3**) and a decrease in MAO-A in the ventricle (**Fig. 4**). We failed to observe any effects of the melatonin treatment in the gene expression of TH (**Fig. 2**) and COMT (**Fig. 5**) in the heart of CUMS rats. Given that NET is responsible for rapidly eliminating noradrenaline from the cardiac synaptic cleft, the elevated expression of NET with the melatonin treatment may explain the greater capacity of noradrenaline uptake in the left atria; a fact which may contribute to the maintenance of atrial function in a stressful situation. An aberrantly high level of cytoplasmic noradrenaline has been linked to oxidative stress and neuronal toxicity. Increased sequestration and reduced deamination of noradrenaline by MAO-A during melatonin treatment produced a decreased generation of reactive oxygens species. Thus, the observed beneficial effects of enhanced uptake and reduced degradation during melatonin treatment were most probably a compensatory mechanism which protects cardiomyocytes from the deleterious effects of noradrenaline overstimulation.

## Conclusions

Additional experiments are needed for a better understanding of the role of melatonin, by identifying the molecular markers involved in chronic stress and thereby ensuring the use of melatonin for therapeutic purposes. The implications of this study may provide insights into the mechanisms of cardiovascular dysfunction that frequently accompany depression. Although melatonin is an effective cardioprotective agent, further investigation is needed to expand its range of therapeutic applications. These findings may provide an insight into the mechanisms underlying depression and cardiovascular disease in humans.

**Acknowledgements.** This work was supported by the Ministry of Education, Scientific and Technological Development of the Republic of Serbia.

## References

**Altun, A., Yaprak, M., Aktoz, M.,Vardar, A., Betul, U.A., Ozbay, G.** 2002: Impaired nocturnal synthesis of melatonin in patients with cardiac syndrome X. *Neuroscience Letters*, 327(2): 143-145.

**Bassani, T.B., Gradowski, R.W., Zaminelli, T., Barbiero, J.K., Santiago, R.M., Boschen, S.L., da Cunha, C., Lima, M.M., Andreatini, R., Vital, M.A.** 2014: Neuroprotective and antidepressant like effects of melatoninin a rotenone-induced Parkinson's disease model in rats. *Brain Research*, 1593: 95-105.

**Carney, R.M., Freedland, K.E., Richard C Veith, R.C.** 2005: Depression, the autonomic nervous system, and coronary heart disease. *Psychosomatic Medicine,* 67: 29-33.

**Dominguez-Rodriguez, A., Abreu-Gonzalez, P., Avanzas, P.** 2012: The role of melatonin in acute myocardial infarction. *Frontiers in Bioscience*, 17: 2433-2441.

**Dominguez-Rodriguez, A., Abreu-Gonzalez, P., Garcia, M.J., Sanchez, J., Marrero, F., Armas-Trujillo, D.** 2002: Decreased nocturnal melatonin levels during acute myocardial infarction. *Journal of Pineal Research*, 33(4): 248-252.

**Dominguez-Rodriguez, A., Abreu-Gonzalez, P., Sanchez-Sanchez, J.J., Kaski, J.C., Reiter, R.J.** 2010: Melatonin and circadian biology in human cardiovascular disease. *Journal of Pineal Research*, 49(1): 14-22.

**Esler, M., Jennings, G., Lambert, G., Meredith, I., Horne, M., Eisenhofer, G.** 1990: Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiological Reviews,* 70(4): 963-985.

**Galano, A., Reiter, R.J.** 2018: Melatonin and its metabolites versus oxidative stress: From individual actions to collective protection. *Journal of Pineal Research*, 65(1): e12514.

**Ganguly, P.K., Dhalla, K.S., Shao, Q., Beamish, R.E., Dhalla, N.S.** 1997: Differential changes in sympathetic activity in left and right ventricles in congestive heart failure after myocardial infarction. *American Heart Journal*, 133(3): 340-345.

**Gavrilovic, L., Spasojevic, N., Tanic, N., Dronjak, S.** 2008: Chronic isolation of adult rats decreases gene expression of catecholamine biosynthetic enzymes in adrenal medulla. *Neuroendocrinology Letters*, 29(6): 1015-1020.

**Gavrilovic, L., Spasojevic, N., Zivkovic, M., Dronjak, S.** 2009: Effect of immobilization stress on gene expression of catecholamine biosynthetic enzymes in heart auricles of socially isolated rats. *Brazilian journal of medical and biological research*, 42(12): 1185-1190.

**Grippo, A.J., Beltz, T.G., Weiss, R.M., Johnson, A.K.** 2006: The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. *Biological Psychiatry,* 59(4): 309-316.

**Grippo, A.J., Moffitt, J.A., Johnson, A.K.** 2002: Cardiovascular alterations and autonomic imbalance in an experimental model of depression. American journal of physiology. *Regulatory, integrative and comparative physiology,* 282(5): R1333-1341.

**Habecker, B.A., Willison, B.D., Shi, X., Woodward, W.R.** 2006: Chronic depolarization stimulates norepinephrine transporter expression via catecholamines. *Journal of Neurochemistry*, 97(4): 1044-1051.

**Hardeland, R., Pandi-Perumal, S.R., Cardinali, D.P.** 2006: Melatonin. *The International Journal of Biochemistry and Cell Biology*, 38(3): 313-316.

**Jovanović, P., Spasojević, N., Puskaš, N., Stefanović, B., Dronjak, S.** 2019: Oxytocin modulates the expression of norepinephrine transporter, $\beta_3$ adrenoceptors and muscarinic $M_2$ receptors in the heart of socially isolated rats. *Peptides*, 111: 132-141.

**Kaludercic, N., Carpi, A., Menabò, R., Di Lisa, F., Paolocci, N.** 2011: Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/ reperfusion injury. *Biochimica et Biophysica Acta*, 1813(7): 1323-1332.

**Kaludercic, N., Mialet-Perez, J., Paolocci, N., Parini, A., Di Lisa, F.** 2014: Monoamine oxidases as sources of oxidants in the heart. *Journal of Molecular and Cellular Cardiology*, 73: 34-42.

**Kuroko, Y., Fujii, T., Yamazaki, T., Akiyama, T., Ishino, K., Sano, S., Mori, H.** 2005: Contribution of catechol O-methyltransferase to the removal of accumulated interstitial catecholamines evoked by myocardial ischemia. *Neuroscience letters*, 388(2): 61-64.

**Laemmli, U.K. 1970:** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

**Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.** 1951: Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193(1): 265-275.

**Mausbach, B.T., Dimsdale, J.E., Ziegler, M.G., Mills, P.J., Ancoli-Israel, S., Patterson, T.L., Grant, I.** 2005: Depressive symptoms predict norepinephrine response to a psychological stressor task in Alzheimer's caregivers. *Psychosoatic Medicine*, 67(4): 638-642.

**Paulis, L., Simko, F., Laudon, M.** 2012: Cardiovascular effects of melatonin receptor agonists. *Expert Opininion on Investigational Drugs*, 21(11): 1661-1678.

**Pechanova, O., Paulis, L., Simko, F.** 2014: Peripheral and central effects of melatonin on blood pressure regulation. *International Journal of Molecular Science*, 15(10): 17920-17937.

**Reiter, R.J., Tan, D.X., Fuentes-Broto, L.** 2010: Melatonin: a multitasking molecule. *Progress in Brain Research*, 181: 127-151.

**Reiter, R.J., Tan, D.X., Paredes, S.D., Fuentes-Broto, L.** 2010: Beneficial effects of melatonin in cardiovascular disease. *Annals of Medicine*, 42(4): 276-285.

**Sack, R.L., Lewy, A.J., Erb, D.L., Vollmer, W.M., Singer, C.M.** 1986: Human melatonin production decreases with age. *Journal of Pineal Research*, 3(4): 379-388.

**Sakotnik, A., Liebmann, P.M., Stoschitzky, K., Lercher, P., Schauenstein, K., Klein, W., Eber, B.** 1999: Decreased melatonin synthesis in patients with coronary artery disease. *European Heart Journal*, 20(118): 1314-1317.

**Sewerynek, E.** 2002: Melatonin and the cardiovascular system. *Neuro endocrinology Letters*, 23: 79-83.

**Shanks, J., Mane, S., Ryan, R., Paterson, D.J.** 2013: Ganglion-specific impairment of the norepinephrine transporter in the hypertensive rat. *Hypertension*, 61(1): 187-193.

**Stefanovic, B., Spasojevic, N., Jovanovic, P., Dronjak, S.** 2019: Melatonin treatment affects changes in adrenal gene expression of catecholamine biosynthesizing enzymes and norepinephrine transporter in the rat model of chronic-stress-induced depression. *Canadian Journal of Physiology and Pharmacology,* 97(7): 685-690.

**Sun, H., Gusdon, A.M., Qu, S.** 2016: Effects of melatonin on cardiovascular diseases: progress in the past year. *Current Opinion in Lipidology*, 27(4): 408-413.

**Tan, D.X., Manchester, L.C., Reiter, R.J., Qi, W.B., Zhang, M., Weintraub, S.T., Cabrera, J., Sainz, R.M., Mayo, J.C.** 1999: Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochimica et Biophysica Acta*, 1472(1-2): 206-214.

**Tarocco, A., Caroccia, N., Morciano, G., Wieckowski, M.R., Ancora, G., Garani, G., Pinton. P.** 2019: Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care. *Cell Death and Disease*, 10(4): 317.

**Veith, R.C., Lewis, N., Linares, O.A., Barnes, R.F., Raskind, M.A., Villacres, E.C., Murburg, M.M., Ashleigh, E.A., Castillo, S., Peskind, E.R., Pascualy, M., Halter, J.B.**1994: Sympathetic nervous system activity in major depression. Basal and desipramine-induced alterations in plasma norepinephrine kinetics. *Arcives of General Psychiatry*, 51(5): 411-422.

**Wehrwein, E.A., Novotny, M., Swain, G.M., Parker, L.M., Esfahanian, M., Spitsbergen, J.M., Habecker, B.A., Kreulenet, D.L.** 2013: Regional changes in cardiac and stellate ganglion norepinephrine transporter in DOCA-salt hypertension. *Autonomic Neuroscience: basic & clinical*, 179(1-2): 99-107.

**Whooley, M.A.** 2006: Depression and cardiovascular disease: healing the broken-hearted. *Journal of the American Medical Association,* 295(24): 2874-2881.

**Willner, P., Muscat, R., Papp, M.** 1992: Chronic mild stress-induced anhedonia. A realistic animal model of depression. *Neuroscience and Biobehavioral Reviews*, 16(4): 525-534.

**Yaprak, M., Altun, A., Vardar, A., Aktoz, M., Ciftci, S., Ozbay, G.** 2003: Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. *International Journal of Cardiology*, 89(1): 103-107.

**Yehuda, R., Siever, L.J., Teicher, M.H., Levengood, R.A., Gerber, D.K., Schmeidler, J., Yang, R.K.** 1998: Plasma norepinephrine and 3-methoxy- 4-hydroxyphenylglycol concentrations and severity of depression in combat posttraumatic stress disorder and major depressive disorder. *Biological Psychiatry*, 44(1): 56-63.