

RESEARCH PAPER

Artificial light at night suppresses the expression of sarco/endoplasmic reticulum Ca²⁺-ATPase in the left ventricle of the heart in normotensive and hypertensive rats

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Funding information

Slovak Research and Development Agency, Grant/Award Number: APVV-17-0178; Scientific Grant Agency of the Ministry of Education of the Slovak Republic, Grant/Award Number: VEGA 1/0492/19

Edited by: Jason Peart

Abstract

Artificial light at night (ALAN) affects the circadian rhythm of the heart rate in normotensive Wistar rats (WT) and spontaneously hypertensive rats (SHR) through the autonomic nervous system, which regulates the heart's activity through calcium handling, an important regulator in heart contractility. We analysed the expression of the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA2) and other selected regulatory proteins involved in the regulation of heart contractility, angiotensin II receptor type 1 (AT₁R), endothelin-1 (ET-1) and tyrosine hydroxylase (TH), in the left ventricle of the heart in WT and SHR after 2 and 5 weeks of ALAN with intensity 1–2 lx. Expression of SERCA2 was decreased in WT (control: 0.53 ± 0.07; ALAN: 0.46 ± 0.10) and SHR (control: 0.72 ± 0.18; ALAN: 0.56 ± 0.21) after 5 weeks of ALAN (*P* = 0.067). Expression of AT₁R was significantly decreased in WT (control: 0.51 ± 0.27; ALAN: 0.34 ± 0.20) and SHR (control: 0.38 ± 0.07; ALAN: 0.23 ± 0.09) after 2 weeks of ALAN (*P* = 0.028) and in SHR after 5 weeks of ALAN. Expression of ET-1 was decreased in WT (control: 0.51 ± 0.27; ALAN: 0.28 ± 0.12) and SHR (control: 0.54 ± 0.10; ALAN: 0.35 ± 0.23) after 5 weeks of ALAN (*P* = 0.015). ALAN did not affect the expression of TH in WT or SHR. In conclusion, ALAN suppressed the expression of SERCA2, AT₁R and ET-1, which are important for the regulation of heart contractility, in a strain-dependent pattern in both WT and SHR.

KEYWORDS

angiotensin II receptor type 1, artificial light at night, rats, sarco/endoplasmic reticulum Ca²⁺-ATPase, the left ventricle of the heart

1 | INTRODUCTION

Circadian rhythms are internal processes in the body that have a periodicity of approximately 24 h and are generated by the circadian system (Black et al., 2019). The circadian system is a hierarchical structure composed of central and peripheral oscillators (Honma, 2018). Outputs of the central oscillator control the phases of the peripheral oscillators, which are located in cells outside the supra-

chiasmatic nucleus and regulate the rhythmicity of at least 8–13% of the expressed genes in the tissues and organs (Martino et al., 2004; Young, 2006).

Disruption of circadian rhythms can result from damage to the central oscillator (Witte et al., 1998b) or impaired entraining of the internal clock with external light and dark cycles, such as phase shifts, which can lead to an internal desynchronization between the central and peripheral oscillators (Molcan et al., 2016; West et al., 2017).

Alterations in circadian rhythms caused by jet lag or phase shifts are associated with many diseases (Khan et al., 2018; Reid & Abbott, 2015), including cardiovascular diseases and structural and functional changes in the heart as observed in *Bmal1*^{-/-} mice (Lefta et al., 2012). In addition to phase shifts, constant dim light of 5 lx (Witte et al., 1998a) and artificial light at night (ALAN) of 2 lx also significantly reduce circadian rhythms of the heart rate in normotensive Wistar rats within a few days (Molcan et al., 2019; Sutovska et al., 2020). Similarly, spontaneously hypertensive rats (SHR), which have elevated sympathetic nervous activity (Fisher & Paton, 2012), experienced a similar decrease in heart rate after ALAN but after a more extended period (Rumanova et al., 2019). The reduction in the difference in cardiovascular parameters between the light and dark phases of the day is associated with a disturbed autonomic nervous system and is a marker of cardiovascular disease (Simko et al., 2016). The autonomic nervous system affects heart activity through changes in the intracellular calcium concentration, a critical regulator of excitation–contraction coupling in cardiomyocytes (El-Armouche & Eschenhagen, 2009). An essential regulator of the calcium cycle in working myocardial cells is the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA2; Kranias & Hajjar, 2012). However, other neurohumoral factors are also involved in the regulation of the calcium cycle in cardiomyocytes, for example, noradrenaline (Engelhardt et al., 2004), angiotensin II (Zhang et al., 2015) and endothelin-1 (ET-1; Zeng et al., 2009).

Therefore, we aimed to evaluate the protein expression of SERCA2, tyrosine hydroxylase (TH), angiotensin II receptor type 1 (AT₁R) and ET-1 in the left ventricle of the heart of normotensive Wistar rats and SHR.

2 | METHODS

2.1 | Ethical approval

The experiments were approved by the Ethical Committee for the Care and Use of Laboratory Animals at the Comenius University in Bratislava, Slovak Republic, and the State Veterinary Authority of the Slovak Republic (Ro-1648/19-221/3) and carried in accordance with the recommendations of the animal care guidelines of the European Union and the journal's principles and regulations (Grundy, 2015). All efforts were made to minimize the number of rats used in this study. At the end of the experiments, rats were anaesthetized with 4% isoflurane in 1 litre O₂ min⁻¹.

2.2 | Animals

Adult male (4.5 months age) Wistar (WT; *n* = 27; 325 ± 18 g) and spontaneously hypertensive (SHR; *n* = 27; 278 ± 16 g) rats were from a breeding station at the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences (Dobrá Voda, Slovak Republic). Animals were housed in transparent plastic cages for three to four animals per cage. Control and experimental rats were housed

New Findings

- **What is the central question of this study?**
Artificial light at night decreases blood pressure and heart rate in rats. Are these changes in heart rate accompanied by changes in protein expression in the heart's left ventricle?
- **What is the main finding and its importance?**
Five weeks of artificial light at night affected protein expression in the heart's left ventricle in normotensive and hypertensive rats. Artificial light at night decreased expression of the sarco/endoplasmic reticulum Ca²⁺-ATPase, angiotensin II receptor type 1 and endothelin-1.

in separate rooms, limiting behavioural synchronization between the groups. Food and water were available *ad libitum* under stable 12 h light–12 h dark (LD) conditions at controlled room temperature (21 ± 2°C) and humidity (55 ± 10%). Before ALAN, all rats were kept for 4 weeks in controlled LD conditions.

2.3 | Experimental protocol

Rats were divided randomly into control (12 h light (150 lx)–12 h dark (0 lx)) and experimental ALAN (12 h light (150 lx)–12 h dim light (1–2 lx)) groups. Light intensity was measured by Sonel LXP 10B (measurement resolution 0.01 lx; Sonel S.A., Świdnica, Poland) at different angles and in different parts of each cage. For ALAN condition, we used warm white light with a colour temperature of 2700 K emitted from the LED bulb Star Classic A60 10 W (Osram GmbH, Munich, Germany; Okuliarova et al., 2021). Rats were weighed at the end of ALAN week 2 and 5. After 2 and 5 weeks of ALAN, samples of heart's left ventricle from control (WT: *n* = 13; SHR: *n* = 13) and experimental (WT: *n* = 14; SHR: *n* = 14) groups were removed. Sampling was performed during the first half of the light phase between ZT03 and ZT06 (ZT00 is the beginning of the light phase of the day). We killed control and experimental rats under isoflurane anaesthesia (4 % isoflurane in 1 litre O₂ min⁻¹) randomly in one day. Samples of the heart's left ventricles were weighed and immediately frozen in liquid nitrogen and stored at –80°C until further processing.

2.4 | Experimental measures

In protein lysates of the heart's left ventricle, we analysed the expression of SERCA2, AT₁R, ET-1 and TH by western blot analysis. Total protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

TABLE 1 Body weight of normotensive rats (WT) and spontaneously hypertensive rats (SHR) exposed to control stable 12 h light to 12 h dark conditions or artificial light at night (ALAN)

	Week of the experiment	Control group	ALAN group	P-value
WT	2	360 ± 29 (7)	348 ± 19 (8)	0.967
SHR	2	301 ± 13 (7)	298 ± 16 (8)	0.999
WT	5	372 ± 27 (6)	371 ± 39 (6)	0.999
SHR	5	324 ± 18 (6)	317 ± 8 (6)	0.999
ANOVA	Strain (WT vs. SHR)			<0.001
	Group (Control vs. ALAN)			0.277
	Interaction (Strain and Group)			0.003

Data were analysed using a two-way ANOVA followed by Tukey's *post hoc* test. Data are presented as the arithmetic mean ± the standard deviation. The values in parentheses represent the number of individuals in the group.

Protein extracts (20 µg) were separated by 12% reducing SDS-polyacrylamide gels and transferred to a Hybond ECL nitrocellulose membrane (Sigma-Aldrich, Steinheim, Germany). Ponceau S staining confirmed the transfer and loading of proteins onto the membranes, which were incubated overnight in Tris-buffered saline containing 0.1% Tween 20 (TBS-T; MP Biomedicals, Eschwege, Germany) and 5% bovine serum albumin (Biowest, Nuaille, France) to block non-specific binding. The next day, the membranes were incubated with primary antibodies: rabbit polyclonal anti-ET-1 (ab117757, Abcam (Cambridge, UK), diluted 1:5000, incubated for 90 min at room temperature), goat polyclonal anti-TH (ab101853, Abcam, diluted 1:1000, incubated for 90 min at room temperature), mouse monoclonal anti-SERCA2 ATPase (ab2861, Abcam, diluted 1:5000, incubated for 120 min at room temperature), rabbit polyclonal anti-AT₁R (ab15552, Merck Millipore (Burlington, MA, USA), diluted 1:1000, incubated for 120 min at room temperature) and mouse monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (MAB374, Merck Millipore, diluted 1:2000, incubated for 60 min at room temperature). After washing with TBS-T, membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibody anti-rabbit (7074, Cell Signaling Technology, Danvers, MA, USA, diluted 1:5000, incubated for 90 min at room temperature), anti-mouse (7076, Thermo Fisher Scientific, diluted 1:2500, incubated for 60 min at room temperature) or anti-goat (SC2020, F180, Santa Cruz Biotechnology, Dallas, TX, USA, diluted 1:5000, incubated for 90 min at room temperature). Signals were detected by chemiluminescence using Clarity Western ECL Substrate (Bio-Rad Laboratories, Hercules, CA, USA) and visualized on Hyperfilms ECL (GE Healthcare Europe GmbH, Freiburg, Germany) and automatically on the Vü-C chemiluminescence imaging system (Pop-Bio Imaging, Milton, UK). The expression of measured proteins was normalized to GAPDH protein expression. Signals were quantified using Quantity One software (4.6.6., Bio-Rad).

2.5 | Data analysis

Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* tests. Differences were considered

statistically significant at $P < 0.05$. Data are presented as the arithmetic mean ± the standard deviation and visualized as bar plots with individual data points. All data analyses and their visualization were performed in R version 3.6.3, package: ggplot2 (R Core Team, 2020; Wickham, 2016).

3 | RESULTS

Normotensive WT rats had significantly ($P < 0.001$) higher body weight in comparison to SHR at the end of ALAN week 2 and 5. The body weights of WT and SHR were not affected by ALAN (Table 1).

SHR had significantly ($P < 0.001$) higher relative weight of the heart's left ventricle than WT. The relative weight of the heart's left ventricle to the body weight of WT and SHR was not affected by ALAN (Figure 1).

We observed significantly ($P < 0.05$) higher expression of SERCA2 in SHR in comparison to normotensive WT rats. Two weeks of ALAN did not affect ($P = 0.463$) SERCA2 expression in WT (control: 0.26 ± 0.07 ; ALAN: 0.28 ± 0.06) and SHR (control: 0.33 ± 0.06 ; ALAN: 0.36 ± 0.14). After 5 weeks of ALAN, SERCA2 expression decreased ($P = 0.067$) in WT (control: 0.53 ± 0.07 ; ALAN: 0.46 ± 0.10) and SHR (control: 0.72 ± 0.18 ; ALAN: 0.56 ± 0.21 ; Figure 2).

AT₁R expression did not differ between WT and SHR. Two weeks of ALAN significantly ($P = 0.028$) decreased expression of AT₁R in the left ventricle of both WT (control: 0.51 ± 0.27 ; ALAN: 0.34 ± 0.20) and SHR (control: 0.38 ± 0.07 ; ALAN: 0.23 ± 0.09). After 5 weeks of ALAN, we observed strain-dependent ($P = 0.056$) changes in AT₁R expression: AT₁R was decreased in SHR (control: 0.85 ± 0.16 ; ALAN: 0.60 ± 0.15) but not in WT (control: 0.75 ± 0.10 ; ALAN: 0.85 ± 0.30 ; Figure 3).

ET-1 expression did not differ between WT and SHR, and 2 weeks of ALAN did not affect ET-1 expression in either WT or SHR. Five weeks of ALAN significantly ($P = 0.015$) decreased ET-1 expression in WT (control: 0.51 ± 0.27 ; ALAN: 0.28 ± 0.12) and SHR (control: 0.54 ± 0.10 ; ALAN: 0.35 ± 0.23 ; Figure 4).

TH expression was not affected by strain ($P = 0.076$) or ALAN ($P = 0.461$) exposure after 2 weeks or after 5 weeks of ALAN ($P = 0.240$; Figure 5).

FIGURE 1 Effect of artificial light at night (ALAN) on the relative weight of the left ventricle in normotensive rats (WT) and spontaneously hypertensive rats (SHR). The relative weight of the left ventricle was normalized to body weight. Data are visualized as the arithmetic mean \pm the standard deviation with individual data points. Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* test. Control: rats exposed to stable 12 h light to 12 h dark conditions; ALAN: rats exposed to 2 weeks (WT control $n = 7$; WT ALAN $n = 8$; SHR control $n = 7$; SHR ALAN $n = 8$) or 5 weeks (WT control $n = 6$; WT ALAN $n = 6$; SHR control $n = 6$; SHR ALAN $n = 6$) of artificial light at night

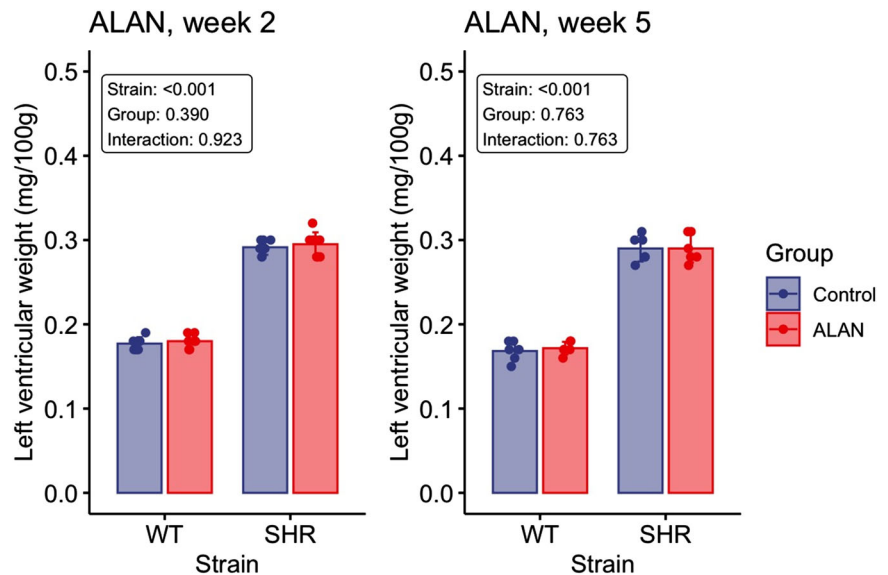
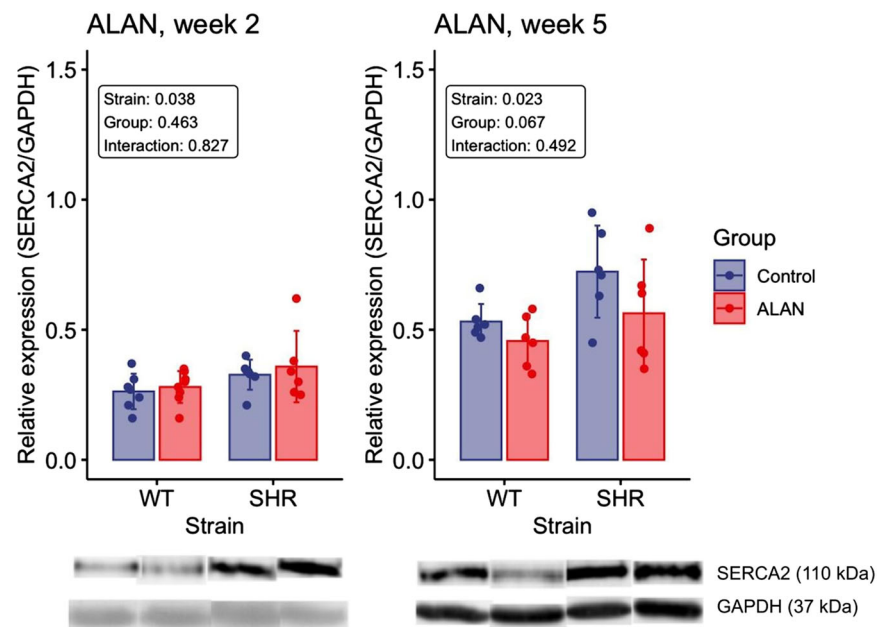


FIGURE 2 Relative expression of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA2) in the left ventricle in normotensive rats (WT) and spontaneously hypertensive rats (SHR). Expression of SERCA2 was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and is visualized as the arithmetic mean \pm the standard deviation with individual data points. Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* test. Control: rats exposed to stable 12 h light to 12 h dark conditions; ALAN: rats exposed to 2 weeks (WT control $n = 7$; WT ALAN $n = 8$; SHR control $n = 7$; SHR ALAN $n = 6$) or 5 weeks (WT control $n = 6$; WT ALAN $n = 6$; SHR control $n = 6$; SHR ALAN $n = 6$) of artificial light at night



4 | DISCUSSION

ALAN exposure is a phenomenon of modern society and affects blood pressure and heart rate in humans (Obayashi et al., 2014, 2019) and rats, probably through the sympathetic nervous system (Molcan et al., 2019; Rumanova et al., 2019; Sutovska et al., 2020). The sympathetic nervous system accelerates heart rate, heart contractility and heart relaxation (lusitropy), which is mediated by SERCA2. Various neuro-humoral factors regulate the activity of SERCA2, such as adrenergic stimulation (Engelhardt et al., 2004), the renin-angiotensin system (Zhang et al., 2015) and ET-1 (Zeng et al., 2009). In the present work, we measured the protein expression of SERCA2, AT_1R , ET-1 and TH in the left ventricle of the hearts of normotensive WT and SHR rats exposed to ALAN.

4.1 | Expression of selected proteins in the left ventricle of WT and SHR

Our study observed a higher expression of SERCA2 in the left ventricle of SHR compared to WT (Figure 2). This is in line with current published data (Silva-Cutini et al., 2019), whereas increased SERCA2 can indicate increased cardiac contractility in SHR (Kobayashi et al., 1995). Increased contractility in SHR is due to adrenergic stimulation and intrinsic cardiomyocyte contractility (Okayama et al., 1998). Published work has described the effect of positive SERCA2-dependent expression on the velocity of relengthening and shortening (Dupont et al., 2012; Okayama et al., 1998; Weisser-Thomas et al., 2007). In SHR, an increase in maximal velocity of relengthening and shortening was observed, which is in line with our results for

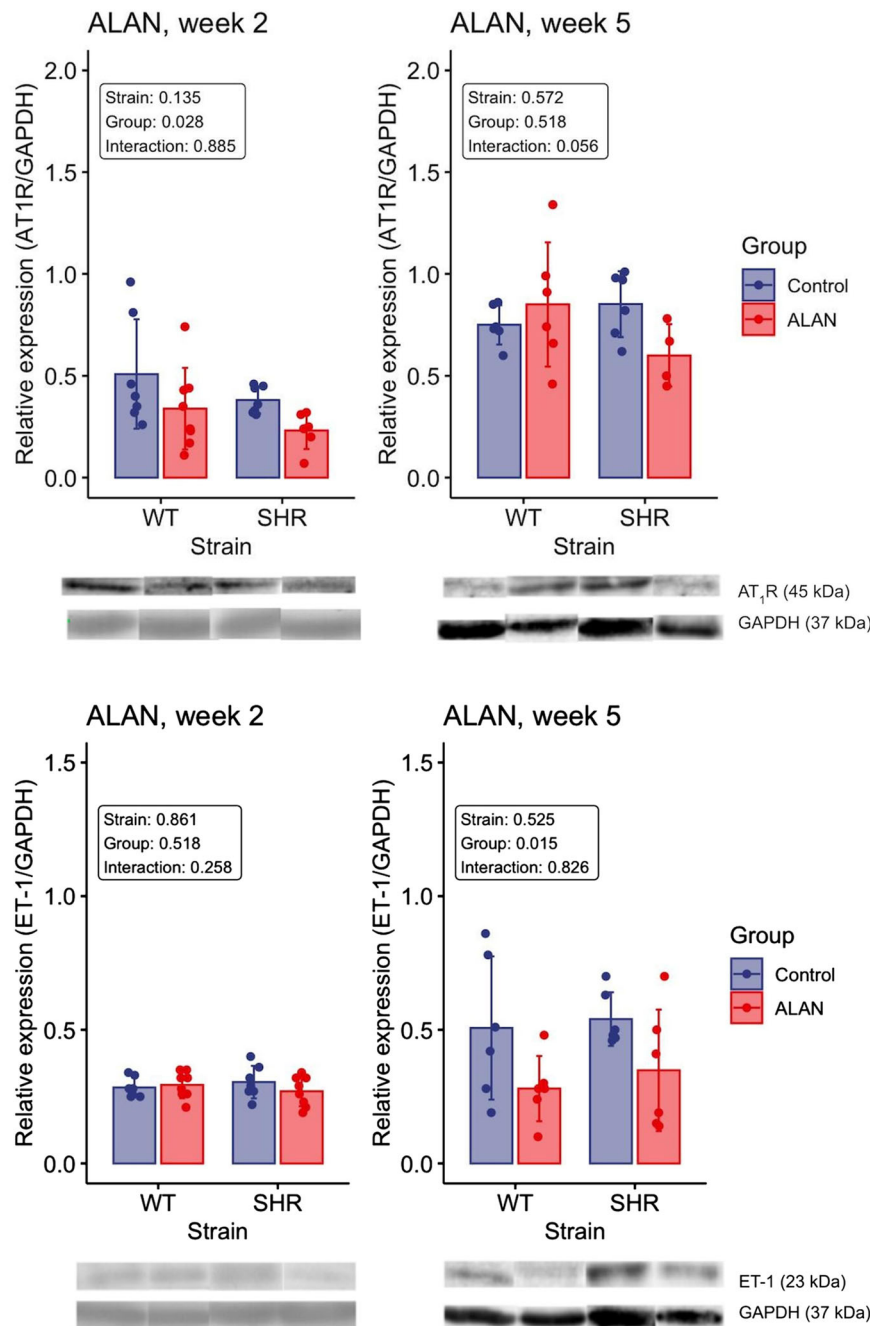


FIGURE 3 Relative expression of angiotensin II receptor type 1 (AT_1R) in the left ventricle in normotensive rats (WT) and spontaneously hypertensive rats (SHR). Expression of AT_1R was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and is visualized as the arithmetic mean \pm the standard deviation with individual data points. Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* test. Control: rats exposed to stable 12 h light to 12 h dark conditions; ALAN: rats exposed to 2 weeks (WT control $n = 7$; WT ALAN $n = 8$; SHR control $n = 7$; SHR ALAN $n = 6$) or 5 weeks (WT control $n = 6$; WT ALAN $n = 6$; SHR control $n = 6$; SHR ALAN $n = 4$) of artificial light at night

FIGURE 4 Relative expression of endothelin-1 (ET-1) in the left ventricle in normotensive rats (WT) and spontaneously hypertensive rats (SHR). Expression of ET-1 was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and is visualized as the arithmetic mean \pm the standard deviation with individual data points. Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* test. Control: rats exposed to stable 12 h light to 12 h dark conditions; ALAN: rats exposed to 2 weeks (WT control $n = 7$; WT ALAN $n = 8$; SHR control $n = 7$; SHR ALAN $n = 8$) or 5 weeks (WT control $n = 6$; WT ALAN $n = 6$; SHR control $n = 6$; SHR ALAN $n = 6$) of artificial light at night

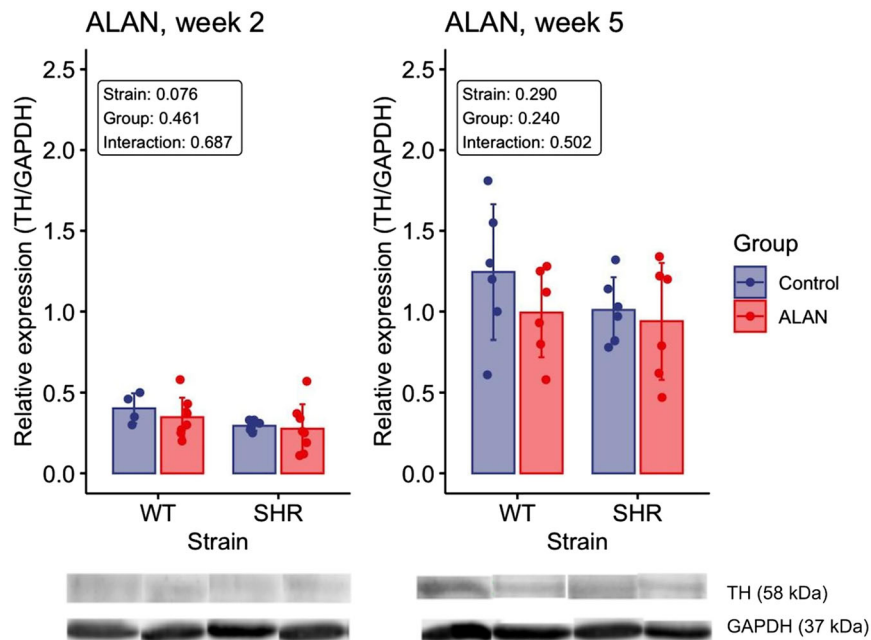
SERCA2 (Kobayashi et al., 1995). Moreover, calcium handling capacity is increased in SHR compared to Wistar-Kyoto rats, due to increased calcium release from the sarcoplasmic reticulum (Okayama et al., 1998), which may be associated with increased expression of SERCA2, an important regulator of calcium handling (Okayama et al., 1998). The activity of SERCA2 is regulated by phospholamban phosphorylation through stimulation of protein kinases A and C triggered by many neurohumoral factors, such as ET-1, angiotensin II and catecholamines, which modulate the activity of the heart (Okumura et al., 2014; Smyrniak et al., 2018).

In our study, the expression of ET-1 in the left ventricle did not differ between SHR and WT. Similarly, expression of ET-1 in SHR did not differ in the mesenteric arteries or was reduced in the thoracic aorta of

SHR compared to WT, probably due to the weakened role of the endothelin system in cardiovascular regulation in the SHR (Larivière et al., 1995). On the other hand, in neurogenic models of hypertension, in deoxycorticosterone acetate (DOCA)-salt rat and DOCA-salt-treated SHR, ET-1 expression was increased compared to normotensive rats (Larivière et al., 1993; Schiffrin et al., 1995; Schiffrin, 2001).

The renin-angiotensin-aldosterone system is upregulated in SHR (Naito et al., 2002). However, we did not observe a difference in AT_1R expression between SHR and WT. AT_1R has a significant 24-h rhythm with higher values during the day's dark, active phase (Naito et al., 2002). In SHR, AT_1R expression decreased during the light phase, ZT08, and fell to values similar to AT_1R expression in Wistar-Kyoto rats (Naito et al., 2002). In our experiment, the left ventricles were

FIGURE 5 Relative expression of tyrosine hydroxylase (TH) in the left ventricle in normotensive rats (WT) and spontaneously hypertensive rats (SHR). Expression of TH was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and is visualized as the arithmetic mean \pm the standard deviation with individual data points. Data were analysed using a two-way ANOVA (factors: strain, groups) followed by Tukey's *post hoc* test. Control: rats exposed to stable 12 h light to 12 h dark conditions; ALAN: rats exposed to 2 weeks (WT control $n = 4$; WT ALAN $n = 8$; SHR control $n = 7$; SHR ALAN $n = 8$) or 5 weeks (WT control $n = 6$; WT ALAN $n = 6$; SHR control $n = 6$; SHR ALAN $n = 6$) of artificial light at night



taken in the middle of the light phase, which may decrease AT_1R expression in SHR.

The expression of TH in the left ventricle did not differ between SHR and WT in our study. TH is a limiting enzyme in the synthesis of catecholamines and its expression depends on the developmental stage of hypertension: 4-week-old prehypertensive SHRs did not differ in TH expression compared to normotensive Wistar-Kyoto rats. On the other hand, 24-week-old SHRs with developed hypertension showed significantly reduced TH expression in the adrenals (Vavřínová et al., 2019). Other studies point to elevated TH levels in 12- to 14-week-old SHRs in the adrenals, LV and plasma compared to Wistar-Kyoto rats (Ren et al., 2017). Similar inconsistent results are found in catecholamine concentrations in SHR (Moura et al., 2005; Vavřínová et al., 2019).

4.2 | Expression of selected proteins in the left ventricle is affected by ALAN

After 2 weeks of ALAN, we did not observe changes in SERCA2 expression. After 5 weeks of ALAN, we observed a decrease in SERCA2 expression in both SHR and WT. It seems that SERCA2 expression is almost constant, with no significant 24-h variability during the LD regimen (Wang et al., 2020). SERCA2 pumps calcium from the cytosol to the sarcoplasmic reticulum, and calcium is released back to the cytosol through ryanodine receptor 2, the major sarcoplasmic reticulum calcium-release channel (Bovo et al., 2017). The expression of ryanodine receptor 2 is directly regulated by clock genes, as knockout *Bmal1*^{-/-} mice show diminished circadian rhythms and decreased expression of ryanodine receptor 2 (Pfeffer et al., 2009). There is a connection between local adrenergic stimulation, circadian clocks in the heart's ventricle and Ca^{2+} levels (Beesley

et al., 2016). Experimental data in rats showed that ALAN affects the amplitude of clock gene expression (Rumanova et al., 2019). Therefore, we hypothesize that ALAN can also be related to a decrease in ryanodine receptor 2 expression. Decreased expression of ryanodine receptor 2 is responsible for reduced calcium release from the sarcoplasmic reticulum to the cytosol, probably due to reduced adrenergic stimulation of the heart after ALAN (Figure 6) (Molcan et al., 2019; Sutovska et al., 2020). Therefore, we propose a long-term decrease in calcium release that can adaptively decrease SERCA2 expression (Oh et al., 2010; Sallinen et al., 2007).

Expression of ET-1 was not affected after 2 weeks of ALAN, while after 5 weeks of ALAN, we observed a decrease in expression of ET-1 in both WT and SHR. ET-1 has a significant 24-h rhythm (Hanai et al., 2005). However, other neurohumoral factors, such as angiotensin II, are predominantly involved in regulating the 24-h rhythm of ET-1 expression (Hahn et al., 1990; Hanai et al., 2005). ALAN exposure inhibits sympathetic nervous system activity (Molcan et al., 2019; Sutovska et al., 2020). The sympathetic nervous system activates the renin-angiotensin-aldosterone system (Schlaich et al., 2009). We assume that the decrease in ET-1 expression after 5 weeks of ALAN can be due to a defective renin-angiotensin-aldosterone system (Doi et al., 2010) and adrenergic stimulation (Molcan et al., 2019; Sutovska et al., 2020).

Expression of AT_1R decreased after 2 weeks of ALAN in WT and SHR and decreased after 5 weeks in SHR. Triple knockout of *Period* gene expression affected AT_1R expression in the aorta and the kidney (Pati et al., 2016), and thus decreased expression of clock genes may be directly related to the observed decrease in AT_1R expression. Cooperation between the clock genes and AT_1R can also be reversed. Angiotensin II acting via AT_1R entrains the clock gene expression in isolated smooth muscle cells from the mouse aorta (Nonaka et al., 2001). Treatment with an antagonist of AT_1R leads to disruption of

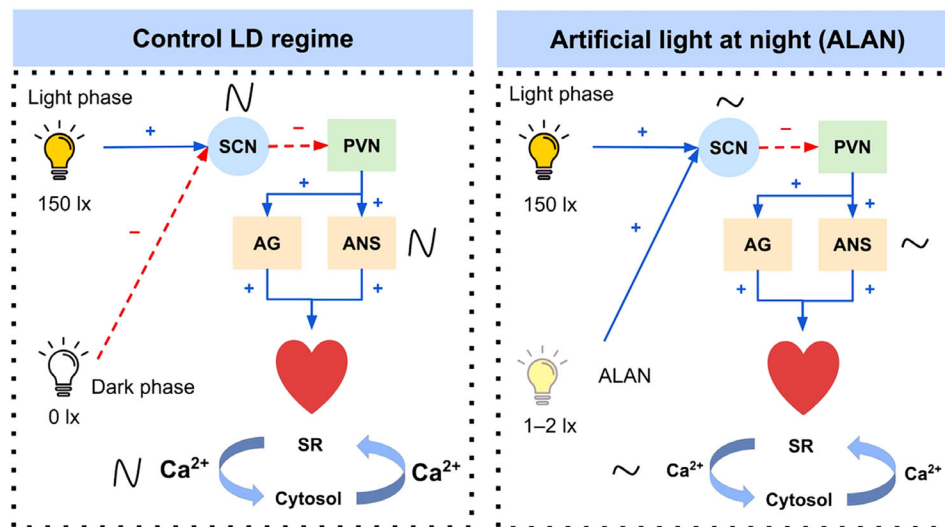


FIGURE 6 Proposed model of the effect of light–dark regime on the regulation of calcium handling in the rat’s heart. The light activates the expression of clock genes in the suprachiasmatic nuclei (SCN). The signal from the SCN is transmitted to the paraventricular nuclei (PVN) via inhibitory neurotransmitters and thus suppresses the activity of the autonomic nervous system (ANS) and function of adrenal glands (AG) in rats during the light phase (Kalsbeek et al., 2012). In contrast, ANS activity is increased during the dark phase in rats. Artificial light at night (≥ 2 lx) also activates the SCN during the dark phase and suppresses the activity of the ANS (Molcan et al., 2019; Sutovska et al., 2020) and the AG, and therefore we assume that ALAN can reduce the calcium load in the rat’s heart. The opposite effects of increased Ca^{2+} load is expected in humans, in whom the activated SCN stimulates the PVN (Kalsbeek et al., 2012)

the synchronizing effects of angiotensin II and diminishes clock gene expression in smooth muscle cells (Nonaka et al., 2001).

In TH expression, we did not observe any significant changes due to ALAN. WT rats exposed to constant light have an altered expression of TH, and other enzymes involved in catecholamine synthesis and *Bmal1* knockout mice result in decreased adrenaline plasma levels (Curtis et al., 2007). In the analysis of adrenaline rhythm in *Bmal1*^{-/-}, significant changes were observed depending on ZT. For example, for ZT06 there was no significant difference in adrenaline concentration between *Bmal1*^{-/-} and *Bmal1*^{+/+} (Curtis et al., 2007). This may explain the changes in TH expression in the middle of the light phase not being observed in our experiment.

Our study did not observe changes in the relative weight of the left ventricle due to ALAN. However, we observed a significant difference in the left ventricle’s relative weight between WT and SHR, as expected (Kokubo et al., 2005). ALAN caused changes in the rat’s left ventricle at the molecular level, which were not accompanied by changes in the rat’s left ventricular mass. On the other hand, in diurnal songbirds, ALAN (1.5 lx) induced cardiac hypertrophy (Alaasam et al., 2021). In elderly people (more than 70 years old), ALAN is associated not only with dysregulated 24-h variability of blood pressure, reduced amplitude and increased night-time blood pressure (Obayashi et al., 2014, 2019) but also with increased carotid artery intima-media thickness, a marker of progression of developing atherosclerosis (Obayashi et al., 2019). Nowadays, the light at night is becoming more frequent, and its effects on the cardiovascular system are measurable. Therefore, it is necessary to understand not only its consequences but also its mechanisms of actions.

In conclusion, ALAN suppressed SERCA2, ET-1 and AT_1R expression in the heart’s left ventricle in normotensive and hypertensive rats. In normotensive rats, the expression of AT_1R was decreased after 2 weeks of ALAN. After 5 weeks of ALAN, expression of SERCA2 and ET-1 was decreased in normotensive rats. In hypertensive rats, expression of AT_1R was decreased after 2 and 5 weeks of ALAN, as was expression of SERCA2, and ET-1 was decreased after 5 weeks of ALAN.

ACKNOWLEDGEMENTS

We thank all the subjects who participated in this study and staff at the Department of Animal Physiology and Ethology, particularly prof. Michal Zeman. The study was supported by the Slovak Research and Development Agency APVV-17-0178 and the Scientific Grant Agency of the Ministry of Education of the Slovak Republic VEGA 1/0492/19.

COMPETING INTERESTS

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

All experiments took place at the Faculty of Natural Sciences, Comenius University in Bratislava. H.S. and L.M. conceived and designed research; H.S., M.M. and L.M. performed experiments; M.M. and L.M. acquired and analysed data; H.S. and L.M. interpreted results of experiments; H.S., M.M. and L.M. prepared figures; H.S. and L.M. drafted the manuscript; H.S., M.M. and L.M. edited and revised the manuscript; H.S., M.M., and L.M. revised it critically for important intellectual content. All authors approved the final version of the

manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those whose qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Sutovska, H., Miklovic, M., & Molcan, L. (2021). Artificial light at night suppresses the expression of sarco/endoplasmic reticulum Ca^{2+} -ATPase in the left ventricle of the heart in normotensive and hypertensive rats. *Experimental Physiology*, 106, 1762–1771. <https://doi.org/10.1113/EP089594>