Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

Original Article

Cite this article: Sutovska H, Molcan L, Koprdova R, Piesova M, Mach M, and Zeman M. (2021) Prenatal hypoxia increases blood pressure in male rat offspring and affects their response to artificial light at night. *Journal of Developmental Origins of Health and Disease* **12**: 587–594. doi: 10.1017/S2040174420000963

Received: 3 April 2020
Revised: 24 July 2020
Accepted: 15 September 2020
First published online: 28 October 2020

Keywords:
Blood pressure; heart rate; prenatal hypoxia; artificial light at night

Address for correspondence:
Lubos Molcan, Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovicova 6, Bratislava, Slovakia.
Email: lubos.molcan@uniba.sk

CAMBRIDGE UNIVERSITY PRESS

Prenatal hypoxia increases blood pressure in male rat offspring and affects their response to artificial light at night

Hana Sutovska[1], Lubos Molcan[1], Romana Koprdova[2], Michaela Piesova[2,3], Mojmír Mach[2] and Michal Zeman[1]

[1]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; [2]Centre of Experimental Medicine SAS, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia and [3]Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Abstract

Prenatal hypoxia (PH) has negative consequences on the cardiovascular system in adulthood and can affect the responses to additional insults later in life. We explored the effects of PH imposed during embryonic day 20 (10.5% O_2 for 12 h) on circadian rhythms of systolic blood pressure (BP) and heart rate (HR) in mature male rat offspring measured by telemetry. We evaluated: (1) stability of BP and HR changes after PH; (2) circadian variability of BP and HR after 2 and 5 weeks of exposure to artificial light at night (ALAN; 1–2 lx); and (3) response of BP and HR to norepinephrine. PH increased BP in the dark (134 ± 2 mmHg vs. control 127 ± 2 mmHg; $p = 0.05$) and marginally in the light (125 ± 1 mmHg vs. control 120 ± 2 mmHg) phase of the day but not HR. The effect of PH was highly repeatable between 21- and 27-week-old PH male offspring. Two weeks of ALAN decreased the circadian variability of HR ($p < 0.05$) and BP more in control than PH rats. After 5 weeks of ALAN, the circadian variability of HR and BP were damped compared to LD and did not differ between control and PH rats ($p < 0.05$). Responses of BP and HR to norepinephrine did not differ between control and PH rats. Hypoxia at the end of the embryonic period increases BP and affects the functioning of the cardiovascular system in mature male offspring. ALAN in adulthood decreased the circadian variability of cardiovascular parameters, more in control than PH rats.

Introduction

Cardiovascular diseases are of a multifactorial origin and can be programmed during prenatal and perinatal development. Unfavourable conditions during prenatal development can have negative consequences on the postnatal development of several organs and affect health in adulthood.[1–3]Prenatal hypoxia (PH) is one example of such a condition. During early stages, the foetus can adapt to a reduced oxygen supply and increase blood distribution to critically important organs, such as the brain and heart, while conversely reducing renal and gastrointestinal perfusion.[4] In a poor intrauterine environment, the foetus develops long-lasting adaptations in cardiovascular and endocrine functions.[5] If PH is present for a prolonged time and during critical periods of development, it can significantly affect the function of the vasculature, heart and kidney, as well as several local[6] and central regulatory mechanisms.[7]

The timing of critical periods, which determine changes in sensitivity of organs to negative stimuli, can differ among stimuli. However, the final days of pregnancy are particularly important.[8,9] In our previous study, we found increased blood pressure (BP) and activation of the sympathetic nervous system in adult male rats exposed prenatally to two 4-h periods of 10.5% of oxygen on prenatal days 19 and 20.[8] PH can contribute to the development of hypertension and higher susceptibility of the cardiovascular system to different environmental conditions, which occur later in development or in adulthood.[10,11]

After birth, organisms are exposed to various environmental conditions, including regular light (L) and dark (D) cycles. Rotation of the light/dark regimen is an important synchronisation factor for central circadian oscillator localised in the suprachiasmatic nucleus of the hypothalamus. These clocks generate circadian rhythms of behavioural and physiological processes, including the daily variability of BP and heart rate (HR).[12,13] Circadian clock outputs are transmitted via the endocrine and autonomic nervous system to the rest of body and entrain their functions to the actual environmental LD cycle.[14] Circadian clocks allow organisms to predict regular changes during the 24-h cycle and help to cope more efficiently with stressors.[15,16] Disruption of circadian rhythms can be induced by several environmental variables, including artificial light at night (ALAN). Low ALAN levels (<10 lx) can disturb the circadian control of different physiological processes, including the cardiovascular system in men.[17] Moreover, low

ALAN levels (1–2 lx) differentially affect the circadian control of BP and HR in normotensive rats[18] in comparison with spontaneously hypertensive rats,[19] which have an activated sympathetic nervous system.

PH increases BP and the activity of the sympathetic nervous system, through which central biological clock outputs are mediated and can be disturbed by ALAN. Therefore, in our present study, we explored the effects of ALAN on circadian rhythms in HR and BP in male offspring exposed to PH. We evaluated: (1) stability of BP and HR changes after PH; (2) circadian variability of BP and HR after 2 and 5 weeks of exposure to ALAN (1–2 lx); and (3) response of BP and HR to norepinephrine in PH offspring.

Materials and methods

Animals

In our study, we examined mature male Wistar rats from nulliparous female Wistar/DV rats ($n = 16$). We induced PH on prenatal day 20 by 12-h exposure of pregnant females ($n = 8$) to 10.5% oxygen in nitrogen. After the hypoxic insult, mothers were returned to their home cages. Control pregnant females ($n = 8$) were placed in the hypoxic chamber on day 20 of gestation for 12 h without inducing hypoxia. Animals were allowed to spontaneously deliver offspring and pups were assigned to the groups after weaning (21 days postpartum) using a single pup per litter to minimise genetic bias as previously described.[8,20] We maintained animals individually in plastic cages with food and water available *ad libitum*. The room temperature (21°C ± 2°C), humidity (55% ± 10%), and regular 12-h L (150 lx):12-h D (0 lx) conditions were automatically controlled. The experiment was 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 Slovak Republic.

Experimental design

Using telemetry, we evaluated daily rhythms of systolic BP and HR in adult, 21- and 27-week-old PH ($n = 7$, 370 ± 8 g) or control ($n = 6$, 384 ± 8 g) male offspring. During this period, rats were exposed to regular 12L:12D conditions (Fig. 1). In 28–32-week-old control and PH rats, we determined the response of daily rhythms of BP and HR to ALAN: 12-h L (150 lx) and 12-h of ALAN phases (dim D; 1–2 lx). We evaluated the consequences of ALAN on circadian rhythms of BP and HR via telemetry after 2 and 5 weeks of ALAN exposure (Fig. 1). In 34-week-old rats, we administered norepinephrine and analysed BP and HR response in the control and PH group (Fig. 1).

Telemetry

We continuously measured BP and HR via telemetry (HD-S10; Data Sciences International, MN, USA). We applied telemetry transmitters to the abdominal aorta.[8,18] After implantation, we treated rats with tramadol (15 mg/kg; SC; Tramal, Stada, Bad Vilbel, Germany) and placed them in a heated room. We allowed the animals to recover for 2 weeks after surgery.

Norepinephrine administration

Norepinephrine (200 µg/kg; SC; arterenol bitartrate hydrate; Calbiochem, Germany) was administered subcutaneously 3 h before the lights were turned off and 3 h before the lights were turned on as we did in our previous experiments. Consequences of norepinephrine and vehicle on BP and HR we analysed previously[15] and therefore this procedure was not repeated in this experiment. During the dark phase, norepinephrine was administered under red light and the procedure lasted maximum 1 min.

Data analysis

We evaluated systolic BP and HR data as 12-h averages separately for the L and D or dim D. We analysed 24-h variability as the difference (delta) between the L and D or dim D. Due to data normalisation, we expressed delta in % (in relation to LD week). We used Chronos-Fit software to analyse parameters of circadian rhythm (%rhythm, amplitude, acrophase and mesor) of BP and HR.[21] % rhythm is a chronobiological term for the coefficient of determination, that is, the squared coefficient of correlation times 100 (%rhythm = $r^2 \cdot 100$). It represents the percentage of variation in the data that is explained by the fitted model. The amplitude is half the range of oscillation of the wave. The acrophase is the peak time of the cosine wave fitted to the data. The mesor is the rhythmically adjusted mean as the mean level of rhythm calculated by cosinor method.[21]

We expressed systolic BP and HR responses to norepinephrine as the difference between the stimulated and basal values. We defined the basal values separately for each rat as the value from the 2-h segment before norepinephrine administration. We calculated the area under the curve from a 90-min norepinephrine response.

We tested the normality of data distribution with the Shapiro–Wilk test and subsequently analysed the data with two-tailed heteroscedastic Student's *t*-test (parameters of the circadian rhythms). Stability of the PH model (Fig. 2) and the response of BP and HR to norepinephrine (Fig. 3) was evaluated by two-way analysis of variance (ANOVA) with repeated measures (factors: group, LD phase) followed by Tukey's test. Exposure of rats to ALAN (Fig. 4) was evaluated by two-way ANOVA with repeated measures (factors: week, LD phase) followed by Tukey's test. BP and HR delta response to ALAN exposure was evaluated by one-way repeated measures ANOVA (factor:week) followed by Tukey's test. Differences were considered statistically significant at $p < 0.05$. Statistical evaluations were performed using the statistical package R, version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented and visualised as the arithmetic mean ± standard error of the mean (SEM; Figs. 4a–f, 3a and b; Excel Office 365, Microsoft, Redmond, USA) or as box plots with individual data points (Figs. 2, 4g–j, 3c–f; R; package: ggplot2). The box representing the range from the first to third quartiles; the band near the middle of the box is the median, and the lines above and below the box indicate the locations of the minimum and maximum value.

Results

Stability of the PH model

Blood pressure

In 21-week-old males, BP was significantly higher in PH compared to control rats (134 ± 2 mmHg vs. 127 ± 2 mmHg; $p = 0.0504$) during the D phase. During the L phase, BP was marginally higher in PH compared to control rats (125 ± 1 mmHg; 120 ± 2 mmHg; $p = 0.0849$). This pattern of BP was also preserved after 6 weeks (Fig. 2). As expected, in both PH ($p < 0.001$) and control ($p < 0.01$) rats, BP was higher during the D compared to the L phase.

https://doi.org/10.1017/S2040174420000963 Published online by Cambridge University Press

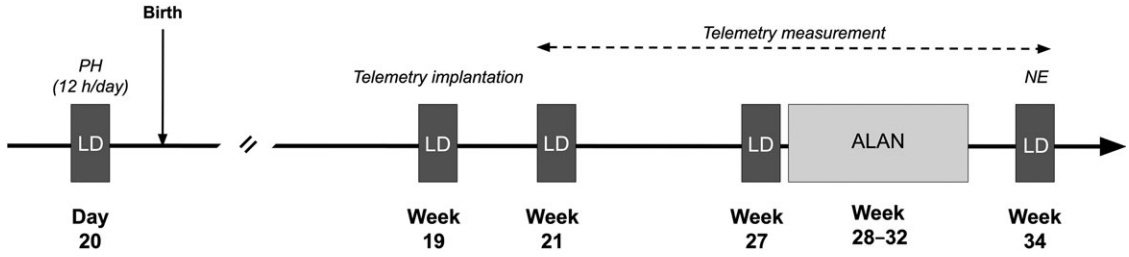

Fig. 1. Experimental design. LD, regular 12-h light:12-h dark regimen; PH, prenatal hypoxia; ALAN, artificial light (1–2 lx) during the dark phase of the day; NE, norepinephrine.

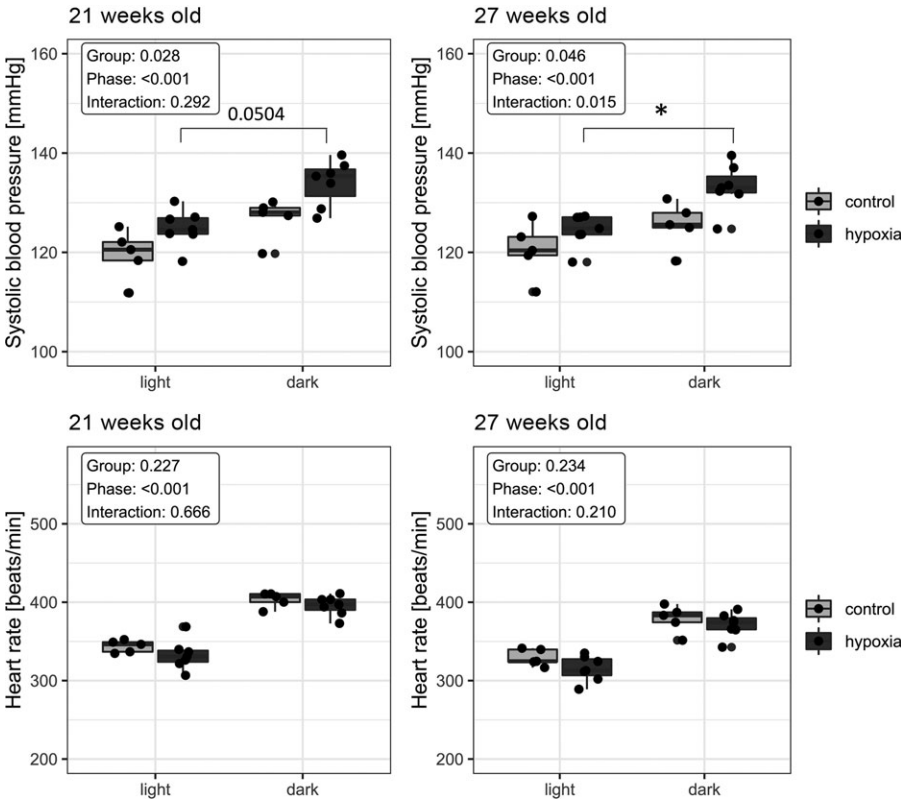

Fig. 2. Effect of prenatal hypoxia on blood pressure and heart rate in mature male offspring. *$p < 0.05$ Data are visualised as box plots with individual data points.

PH did not affect parameters of the circadian rhythm of BP (%rhythm, acrophase, amplitude, and mesor; Table 1).

Heart rate

In 21-week-old males, PH did not affect HR. We did not observe differences between control and PH males (Fig. 2). In both PH ($p < 0.001$) and control ($p < 0.001$) rats, HR was significantly higher during the D compared to the L phase of the day. PH did not affect the parameters of the circadian rhythm of HR (Table 2).

Exposure of PH male offspring to ALAN

Blood pressure

In control rats, BP was affected by both LD phase ($p < 0.001$) and ALAN ($p = 0.035$; Fig. 4g). Delta, 24-h variability, was affected by ALAN in control ($p = 0.033$; Fig. 4g), but not in PH ($p = 0.227$; Fig. 4i) rats. In control rats, the difference between L and dim D decreased significantly after 2 weeks of ALAN ($p = 0.021$; −60.5% ± 11.8%) and was recovered after 5 weeks of ALAN (−16.7% ± 24.8%; $p = 0.667$) compared to control LD conditions (Fig. 4g). In both control and PH rats, ALAN did not affect BP during the L phase of the day (Fig. 4g, i).

After 2 weeks of ALAN, the circadian rhythm of BP was disrupted in control rats: only two of six rats exhibited significant 24-h variability. After 5 weeks of ALAN, the circadian rhythm of BP was restored in five of six rats; however, %rhythm was still significantly lower ($p = 0.029$) compared to the LD regimen. In PH rats, the circadian rhythm was disrupted after 5 weeks of ALAN; %rhythm was decreased ($p = 0.042$; Table 1). As demonstrated with the actograms (Fig. 5), daily rhythmicity was still present for all recorded parameters after 2 and 5 weeks of ALAN in control and PH males.

Heart rate

In both control (Fig. 4h) and PH (Fig. 4j) rats, HR was affected by LD phase and ALAN.

The LD difference decreased by 26.4% ± 7.8% in control ($p = 0.086$) and by 15.8% ± 3.8% in PH ($p = 0.049$) rats after

https://doi.org/10.1017/S2040174420000963 Published online by Cambridge University Press

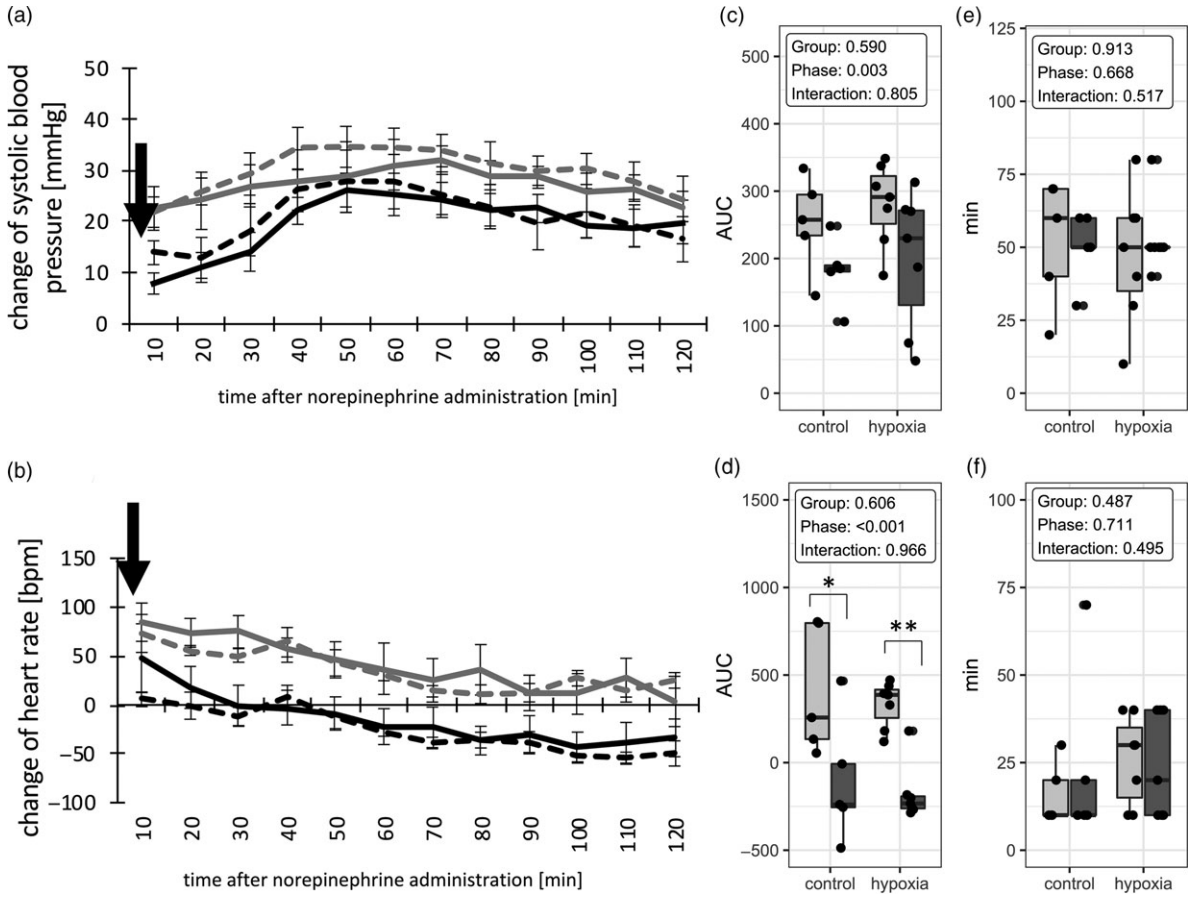

Fig. 3. (a) Blood pressure and (b) heart rate responses on norepinephrine administration. Responses of (c) blood pressure and (d) heart rate on norepinephrine analysed as the area under the curve (AUC). Maximal time in minutes of (e) blood pressure, and (f) heart rate responses on norepinephrine. *$p < 0.05$, **$p < 0.01$. Data are visualised as the arithmetic mean ± standard error of the mean (a, b) and normalised to the value before norepinephrine administration or as box plots with individual data points (c–f).
Notes: dashed grey line, prenatal hypoxia mature male offspring in the light phase of the day; dashed black line, prenatal hypoxia mature male offspring in the dark phase of the day; grey line, control rats in the light phase; black line, control rats in the dark phase of the day; light grey box plots, light phase of the day; dark grey box plots, dark phase of the day.

2 weeks of ALAN. After 5 weeks of ALAN, the LD difference was restored in control ($p = 0.721$ vs. LD regimen, Fig. 4h) rats, while in PH rats it was continuously decreased (−28.7% ± 4.3%; $p < 0.001$, Fig. 4j) compared to the LD regimen.

Two weeks of ALAN suppressed %rhythm ($p = 0.011$), amplitude ($p = 0.004$), and mesor ($p = 0.008$) in control but not PH males. After 5 weeks of ALAN, the %rhythm was significantly decreased in control ($p = 0.004$) and PH ($p = 0.016$) rats (Table 2).

Response to norepinephrine

Response to norepinephrine administration was significantly higher during the L compared to the D phase of the day in both BP ($p = 0.003$) and HR ($p < 0.001$; Fig. 3). PH and control males did not differ in the BP and HR response to norepinephrine (Fig. 3).

Discussion

Hypoxic conditions during embryonic life can contribute to hypertension in adulthood and change the response of offspring to environmental challenges. In the current study, we found that: (1) half-day hypoxia on prenatal day 20 consistently increased BP in mature (21 and 27 weeks old) male offspring; (2) PH rats were less sensitive to ALAN compared to control rats after 2-week exposure, but after 5-week exposure, the circadian control of HR and BP variability was equally suppressed in both groups in comparison with LD; and (3) BP and HR responses to norepinephrine administration were not different between PH and control rats.

PH can affect the development of the foetus and control mechanisms that act postnatally.[7] The extent of functional changes caused by negative prenatal conditions depends on their intensity and time when they act.[22] Our results showed that 12-h hypoxia during prenatal day 20 resulted in higher BP but not HR compared to control adult male rats. It is necessary to mention that changes in BP and HR caused by 12-h prenatal hypoxia were stable and did not differ between 21- and 27-week-old rats. These results are consistent with our previous data demonstrating that even shorter (4 h) durations of hypoxic conditions during prenatal days 19 and 20 can increase BP in adulthood.[8] In contrast, the same level of hypoxia (10.5%) applied in the middle of the embryonic development (days 8–14) did not increase BP in adult male rats (our unpublished data). PH can affect different regulatory areas in the brain and alter the expression of adrenergic receptors in the heart and arteries.[23–25] These changes can lead to a modified function of the autonomic nervous system and increase sympathetic nervous system activity.[8] Long-lasting PH can affect both the central and peripheral control mechanisms mediated by transcriptomic and epigenomic mechanisms.[26] In our experiment, we

https://doi.org/10.1017/S2040174420000963 Published online by Cambridge University Press

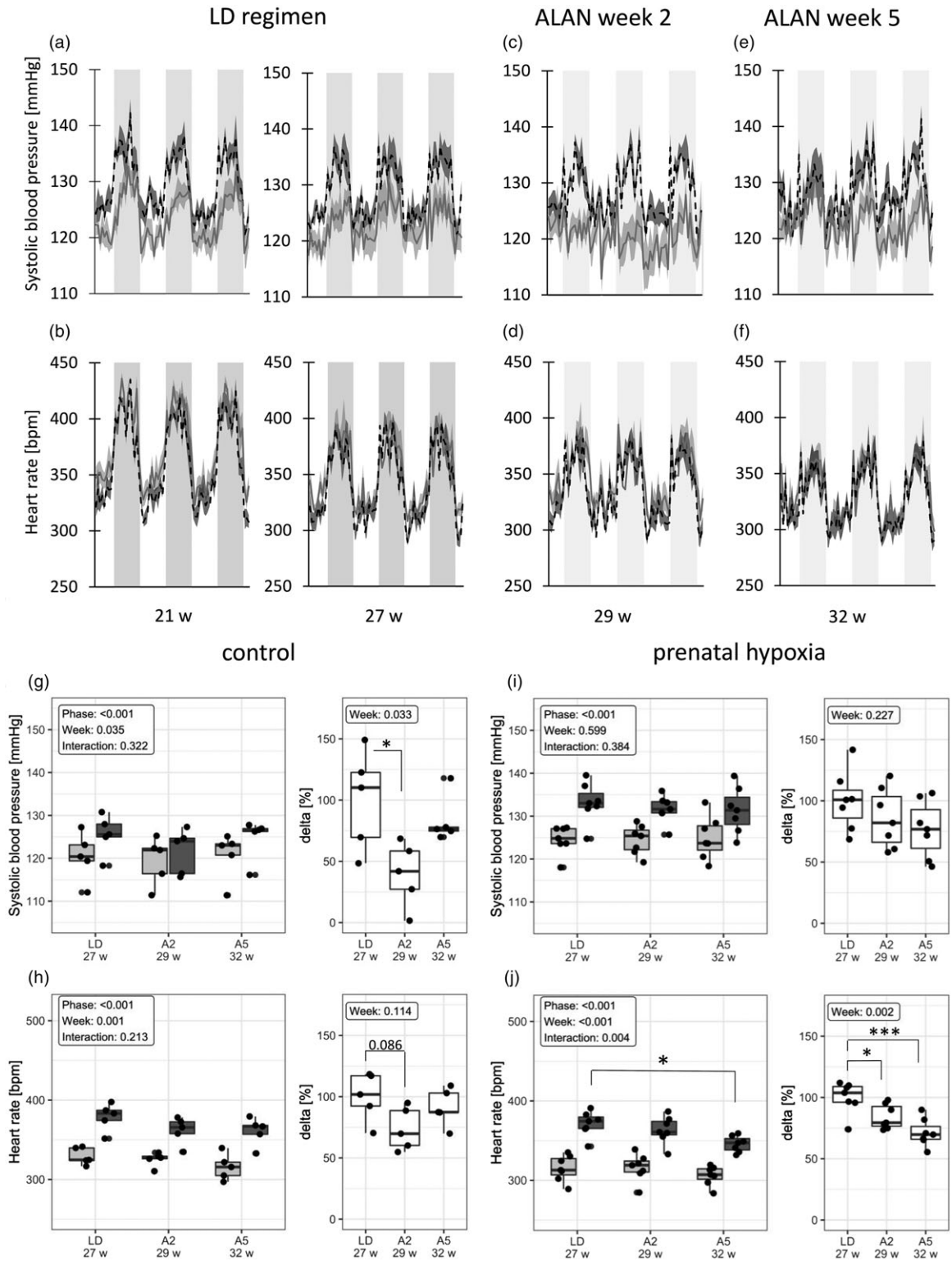

Fig. 4. Effect of artificial light at night (ALAN) on blood pressure and heart rate in control and prenatal hypoxia affected rats. Original data from telemetry: (a) blood pressure and (b) heart rate in 21- and 27-week-old (21 week, 27 week) rats during the LD regimen (L light phase 150 lx, D dark phase 0 lx). (c) Blood pressure and (d) heart rate in 29-week-old (29 week) rats during the second week of the ALAN regimen (A2). (e) Blood pressure and (f) heart rate in 32-week-old (32 week) rats during the fifth week of ALAN (A5). The dashed black line indicates prenatal hypoxia mature male offspring, while the grey line represents control rats. Grey and white vertical bars represent the D and L phase, respectively. Data are visualised as the arithmetic mean ± standard error of the mean. Mean values of (g) blood pressure and (h) heart rate and their differences between L and D phases of days in % (delta) in control rats. Mean values of (i) blood pressure and (j) heart rate and their differences between L and D phases of days in % (delta) in prenatal hypoxia mature male offspring. Data are visualised as box plots with individual data points. $^{*}p < 0.05$, $^{***}p < 0.001$.

https://doi.org/10.1017/S2040174420000963 Published online by Cambridge University Press

Table 1. Parameters (mean ± standard error of the mean [SEM]) of the circadian rhythm in blood pressure after 2 and 5 weeks of artificial light at night (ALAN) exposure

	LD, control week		ALAN, week 2		ALAN, week 5	
	Control $n = 6$	Prenatal hypoxia $n = 7$	Control $n = 6$	Prenatal hypoxia $n = 7$	Control $n = 6$	Prenatal hypoxia $n = 7$
Nr rhythmic animals	5/6	7/7	2/6	7/7	5/6	7/7
%rhythm	21.11 ± 2.07	21.35 ± 2.21	5.56 ± 1.47**	16.13 ± 2.97***	12.32 ± 1.74*	13.87 ± 2.21*
Acrophase	18.30 ± 0.68	17.33 ± 0.26	15.47 ± 3.91	17.81 ± 0.44	18.83 ± 0.44	18.13 ± 0.29
Amplitude	4.30 ± 0.48	6.00 ± 0.60	3.16 ± 0.33	4.91 ± 0.40	2.91 ± 0.30	4.28 ± 0.29
Mesor	126 ± 3	129 ± 1	123 ± 3	128 ± 1	125 ± 3	129 ± 2

*$p < 0.05$ vs. LD; **$p < 0.01$ vs. LD; ***$p < 0.05$ control vs. prenatal hypoxia in the same week.

Table 2. Parameters (mean ± standard error of the mean [SEM]) of the circadian rhythm in heart rate after 2 and 5 weeks of artificial light at night (ALAN) exposure

	LD, control week		ALAN, week 2		ALAN, week 5	
	Control $n = 6$	Prenatal hypoxia $n = 7$	Control $n = 6$	Prenatal hypoxia $n = 7$	Control $n = 6$	Prenatal hypoxia $n = 7$
Nr rhythmic animals	6/6	7/7	6/6	7/7	6/6	7/7
%rhythm	37.55 ± 3.28	35.36 ± 3.22	21.28 ± 2.61*	31.79 ± 3.44***	27.67 ± 2.73*	24.77 ± 2.15**
Acrophase	17.10 ± 0.25	16.73 ± 0.17	17.42 ± 0.25	17.31 ± 0.31*	17.75 ± 0.21	17.53 ± 0.22 *
Amplitude	37.99 ± 3.46	35.10 ± 5.30	23.53 ± 3.34**	33.36 ± 2.13***	32.54 ± 2.93*	25.44 ± 1.97
Mesor	352 ± 5	311 ± 34	342 ± 5**	340 ± 6	335 ± 6**	324 ± 4

*$p < 0.05$ vs. LD; **$p < 0.01$ vs. LD; ***$p < 0.05$ control vs. prenatal hypoxia in the same week.

exposed animals to PH for 12 h, and even such short exposure can change endothelial mechanisms responsible for higher BP in adult rats.[27] PH leads to endothelial dysfunction in systemic resistance arteries and affects the acute regulation of BP,[5,28] while chronic regulation of BP is mediated by the kidney. Prenatal adverse condition reduces foetal kidney growth and reduces the number of nephrons[5] and glomeruli,[29] an alteration that is often associated with later development of hypertension.[5] It leads in the kidney to upregulation of renin–angiotensin–aldosterone system.[28]

While daily rhythms of recorded parameters persisted after ALAN exposure, the treatment suppressed circadian variability (amplitude, %rhythm) of BP and HR in both PH and control rats. We analysed the stability of circadian rhythms by Cosinor analysis and as the difference between the L and D or dim D phase. In the control group, ALAN suppressed considerably 24-h variability of BP and HR after 2 weeks of exposure. After 5 weeks of ALAN, 24 h variability of BP and HR was restored. We identified a different pattern in the PH group: we did not find such pronounced suppression of circadian variability of BP and HR after 2 weeks of ALAN as in the control group. However, the light–dark variability of both parameters continuously decreased after 5 weeks of ALAN in PH rats, and there was no difference between the groups at that time. These results are in line with our previous study,[18] in which ALAN of the same intensity suppressed circadian variability of BP and HR in rats together with changes of spontaneous baroreflex sensitivity. On the other hand, in spontaneously hypertensive rats,[19] the consequences of ALAN resembled those in PH rats found in this study. These data show that even low-intensity ALAN can act as a mild circadian disruptor and, consequently, interfere with circadian rhythms in cardiovascular parameters. It is necessary to mention that disruption of 24-h oscillations of cardiovascular parameters, especially BP, is associated with cardiovascular risk in humans and is used as a prognostic factor.[12] Our data suggest that PH can change regulatory mechanisms that control the cardiovascular system and responses to environmental stimuli. The nature of these changes is still unknown, but the autonomic nervous system can be involved because PH and spontaneously hypertensive rats have up-regulated activity of the sympathetic nervous system.[8,30]

Light is received by the retina and intrinsically photosensitive ganglion cells transmit the information to the central circadian oscillator localised in the suprachiasmatic nucleus of the hypothalamus. From there, the entrained rhythmic signals are passed to the paraventricular nucleus (PVN) of the hypothalamus, which controls the endocrine and sympathetic system.[31] Among the hormonal signals, the circadian rhythm of melatonin synthesis in the pineal gland can be of primary importance because it represents a direct output of the central circadian clocks and melatonin has a pleiotropic action. The circadian rhythm of plasma melatonin concentrations was eliminated in our previous study by the same low light illuminance[18] as we applied in the present experiment. Melatonin can influence the brain structures involved in the BP control and reactivity of peripheral vessels.[32]

In addition to its role in the control of the endocrine system, the PVN is involved in the regulation of the autonomic nervous system.[33] Morphological and electrophysiological studies have shown that PVN is reciprocally connected to regions of the brain that are involved in cardiovascular regulation. Importantly, the PVN projects to both the intermediolateral cell column of the spinal cord and the rostral ventrolateral medulla, regions critical in the control of the sympathetic nervous system and the cardiovascular system.[34]

https://doi.org/10.1017/S2040174420000963 Published online by Cambridge University Press

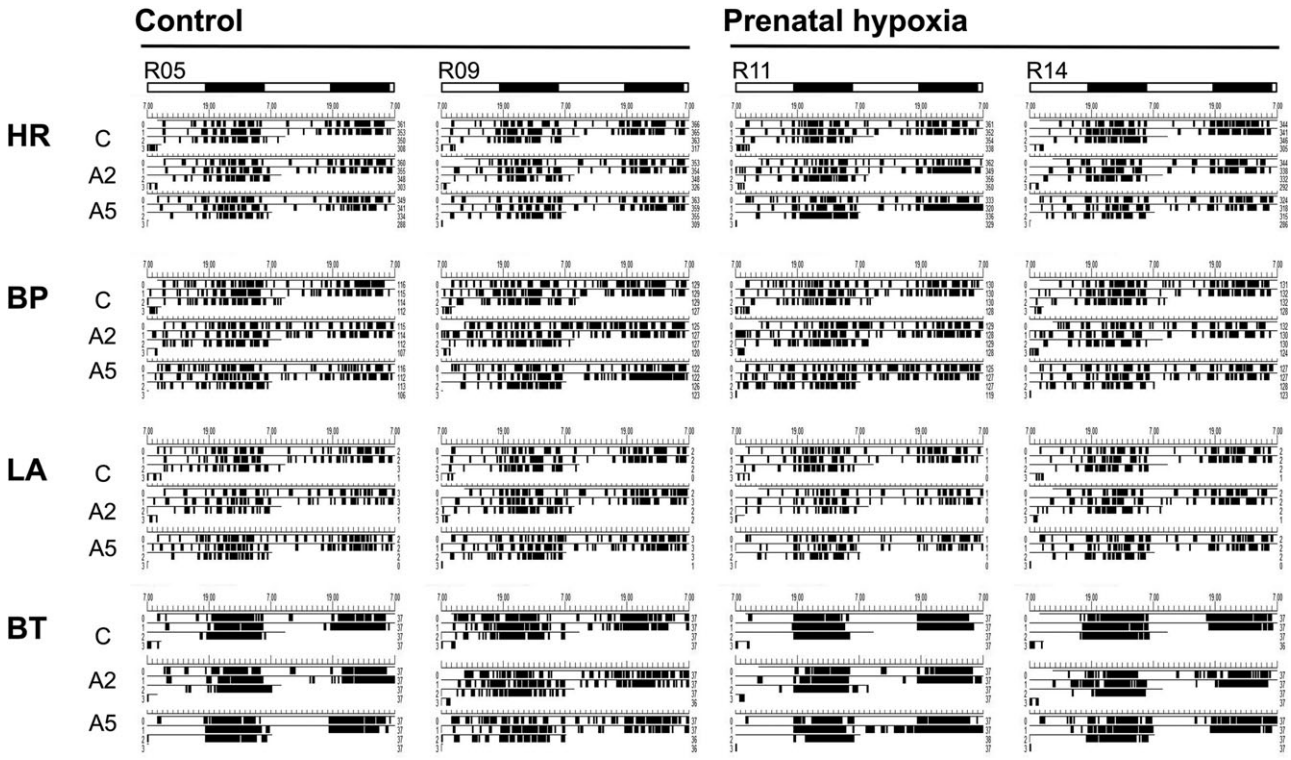

Fig. 5. Representative individual actograms of heart rate (HR), systolic blood pressure (BP), locomotor activity (LA) and body temperature (BT) in freely moving control and mature offspring exposed to prenatal hypoxia during regular 12-h light:12-h dark (C) and second (A2) and fifth (A5) week of the artificial light at night exposure. Actograms were made from raw data by Chronos-Fit. Data for each week is from a 3-day measurement.

Gestational intermittent hypoxia,[35] as well as perinatal hypoxia (E19 to PD14),[36] can upregulate sympathetic outflow to the heart and vessels. Moreover, hypoxia increases expression of α_1-adrenergic receptors in arteries,[23] but not β_1-adrenergic receptors in the heart[24] and chronic PH causes postnatal desensitization of β-adrenoceptors in the heart.[37] Therefore, PH in our study could increase BP but not HR. The centrally increased sympathetic activity can at least partially explain the increased resistance of PH animals to ALAN. We hypothesise that the activated sympathetic nervous system in PH rats can stabilise the outflow to the rostral ventrolateral medulla and protect against disturbing effects of ALAN after 2 weeks of ALAN in comparison with control animals.

In the last part of our study, we tested the significance of α-adrenergic signalling on vessels in rats by evaluating the response of BP to norepinephrine administration. The response to norepinephrine was phase-dependent; both control and PH groups showed higher response during the L in comparison with the D phase of the day. These data are in line with our previous studies.[8,15] The absence of differences in the BP response to norepinephrine administration between PH and control rats does not exclude the participation of the sympathetic nervous system in their different response to ALAN at the central level because peripherally administered norepinephrine is unable to penetrate the blood-brain barrier.[38] Furthermore, the expression of adrenergic receptors in the heart and blood vessels[23–25] can be different and should be further studied.

Conclusions

Prenatal hypoxia increased BP in mature male rats and permanently affected regulatory mechanisms controlling the cardiovascular system. Daily rhythms of recorded parameters were dumped after ALAN exposure in both groups and the response of BP to ALAN was more pronounced in the control compared to PH rats after 2 weeks of exposure. The higher resistance of PH animals to ALAN can be explained at least partially by centrally increased sympathetic activity and supressed melatonin rhythmicity.

Acknowledgements. We would like to thank Proof Reading Servis.com, the UK for the professionally proofread of the manuscript.

Financial Support. 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, VEGA 2/0154/20).

Conflicts of Interest. The authors declare that there is no conflict of interest.

Ethical Standards. The experiment was 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 Slovak Republic.

References

1. Giussani DA, Davidge ST. Developmental programming of cardiovascular disease by prenatal hypoxia. *J Dev Orig Health Dis.* 2013; 4, 328–337.
2. Kumar P, Morton JS, Shah A, *et al.* Intrauterine exposure to chronic hypoxia in the rat leads to progressive diastolic function and increased aortic stiffness from early postnatal developmental stages. *Physiol Rep.* 2020; 8, e14327.
3. Andraweera PH, Gatford KL, Care AS, *et al.* Mechanisms linking exposure to preeclampsia in utero and the risk for cardiovascular disease. *J Dev Orig Health Dis.* 2020; 1–8.

4. Giussani DA, Camm EJ, Niu Y, *et al.* Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. *PLoS ONE.* 2012; 7, e31017.
5. Verschuren MTC, Morton JS, Abdalvand A, *et al.* The effect of hypoxia-induced intrauterine growth restriction on renal artery function. *J Dev Orig Health Dis.* 2012; 3, 333–341.
6. Alma LJ, De Groot CJM, De Menezes RX, *et al.* Endothelial dysfunction as a long-term effect of late onset hypertensive pregnancy disorders: high BMI is key. *Eur J Obstet Gynecol Reprod Biol.* 2018; 225, 62–69.
7. Tain Y-L, Huang L-T, Hsu C-N. Developmental programming of adult disease: reprogramming by melatonin? *Int J Mol Sci.* 2017; 18, 426.
8. Svitok P, Molcan L, Stebelova K, *et al.* Prenatal hypoxia in rats increased blood pressure and sympathetic drive of the adult offspring. *Hypertens Res.* 2016; 39, 501–505.
9. Van den Bergh BR, van den Heuvel MI, Lahti M, *et al.* Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. *Neurosci Biobehav Rev*. Epub ahead of print 28 July 2017. doi: 10.1016/j.neubiorev.2017.07.003.
10. Shah A, Matsumura N, Quon A, *et al.* Cardiovascular susceptibility to in vivo ischemic myocardial injury in male and female rat offspring exposed to prenatal hypoxia. *Clin Sci.* 2017; 131, 2303–2317.
11. Zeman M, Okuliarova M. Sex-specific cardiovascular susceptibility to ischaemic myocardial injury following exposure to prenatal hypoxia. *Clin Sci.* 2017; 131, 2791–2794.
12. Smolensky MH, Hermida RC, Portaluppi F. Circadian mechanisms of 24-hour blood pressure regulation and patterning. *Sleep Med Rev.* 2017; 33, 4–16.
13. Black N, D'Souza A, Wang Y, *et al.* Circadian rhythm of cardiac electrophysiology, arrhythmogenesis, and the underlying mechanisms. *Heart Rhythm.* 2019; 16, 298–307.
14. Coomans CP, Ramkisoensing A, Meijer JH. The suprachiasmatic nuclei as a seasonal clock. *Front Neuroendocrinol.* 2015; 37, 29–42.
15. Molcan L, Vesela A, Zeman M. Repeated phase shifts in the lighting regimen change the blood pressure response to norepinephrine stimulation in rats. *Physiol Res.* 2014; 63, 567–575.
16. Chellappa SL, Vujovic N, Williams JS, Scheer FA. Impact of circadian disruption on cardiovascular function and disease.*Trends Endocrinol Metab.* 2019; 30, 767–779.
17. Obayashi K, Saeki K, Iwamoto J, Ikada Y, Kurumatani N. Association between light exposure at night and nighttime blood pressure in the elderly independent of nocturnal urinary melatonin excretion. *Chronobiol Int.* 2014; 31, 779–786.
18. Molcan L, Sutovska H, Okuliarova M, *et al.* Dim light at night attenuates circadian rhythms in the cardiovascular system and suppresses melatonin in rats. *Life Sci.* 2019; 231, 116568.
19. Rumanova VS, Okuliarova M, Molcan L, Sutovska H, Zeman M. Consequences of low-intensity light at night on cardiovascular and metabolic parameters in spontaneously hypertensive rats. *Can J Physiol Pharmacol.* 2019; 97, 863–871.
20. Ujhazy E, Dubovicky M, Navarova J, *et al.* Subchronic perinatal asphyxia in rats: embryo–foetal assessment of a new model of oxidative stress during critical period of development. *Food Chem Toxicol.* 2013; 61, 233–239.
21. Zuther P, Gorbey S, Lemmer B. Chronos-Fit 1.06. Chronos-Fit. 2009. http://www.ma.uni-heidelberg.de/inst/phar/lehre/chrono.html.
22. Schulz LC. The Dutch Hunger Winter and the developmental origins of health and disease. *Proc Natl Acad Sci.* 2010; 107, 16757–16758.
23. Eckhart AD, Zhu Z, Arendshorst WJ, Faber JE. Oxygen modulates alpha 1B-adrenergic receptor gene expression by arterial but not venous vascular smooth muscle. *Am J Physiol-Heart Circ Physiol.* 1996; 271, H1599–H1608.
24. Hutchinson DS, Brew N, Vu T, *et al.* Effects of hypoxia-ischemia and inotropes on expression of cardiac adrenoceptors in the preterm fetal sheep. *J Appl Physiol.* 2018; 125, 1368–1377.
25. Moretta D, Papamatheakis DG, Morris D, *et al.* Long-term high altitude hypoxia and alpha adrenoceptor-dependent pulmonary arterial contractions in fetal and adult sheep. *Front Physiol.* 2019; 10, 1032.
26. Chen X, Zhang L, Wang C. Prenatal hypoxia-induced epigenomic and transcriptomic reprogramming in rat fetal and adult offspring hearts. *Sci Data.* 2019; 6, 1–8.
27. Chen X, Qi L, Fan X, *et al.* Prenatal hypoxia affected endothelium-dependent vasodilation in mesenteric arteries of aged offspring via increased oxidative stress. *Hypertens Res.* 2019; 42, 863–875.
28. Walton SL, Bielefeldt-Ohmann H, Singh RR, *et al.* Prenatal hypoxia leads to hypertension, renal renin-angiotensin system activation and exacerbates salt-induced pathology in a sex-specific manner. *Sci Rep.* 2017; 7, 1–13.
29. Svitok P, Husková Z, Červenková L, *et al.* The exaggerated salt-sensitive response in hypertensive transgenic rats (TGR mRen-2) fostered by a normotensive female. *Hypertens Res.* 2019; 42, 459–468.
30. Yang K, Wang Y, Ding Y, *et al.* Valsartan chronotherapy reverts the non-dipper pattern and improves blood pressure control through mediation of circadian rhythms of the renin-angiotensin system in spontaneous hypertension rats. *Chronobiol Int.* 2019; 36, 1058–1071.
31. Thosar SS, Butler MP, Shea SA. Role of the circadian system in cardiovascular disease. *J Clin Invest.* 2018; 128, 2157–2167.
32. Nishi EE, Almeida VR, Amaral FG, *et al.* Melatonin attenuates renal sympathetic overactivity and reactive oxygen species in the brain in neurogenic hypertension. *Hypertens Res Off J Jpn Soc Hypertens.* 2019; 42, 1683–1691.
33. Coote JH. A role for the paraventricular nucleus of the hypothalamus in the autonomic control of heart and kidney. *Exp Physiol.* 2005; 90, 169–173.
34. Pyner S, Coote JH. Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. *Neuroscience.* 2000; 100, 549–556.
35. Fan J-M, Wang X, Hao K, *et al.* Upregulation of PVN CRHR1 by gestational intermittent hypoxia selectively triggers a male-specific anxiogenic effect in rat offspring. *Horm Behav.* 2013; 63, 25–31.
36. Raff H, Jacobson L, Cullinan WE. Augmented hypothalamic corticotrophin-releasing hormone mRNA and corticosterone responses to stress in adult rats exposed to perinatal hypoxia. *J Neuroendocrinol.* 2007; 19, 907–912.
37. Lindgren I, Altimiras J. Chronic prenatal hypoxia sensitizes β-adrenoceptors in the embryonic heart but causes postnatal desensitization. *Am J Physiol-Regul Integr Comp Physiol.* 2009; 297, R258–R264.
38. Kostrzewa RM. The blood-brain barrier for catecholamines — Revisited. *Neurotox Res.* 2007; 11, 261–271.