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ORIGINAL ARTICLE

Prenatal hypoxia in rats increased blood pressure and sympathetic drive of the adult offspring

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Decreased oxygenation during pregnancy and early periods of ontogeny can affect normal body development and result in diseases in adulthood. The aim of this study was to use the model of prenatal intermittent hypoxia (PIH) and evaluate the effects of short-term hypoxia at the end of gestation on blood pressure (BP) control in adulthood. Wistar rats were exposed daily to PIH for 4 h during gestational day 19 and 20. In adult male rats, heart rate (HR), systolic BP and pulse pressure (PP) were acquired by radiotelemetry during 1 week. On the basis of HR variability and BP variability, sympathovagal balance (LF/HF) and spontaneous baroreflex sensitivity (sBRS) were evaluated. Systolic BP and PP were significantly elevated in PIH rats in comparison with control rats during the light and dark phase of the day, while LF/HF increased only during the light phase of the day. In contrast, sBRS tended to decrease only during the dark phase in PIH rats. In all measured and calculated parameters, significant circadian rhythms were present and were not affected by PIH. In conclusion, our data suggest that short intermittent hypoxia at the end of gestation can increase BP and PP via significant changes in LF/HF, which occur especially during the passive phase of the day. Results suggest that minor changes in the autonomous nervous system activity induced by environmental conditions during the perinatal period may contribute to development of hypertension in adulthood.
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INTRODUCTION

Hypertension is an important public health problem since it represents a strong independent risk factor for the development of cardiovascular and cerebrovascular morbidities. Hypertension is a multifactorial disease and is caused by several genetic, environmental and behavioral factors, which can vary in different parts of the world.[1,2] Adverse conditions during pregnancy[3] and in the early periods of ontogeny could also contribute to the development of hypertension in adulthood.[4]

This phenomenon results from developmental plasticity. During critical periods of development, physiological systems are more sensitive to external factors and later, after the loss of plasticity and the fixation of functional abilities, adaptation makes an organism more prepared for the external environment and increases its chances of survival.[5] However, some inadequate environmental conditions during early ontogeny may cause inadequate adaptation and long-lasting changes in the structure and function of organs, which can lead to diseases in adulthood.[6]

An adequate oxygen supply to the fetus is of key importance for the proper organ development. Epidemiological data demonstrate a high incidence of perinatal asphyxia in both resource-rich and resource-poor countries, 1/1000 and 5–10/1000 live births, respectively.[7] Several animal models are used to study effects of low oxygenation during *in utero* development, mostly using long-lasting chronic prenatal hypoxia[8–14] and oxidative stress is expected to have a pivotal role here.[9,11,12] However, even short asphyxial periods during the perinatal period can cause developmental changes in different brain areas.[15] In rats, gestational days 19 and 20 represent an important period for neuronal proliferation, differentiation and functional organization of many brain regions[16] and can be sensitive for hypoxic insults and subsequent oxidative stress.

In the present study, we used our previously developed model of prenatal intermittent hypoxia consisting of two 4 -h periods of low oxygen environment (10.5% of oxygen) during gestational day 19 and 20.[17] Results obtained with this model show that such treatment can affect normal brain development[17] and anxiety- and depression-like behaviors in rat offspring.[18]

Chronic prenatal hypoxia can modify processes controlling blood pressure (BP)[9] and central brain mechanisms can be involved.[12,19] Therefore, we measured BP and heart rate (HR) in mature rats by radiotelemetry, in conscious, free-moving animals with very high sampling resolution over a long time period,[20] which enables us to

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reveal minor changes during 24-h period that are not traceable with the tail-cuff method. This method allows us to calculate heart rate variability and evaluate the activity of the autonomous nerve system and spontaneous baroreflex sensitivity. As chronic prenatal hypoxia was suggested to have long-lasting consequences on the functional output of biological clocks,[21] we evaluated also daily changes in measured cardiovascular parameters.

Therefore, the aim of our study was to assess an impact of intermittent hypoxia at the end of gestation on BP regulation and circadian rhythm control in mature rats.

MATERIALS AND METHODS

Virgin female Wistar/DV rats (weight 200–220 g; age 3–4 months; $n=40$) were obtained from the breeding station Dobrá Voda (Slovak Republic, reg. no. SK CH 24011). The animals were kept in plastic cages under controlled environmental conditions (light:dark regime 12:12; temperature 22 ± 2 °C; humidity 50–70%; food and water *ad libitum*). After 1 week of adaptation, females were mated with males in the ratio 3:1 and the presence of spermatozoa in a vaginal smear determined the gestational day 0. The experiments were approved by the Ethical committee of the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences and the by the State Veterinary and Food Authority of the Slovak Republic.

Experimental procedures

The prenatal intermittent hypoxia (PIH) was induced by the exposure of pregnant rats to a low oxygen environment, consisting of 10.5% O_2 in 89.5% N_2 for 4 h per day during gestational day 19 and 20. More details about the procedures and the consequences for females and offspring are given elsewhere.[17] Male offspring were kept with their mothers during the suckling periods and from day 22 separated by sex in group cages. Adult males (3 months), five control (377 ± 8 g) and six PIH exposed rats (384 ± 9 g), each from a different mother, were used for radiotelemetry measurements of BP and HR.

Blood pressure and heart rate measurement

The cardiovascular parameters were measured by radiotelemetry (Data Science International, St Paul, MN, USA) allowing continuous acquisition of BP, HR and locomotor activity (LA) in freely moving animals. Anesthesia was induced with 4% isoflurane in 100% oxygen and maintained with 1.5–2% isoflurane in 100% oxygen. The abdominal cavity was then opened and sterile moistened gauze was used to retract the intestines. The pressure radiotelemetric transmitter TA11PA-C40 (DSI, St Paul, MN, USA) was surgically implanted rostrally into the abdominal aorta just above its bifurcation.[22] The catheter was stabilized in the aorta with tissue glue (3M Vetbond; DSI) and a cellulose patch (Cellulose Patch Kit—Small Animals; DSI). After the operation, abdominal wall and the skin were sutured with sterile cotton thread. The rats were treated postoperatively with ampicillin (100 mg kg^{-1}; SC; BB Pharma a.s., Prague, Czech Republic) and tramadol (15 mg kg^{-1}; SC; TRAMAL, STADA, Bad Vilbel, Germany). Telemetry data from implanted animals were collected 2 weeks after the surgical procedure, when circadian rhythms of BP, HR and LA were presented.

Data collection and analyses

Data were acquired and calculated for HR, systolic BP, pulse pressure (PP) and LA by the Dataquest A.R.T. 4.1 Gold system (DSI) with scheduled sampling intervals every 15 min with 300 -s (500 Hz) segment duration during a period of 1 week. HRV (HR variability) and BPV (BP variability) were calculated from

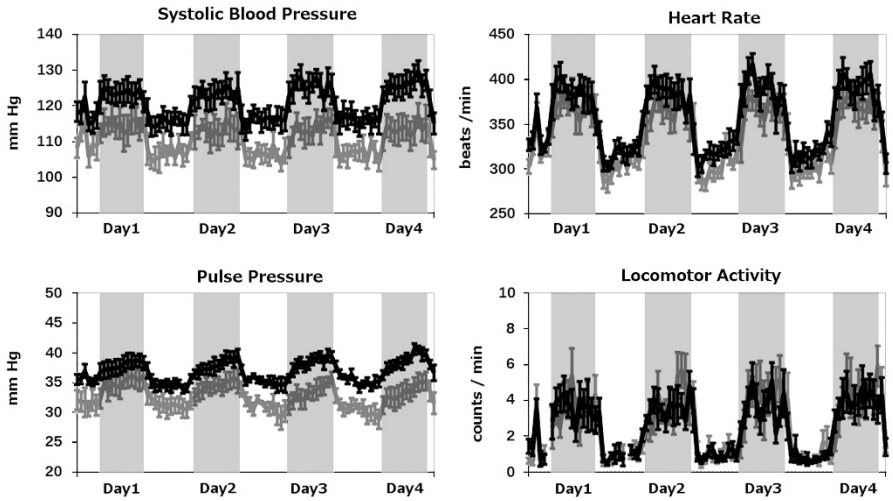

Figure 1 Light/dark differences of basic cardiovascular parameters and locomotor activity in control rats (gray line; $n=5$) and rats exposed to prenatal intermittent hypoxia (black line; $n=6$) during a period of 4 days. Original data are expressed as 1 h means ± s.e.m.

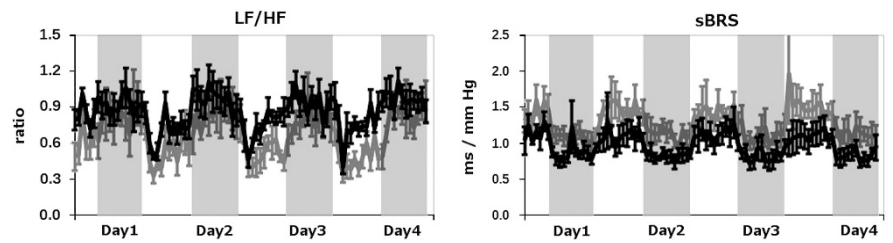

Figure 2 Light/dark differences of sympathovagal balance (LF/HF) and spontaneous baroreflex sensitivity (sBRS) in control rats (gray line; $n=5$) and rats exposed to prenatal intermittent hypoxia (black line; $n=6$) during 4 days. Original data are expressed as 1 h means ± s.e.m. of mean. HF, high frequency; LF, low frequency.

Table 1 Effects of PIH on HR, SBP, PP and LA in conscious telemetry monitored animals

	Light phase			*Dark phase*		
	Control (n = 5)	*PIH* (n = 6)	P-*value*	*Control* (n = 5)	*PIH* (n = 6)	P-*value*
HR (beats per min)	306 ± 8	321 ± 8	0.21	368 ± 12	388 ± 11	0.25
SBP (mm Hg)	107 ± 2	116 ± 3	0.03	114 ± 3	125 ± 3	0.03
PP (mm Hg)	31 ± 1	35 ± 1	0.01	34 ± 1	38 ± 1	0.02
LA (counts per min)	1.2 ± 0.2	1.1 ± 0.2	0.82	3.9 ± 0.5	3.7 ± 0.5	0.77
LF/HF (ratio)	0.52 ± 0.08	0.74 ± 0.06	0.04	0.81 ± 0.11	0.95 ± 0.10	0.34
sBRS (ms (mm Hg)$^{-1}$)	1.45 ± 0.15	1.10 ± 0.15	0.13	1.13 ± 0.11	0.85 ± 0.08	0.07

Abbreviations: HF, high frequency; HR, heart rate; LA, locomotor activity; LF, low frequency; PIH, prenatal intermittent hypoxia; PP, pulse pressure; SBP, systolic blood pressure.
From beat-to-beat data of HR, sympathovagal balance (LF/HF) and spontaneous baroreflex sensitivity (sBRS) were calculated. Values are expressed as mean ± s.e.m.

Table 2 Comparison of n.u. of nLF and nHF frequency bands obtained by the Lomb–Scargle periodogram in control and hypoxic rats expressed by mean ± s.e.m. separately during light (L) and dark (D) phase of the day

	Control			*Hypoxia*			
Parameter	*Mean*	*s.e.m.*	N	*Mean*	*s.e.m.*	N	P-*value*
nLF_L (n.u.)	0.316	0.034	5	0.403	0.017	6	0.066
nLF_D (n.u.)	0.418	0.036	5	0.469	0.025	6	0.281
nHF_L (n.u.)	0.684	0.034	5	0.597	0.017	6	0.066
nHF_D (n.u.)	0.582	0.036	5	0.531	0.025	6	0.281

Abbreviations: nHF, normalized unit of high frequency band; nLF, normalized unit of low frequency band; n.u., normalized unit.

300-s segments that were processed for ectopic beats detection (data segments with more than 5% of ectopic beats were removed from a data cluster) and trends were removed by wavelet transformation. Segments were interpolated (5 Hz) with 50% windows overlapping to minimize a spectral leakage.[23] A Lomb–Scargle periodogram was used to obtain the power spectrum of the fluctuations using HRV Analysis Software.[24] Frequency domains were divided into low frequency (LF; 0.2–0.75 Hz) and high frequency (HF; 0.75–2.5 Hz) bands and their ratio (LF/HF) was used as an index of sympathovagal balance.[25] Spontaneous baroreflex sensitivity (sBRS) was computed as α-index within the LF range ($(LF_{HRV}/LF_{BPV})^{0.5}$).[26] Circadian amplitude (the difference between the peak and the mean value of a wave), acrophase (the time at which the peak of a rhythm occurs) and percentage of rhythmicity (the coefficient of determination; represents the percentage of variation in the data that is explained by the fitted model) of cardiovascular parameters[27,28] were calculated from the original data using Chronos-Fit software.[29]

Statistical analysis

The normality of the data distribution was tested using the Kolmogorov–Smirnov test. Comparison of light and dark phases was performed using a paired *t*-test while comparison of PIH and control groups was done using a non-paired *t*-test. Calculated data are expressed as arithmetic means ± s.e.m.

RESULTS

Light–dark differences

Control rats exposed to regular light–dark conditions exhibited a significant daily pattern of measured parameters for HR ($P<0.001$), systolic BP ($P<0.01$), PP ($P<0.01$) and LA ($P<0.01$; Figure 1). The calculated parameters LF/HF ($P=0.013$) and sBRS ($P=0.053$) also showed differences between the light and dark phases of the day (Figure 2). Similarly, PIH rats revealed a strong daily pattern in HR ($P<0.001$), systolic BP ($P<0.001$), PP ($P<0.01$), LA ($P<0.001$; Figure 1), LF/HF ($P<0.01$) and sBRS ($P<0.05$; Figure 2).

Table 3 Circadian changes in cardiovascular parameters and locomotor activity in control and PIH exposed rats

	Control (n = 5)	*PIH* (n = 6)	P-*value*
Percentage of the rhythm			
HR	44 ± 3	48 ± 3	0.34
SBP	19 ± 4	29 ± 4	0.09
PP	32 ± 1	41 ± 9	0.35
LA	29 ± 3	25 ± 2	0.25
LF/HF	23 ± 4	18 ± 3	0.31
sBRS	15 ± 4	17 ± 4	0.64
Amplitude			
HR (beats per min)	43 ± 5	46 ± 4	0.64
SBP (mm Hg)	4.8 ± 0.7	5.5 ± 0.5	0.41
PP (mm Hg)	2.2 ± 0.3	2.3 ± 0.4	0.86
LA (counts per min)	2.0 ± 0.2	1.8 ± 0.3	0.58
LF/HF (ratio)	0.21 ± 0.05	0.48 ± 0.30	0.43
sBRS (ms (mm Hg)$^{-1}$)	0.24 ± 0.07	0.19 ± 0.05	0.43
Acrophase (hh:mm; ZT)			
HR	18:07 ± 00:19	18:17 ± 00:36	0.81
SBP	18:42 ± 00:32	18:31 ± 00:19	0.76
PP	20:36 ± 00:15	20:26 ± 00:23	0.72
LA	19:25 ± 00:20	18:43 ± 00:16	0.13
LF/HF	17:58 ± 04:25	15:41 ± 0:55	0.07
sBRS	07:25 ± 01:01	07:43 ± 00:42	0.82

Abbreviations: HR, heart rate; LA, locomotor activity; LF/HF, sympathovagal balance (low frequency/high frequency); PIH, prenatal intermittent hypoxia; PP, pulse pressure; SBP, systolic blood pressure; sBRS, spontaneous baroreflex sensitivity; ZT, Zeitgeber time.
Values are expressed as mean ± s.e.m.; ZT00 = 06:00 (beginning of light phase of the day); ZT12 = 18:00 (beginning of dark phase of the day).

Effects of prenatal hypoxia

Systolic BP ($P<0.05$) and PP ($P<0.01$) was significantly increased in PIH in comparison with control rats during both the light and dark phases (Table 1). The sympathovagal balance (LF/HF ratio) was higher ($P<0.05$) in PIH than in control rats but only during the light (passive) phase of the day. This reflects the trend for nLF to be higher and nHF to be lower in the PIH group than in controls, but only during the light phase of the day (Table 2). An opposite pattern was found in the sBRS where we observed a trend ($P=0.07$) to lower values in PIH in comparison with control rats (Table 1) during the dark (active) phase. Other parameters, such as HR and LA, did not differ between PIH and control rats during both phases of the day.

Circadian changes in cardiovascular parameters and locomotor activity

Prenatal hypoxia did not affect circadian rhythms of measured traits in adult offspring. The percentage of the rhythm, amplitude and acrophase of circadian rhythms were not affected. As expected, HR, systolic BP, PP, LA and LF/HF had maximal values of their rhythms in the middle of the dark phase and sBRS in the middle of the light phase (Table 3).

DISCUSSION

In humans, perinatal asphyxia is a major cause of death and acquired brain damage in newborn infants,[7] however, there are limited data on long-term outcomes of intermittent oxygen insufficiency during perinatal period. In our study, we used the model of prenatal intermittent hypoxia characterized by short hypoxic periods at the end of gestation to explore its consequences on the blood pressure control. Exposure of rats to a low oxygen environment during gestation is associated with abnormal development of fetuses, usually accompanied by malformations of the central nervous system[30] and several functional abnormalities in different physiological systems,[10] including the cardiovascular system.[9]

Experimental studies show that chronic prenatal hypoxia can change the development of several mechanisms involved in BP regulation,[11] but there is limited amount of references confirming prenatal hypoxia as a risk factor for the arterial BP increase during basal conditions.[19] In our study, we observed higher values of systolic BP and PP in the PIH group in comparison with control rats, with no changes in HR. We hypothesize that the short-term exposure to hypoxia (gestational day 19–20) induced an increase in sympathetic activity in mature rats, which have a dominant role in BP increase in our experiments. Postnatally, acute hypoxia activates peripheral chemoreceptors, which directly increase the sympathetic outflow.[31] Chronic prenatal hypoxia can program offspring to the higher sympathovagal balance,[12] increased sympathetic innervation[19] and elevated BP as a result of increased sympathetic tone.[19] In our experiments, we evaluated sympathovagal balance and spontaneous baroreflex sensitivity in conscious rats over a long period. Sympathovagal balance was increased in PIH in comparison with control rats mainly during the light (passive) phase of the day, whereas spontaneous baroreflex sensitivity showed a strong tendency to be decreased in PIH in comparison with control rats during the dark phase of the day. Our results are in line with induced expression of tyrosine hydroxylase mRNA during the first postnatal week in the ventral medulla and lower levels of phenylethanolamine *N*-methyltransferase protein during the second postnatal week in the dorsal medulla after prenatal hypoxia (gestational day 18–21).[32] The observed imbalance in catecholamine synthesis may later contribute to autonomic nervous system disorders[19] and BP increase.[33]

Baroreflex sensitivity provides an insight into the responsiveness of the cardiovascular system and can be linked to several health complications.[34] Our results showed a strong tendency to a decline of sBRS in the PIH group mainly during the dark phase of the day. In adult conscious rats, intermittent hypoxia induced an elevation of BP and sympathetic outflow followed by a decrease in the baroreflex sensitivity without changes in HR.[31] Similarly, in young humans, decreased baroreflex sensitivity is linked with a prehypertensive state and can contribute to the development of cardiovascular diseases.[35] Moreover, a tendency to reduce the baroreflex sensitivity suggests changes in the central blood pressure control as a result of short intermittent hypoxia in utero.

In addition to activation of the sympathetic system, possible endothelial dysfunction[36] can explain increased BP induced by augmented conversion of inactive endothelin-1 to its active form in male rats.[37] It is well known that endothelial dysfunction is connected with hypertension[38] or BP and PP increase.[39] Moreover, prenatal hypoxia (gestational day 15–21) reduced the intrauterine growth and rats are more vulnerable to environmental stressors that affect endothelial function.[13]

Evaluation of parameters describing circadian rhythms such as acrophase and amplitude did not reveal significant differences between the PIH and control rats under synchronized LD conditions. Long-term prenatal hypoxia induces alterations of the functional organization of the circadian system in adult rats and a decreased sensitivity of the biological clock to light.[21] Prenatal intermittent hypoxia in our study did not induce changes in circadian rhythms of cardiovascular traits in PIH rats indicating that the suprachiasmatic nucleus, as the central hypothalamic circadian oscillator, is less prone to hypoxic insults than brain structures involved in BP control, or this period is not critical for its development. As our rats were not exposed to constant darkness, we cannot estimate effects of the treatment on the endogenous free running period.

In conclusion, our data show for the first time that short hypoxic periods at the end of gestation can increase BP and PP via significant changes in LF/HF ratio, which occur especially during the passive phase of the day. Results suggest that minor changes in the autonomous nervous system activity induced by environmental conditions during the perinatal period may contribute to the development of hypertension in adulthood.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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