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Sustained hypoxia in mice increases parasympathetic but not sympathetic tone

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ABSTRACT

The autonomic profile of mice submitted to sustained hypoxia (SH) was not yet fully evaluated. Herein, we characterized the cardiovascular and autonomic profile of conscious freely moving mice submitted to SH using two sequential experimental protocols to evaluate the parasympathetic and sympathetic tone to the heart and the sympathetic tone to the vascular resistance. In the first protocol the sequence of antagonists was methyl-atropine followed by propranolol and then by prazosin, while in the second protocol the sequence was propranolol followed by methyl-atropine and then by prazosin. In SH the baseline heart rate was significantly lower than in control mice and the antagonism of the parasympathetic and sympathetic tone to the heart in both experimental protocols indicated an increased parasympathetic tone in SH mice and no changes in the sympathetic tone. Antagonism of the sympathetic tone to the vascular resistance with prazosin produced similar changes in arterial pressure in control and SH mice. Altogether these findings support the concept that mice submitted to SH present a significant increase in the parasympathetic but not in the sympathetic tone, which may explain why the baseline arterial pressure was not increased in SH mice.

1. Introduction

The assessment of cardiovascular autonomic control in mice has been studied using the analysis of heart rate (HR) and arterial pressure (AP) variability in the frequency-domain (spectral analysis) and by means of pharmacological autonomic blockades (Just et al., 2000; Janssen and Smits, 2002; Chen et al., 2005; Fazan et al., 2005; Baudrie et al., 2007; Laude et al., 2008; Rodrigues et al., 2011). Several studies reported a sympathetic predominance in the control of baseline HR in mice and also an important role of vagal tone under resting conditions (Ishii et al., 1996; Gehrmann et al., 2000; Janssen et al., 2000; Just et al., 2000; Janssen and Smits, 2002; Fazan et al., 2005; Rodrigues et al., 2011). However, the relative role of the parasympathetic and sympathetic in determining the baseline HR in mice are controversial because some studies raised questions about the predominant role of the sympathetic tone (Baudrie et al., 2007; Laude et al., 2008; Swoap et al., 2008; Lujan et al., 2016). These controversies are related to different factors surrounding the experimental design, such as the in vivo cardiovascular recordings conditions, the recovery period between the surgery for catheter/telemetric devices implantation and the recording in behaving mice, the impacts of room temperature on the metabolic state of animals, and the different frequency-domain indexes used in the spectral analysis between the studies (Stauss, 2007).

It is also important to consider that the autonomic control to the cardiovascular system in mice depends upon the activity state, age, and genetic background of mice (Janssen and Smits, 2002; De Angelis et al., 2004; Axsom et al., 2020; Piantoni et al., 2021). In this context, it was demonstrated that some pathophysiological conditions, such as myocardial infarction, diabetes, and psychosocial stress can change the cardiac autonomic balance to the heart of mice (Costoli et al., 2004; Gross et al., 2008; Durand et al., 2014). However, the autonomic profile of conscious freely moving mice previously submitted to sustained hypoxia was not yet evaluated.

In a recent study, we showed that the changes in autonomic and respiratory patterns in mice submitted to SH are different in relation to that observed in rats submitted to the same protocol of hypoxia (Rodrigues et al., 2021). These previous studies suggested an autonomic imbalance in favor of the parasympathetic component in mice submitted to SH mainly related to changes in the respiratory pattern. However, from the observed increase in the vagus nerve activity in the *in situ* working heart-brainstem preparation of SH mice, it was not possible to correlate it with the significant reduction in the baseline HR of conscious

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freely moving mice. For this reason, the present study was performed in conscious freely moving mice in order to evaluate the autonomic balance to the heart (parasympathetic vs sympathetic tone) and the sympathetic tone to the vascular resistance in mice previously submitted to SH. To reach these goals we performed a pharmacological evaluation of the autonomic drive to the cardiovascular system using sequential antagonisms of the parasympathetic tone to the heart (methyl-atropine) and the sympathetic tone to the heart (propranolol) and to the vascular resistance (prazosin) in conscious freely moving mice. A second experimental protocol using the sequence propranolol followed by methyl-atropine and prazosin was also used for a full characterization of the autonomic balance to the heart of SH mice.

2. Materials and methods

Male C57BL/6 mice (7–8 weeks old, ~25g, n = 30) provided by the Animal Care Facility of the University of São Paulo, Campus of Ribeirão Preto, were used in this study. Before the experimental protocols, mice were maintained in standard environmental conditions (23 ± 1 °C, 12h–12h light-dark cycle) with chow and water provided *ad libidum*. All experimental protocols used in this study were approved by the Institutional Ethics Committee on Animal Experimentation (CEUA#140/2019).

Under anesthesia with isoflurane (Isoforine®, Cristália Produtos Químicos Farmacêuticos Ltda., Itapira, SP, Brazil) at a rate of 5% for induction and 1–2% for maintenance, mice had a saline-filled catheter [(MRE-025) Braintree Scientific, city, MA, USA] inserted into the femoral artery and a polyethylene saline-filled catheter [(LDPE-PE/05) Scientific Commodities, Lake Havasu City, AZ, USA] inserted into the jugular vein for measurement of pulsatile arterial pressure (PAP) and drugs administration, respectively (Rodrigues et al., 2021). Both catheters were exteriorized subcutaneously in the back of the neck and mice were allowed to recover for 5 days before the cardiovascular recordings. After the surgery, mice received an antibiotic [Pentabiotic, Fort Dodge Saúde Animal Ltda., Campinas, Brazil (0.2 ml of 1.2 million UI, I.M)].

On the fourth day after the surgery for arterial and venous cannulation mice were submitted to SH or normoxic protocol, as previously described by Rodrigues et al. (2021). SH mice remained 24 h inside the chamber at a fraction of inspired O2 (FiO2) of 0.1 while control mice remained in another chamber under normoxic condition (FiO2 = 0.208) for the same period of time.

At the end of SH or control protocol, the arterial catheter was connected to a pressure transducer (MLT0380; ADInstruments, Bella Vista, NSW, Australia) linked to an amplifier (Bridge Amp, ML221; ADInstruments) and to the recording system. Heart rate (HR) was counted from the Pulsatile Arterial Pressure (PAP) signals acquired by a computerized system (PowerLab 4/25 ML845; ADInstruments) and sampled (1 kHz) on a computer using an acquisition software (LabChart 5, ADInstruments). Baseline PAP, mean arterial pressure (MAP), and HR were recorded for 60 min in behaving mice, and the values obtained in the first 30 min were not considered due to the expected stress associated with handling the animals.

After 30 min of baseline cardiovascular recordings, the tachycardic response to methyl-atropine [a muscarinic receptors antagonist, 1 mg/kg, i.v., Fazan et al., 2005; (Sigma, St Louis, MO, USA)] was used as an index to indirectly evaluate the baseline parasympathetic tone to the heart. The changes in baseline MAP and HR in response to methyl-atropine recorded for 10 min after the injection and quantified 1 min during the maximal response were compared with the baseline values prior to the injection in SH and control mice.

Ten minutes after methyl-atropine injection, the bradycardic response to propranolol [β adrenoceptors antagonist, 3 mg/kg, i.v., Fazan et al., 2005 (Sigma, St Louis, MO, USA)] was used as an index to indirectly evaluate the baseline sympathetic tone to the heart. The changes in baseline MAP and HR in response to propranolol recorded for 10 min after the injection and quantified 1 min during the maximal response were compared with the baseline values prior to the injection of propranolol in SH and control mice. The antagonism of muscarinic receptors with methyl-atropine followed by the antagonism of beta-adrenoceptors provided the intrinsic heart rate, which correspond to the frequency discharge of the cardiac pacemaker without the inputs of the autonomic nervous system.

Ten minutes after the injection of propranolol, when both parasympathetic and sympathetic branches to the heart were blocked, the fall in the baseline MAP in response to prazosin [α-1 adrenoceptors antagonist, 1 mg/kg; Fazan et al., 2005 (Sigma, St Louis, MO, USA)] was used as an index to indirectly evaluate the baseline sympathetic tone to the vessels in charge of the vascular resistance. The magnitude of fall in MAP in response to prazosin recorded for 10 min after the injection and quantified 1 min during the maximal response was compared with the baseline MAP prior to the prazosin injection in SH and control mice. In distinct groups of control and SH mice, we used an experimental protocol similar to that described above, but the sequence of the injections of the antagonists was initiated with propranolol, followed by methyl-atropine, and then prazosin.

At the end of the experimental protocol, mice were killed using a high concentration of the anesthetic urethane (2 g/kg, i.v; Sigma-Aldrich, St Louis, MO, USA).

Data are expressed as means ± standard deviation (SD). The normality of residuals data was tested using the Shapiro-Wilk normality test and analyzed using one-way or two-way ANOVA followed by the Bonferroni *post hoc* test. Differences were considered statistically significant when $P \leq 0.05$. All graphics and statistical analyses were performed using GraphPad Prism 8.

3. Results

3.1. Baseline cardiovascular parameters in control and SH mice

Representative tracings of baseline cardiovascular parameters in control and SH mice of the two groups of SH mice (n = 15) and the two groups of control mice (n = 15) are presented in Fig. 1A. SH mice (n = 15) presented no significant changes in baseline MAP (116 ± 7 vs 112 ± 8 mmHg, P = 0,1617, Fig. 1B) but presented a significant decrease in baseline HR (470 ± 78 vs 602 ± 62 beats. min^{-1}; P < 0,0001, Fig. 1C) in comparison with control mice (n = 15).

3.2. Cardiovascular responses to sequential autonomic blockade with methyl-atropine followed by propranolol and prazosin

We indirectly evaluated the parasympathetic component to the heart with methyl-atropine in mice from control (n = 7) and SH groups (n = 8). Representative tracings of changes in PAP, MAP, and HR in response to cardiac parasympathetic antagonism of a control and SH mice are present in Fig. 2A. The baseline MAP evaluated before the injection of methyl-atropine (111 ± 8 vs 115 ± 11 mmHg, P > 0,9999, Fig. 2B) and the MAP after methyl-atropine (105 ± 10 vs 106 ± 20 mmHg, P > 0,9999, Fig. 2B) were not different between the groups (SH vs Control). Similarly, the MAP before and after methyl-atropine was not different in control (106 ± 20 vs 115 ± 11 mmHg, P = 0,4499, Fig. 2B) and SH group (105 ± 10 vs 111 ± 8 mmHg, P = 0,7109, Fig. 2B). Therefore, control and SH mice presented similar changes in MAP (ΔMAP; −6 ± 8 vs −8 ± 12 mmHg, P = 0.6583, data not shown).

The baseline HR evaluated before the injection of methyl-atropine of SH group was significantly decreased (P = 0,0017) in comparison with the control group (457 ± 86 vs 614 ± 82 beats. min^{-1}; Fig. 2C). The HR after methyl-atropine (640 ± 89 vs 709 ± 52 beats. min^{-1}; P = 0,2143; Fig. 2C) was not different between the groups (SH vs Control). SH mice presented a significant increase (P = 0,0002) in HR after methyl-atropine in comparison with baseline values (640 ± 89 vs 457 ± 86 beats. min^{-1}; Fig. 2C), while in control mice the HR before and after methyl-atropine was not different (709 ± 52 vs 614 ± 82 beats. min^{-1};

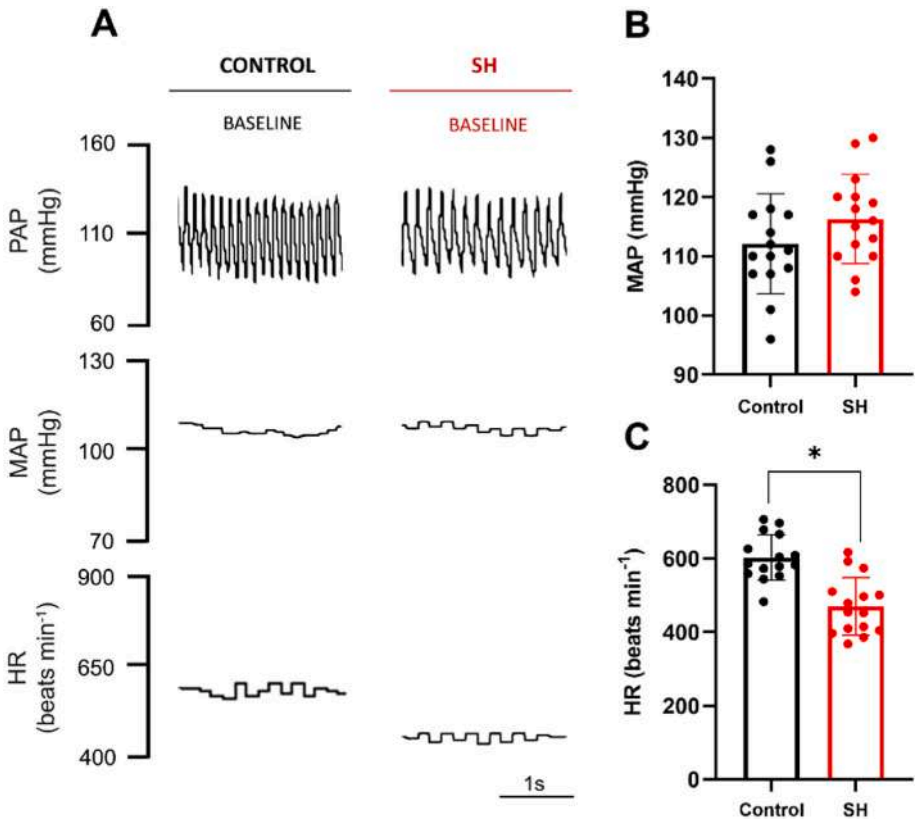

Fig. 1. Baseline cardiovascular parameters of mice from control (n = 15) and SH (n = 15) groups. Representative traces of pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) of a control and SH mice (Panel A). Average values of MAP (Panel B) and HR (Panel C) in control and SH groups. * $P < 0.05$, unpaired *t*-test.

P = 0,0694; Fig. 2C). Therefore, the magnitude of tachycardic response to methyl-atropine was greater (P = 0.0359) in SH than in control mice (ΔHR; 182 ± 87 vs 95 ± 49 beats. min^{-1}, data not shown).

We indirectly evaluated the sympathetic component to the heart with propranolol in mice from control (n = 7) and SH groups (n = 8). Representative tracings of changes in PAP, MAP, and HR in response to cardiac sympathetic antagonism of a control and SH mice are present in Fig. 3A. The baseline MAP evaluated before the injection of propranolol (102 ± 8 vs 101 ± 20 mmHg, P > 0,9999, Fig. 3B) and the MAP after propranolol (91 ± 9 vs 87 ± 14 mmHg, P > 0,9999, Fig. 3B) were not different between the groups (SH vs Control). Similarly, the MAP before and after propranolol were not different in control (87 ± 14 vs 101 ± 20 mmHg, P = 0,1528, Fig. 3B) and SH group (91 ± 9 vs 102 ± 8 mmHg, P = 0,2344, Fig. 3B). Therefore, control and SH mice presented similar changes in MAP in response to propranolol (ΔMAP; −11 ± 7 vs −13 ± 12 mmHg, P = 0,6376, data not shown).

The baseline HR evaluated before the injection of propranolol (617 ± 116 vs 659 ± 67 beats. min^{-1}, P = 0,7071, Fig. 3B) and the HR after propranolol (417 ± 57 vs 467 ± 88 beats. min^{-1}, P = 0,5365, Fig. 3B) were similar between the groups (SH vs Control). The HR after the double antagonism of parasympathetic and sympathetic tone to the heart (HR after propranolol) represents the intrinsic frequency discharge of the cardiac pacemaker. In both SH (417 ± 57 vs 617 ± 116 beats. min^{-1}, P = 0,0002, Fig. 3B) and control group (467 ± 88 vs 659 ± 67 beats. min^{-1}, P = 0,0006, Fig. 3B) the HR after propranolol was significantly reduced in comparison with the baseline values, in a way that controls and SH mice presented similar changes in HR (ΔHR; −200 ± 90 vs −192 ± 68 beats. min-1, P = 0.5358, data not shown) to cardiac sympathetic antagonism with propranolol.

We indirectly evaluated the sympathetic tone to the vascular resistance with prazosin in mice from control (n = 7) and SH groups (n = 8). Representative tracings of changes in PAP and MAP in response to the blockade of the sympathetic vasomotor tone of a control and SH mice are present in Fig. 4A. The baseline MAP evaluated before the injection of prazosin (91 ± 9 vs 90 ± 12 mmHg, P > 0,9999, Fig. 4B) and the MAP after prazosin (77 ± 5 vs 74 ± 12 mmHg, P > 0,9999, Fig. 4B) were similar between the groups (SH vs Control). In both SH (77 ± 5 vs 91 ± 9 mmHg, P = 0,0186, Fig. 3B) and control group (74 ± 12 vs 90 ± 12 mmHg, P = 0,0142, Fig. 4B) the MAP after prazosin was significantly reduced in comparison with the baseline values in a way that controls and SH mice presented similar changes in MAP (ΔMAP; −14 ± 7 vs −16 ± 7 mmHg, P = 0.5835, data not shown) to the blockade of vasomotor sympathetic tone with prazosin.

3.3. *Cardiovascular responses to sequential autonomic blockade with propranolol followed by methyl-atropine and prazosin*

In the second experimental protocol, we indirectly evaluated the sympathetic component to the heart with propranolol in mice from control (n = 8) and SH groups (n = 7). The baseline MAP evaluated before the injection of propranolol (112 ± 3 vs 106 ± 6 mmHg, P = 0,06, Fig. 5A) and the MAP after propranolol (117 ± 8 vs 110 ± 8 mmHg, P = 0,1186, Fig. 5A) were not different between the groups (SH vs Control). Similarly, the MAP before and after methyl-atropine were not different in control (110 ± 8 vs 106 ± 6 mmHg, P = 0,6142, Fig. 5A) and SH group (117 ± 8 vs 112 ± 3 mmHg, P > 0,9999, Fig. 5A). Therefore, control and SH mice presented similar changes in MAP (ΔMAP; 5 ± 6 vs 4 ± 8 mmHg, P = 0,8208, data not shown).

The baseline HR evaluated before the injection of propranolol in the SH group was significantly decreased (P < 0,0001) in comparison with the control group (448 ± 72 vs 605 ± 39 beats. min^{-1}; Fig. 5B). Similarly, the HR after propranolol of SH group was significantly decreased (P = 0,0004) in comparison with the control group (368 ± 38 vs 470 ± 22 beats. min^{-1}, Fig. 5B). In both SH (368 ± 38 vs 448 ± 72 beats.

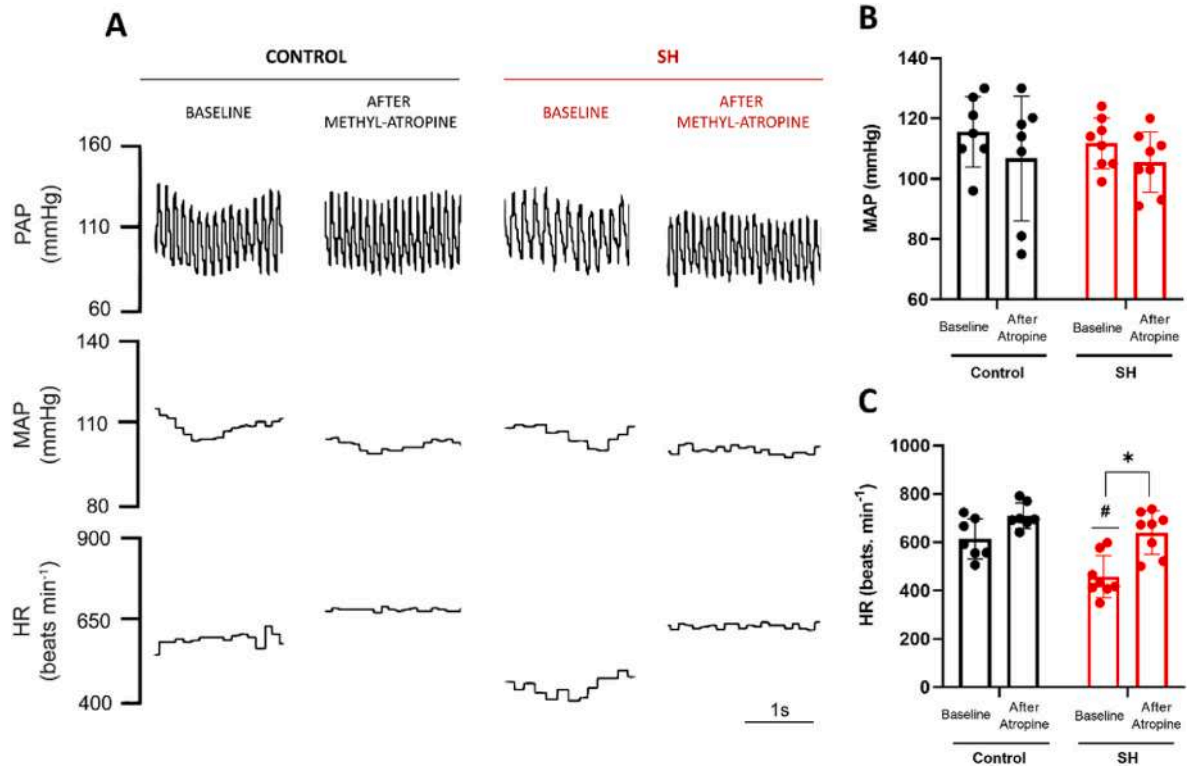

Fig. 2. Changes in the cardiovascular parameters in response to the antagonism of the parasympathetic drive to the heart in mice from control (n = 7) and SH (n = 8) groups. Representative traces of changes in pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in response to the antagonism of parasympathetic tone to the heart with methyl-atropine of a control and SH mice (Panel A). Average values of MAP (Panel B) and HR (Panel C) before and after the injection of methyl-atropine in control and SH groups. * Different within the same group ($P < 0.05$), one-way ANOVA. #Different from control group ($P < 0.05$), two-way ANOVA.

min^{-1}, P = 0,0062, Fig. 5B) and control group (470 ± 22 vs 605 ± 39 beats. min^{-1}, P < 0,0001, Fig. 5B) the HR after propranolol was significantly reduced in comparison with the baseline values. Therefore, control and SH mice presented similar changes in HR (ΔHR; −79 ± 57 vs −134 ± 47 beats. min^{-1}, P = 0,0622, data not shown) to cardiac sympathetic antagonism with propranolol.

We indirectly evaluated the parasympathetic component to the heart with methyl-atropine in mice from control (n = 8) and SH groups (n = 7). The baseline MAP evaluated before the injection of methyl-atropine (120 ± 3 vs 116 ± 9 mmHg, P = 0,3985, Fig. 5C) was not different between the groups (SH vs Control). The MAP after methyl-atropine of SH group was significantly increased (P = 0,0433) in comparison with the control group (127 ± 6 vs 119 ± 3 mmHg, Fig. 5C). The MAP before and after methyl-atropine was not different in control (119 ± 3 vs 116 ± 9 mmHg, P = 0,8348, Fig. 5C) and SH group (127 ± 6 vs 120 ± 3 mmHg, P = 0,1203, Fig. 5C). The magnitude of changes in MAP (ΔMAP; 5 ± 4 vs 3 ± 7 mmHg, P = 0,3800, data not shown) in response to methyl-atropine was similar in both groups (Control vs SH).

The baseline HR evaluated before the injection of methyl-atropine in the SH group was significantly decreased (P < 0,0001) in comparison with the control group (392 ± 26 vs 493 ± 37 beats. min^{-1}; Fig. 5D). Similarly, the HR after methyl-atropine (the intrinsic heart rate) was significantly decreased in SH in comparison with controls (478 ± 26 vs 520 ± 30 beats. min^{-1}; P = 0,0263; Fig. 5D). SH mice presented a significant increase (P < 0,0001) in HR after methyl-atropine in comparison with baseline values (478 ± 25 vs 392 ± 26 beats. min^{-1}, Fig. 5D), while in control mice the HR before and after methyl-atropine was not different (520 ± 30 vs 493 ± 37 beats. min^{-1}; P = 0,1770, Fig. 5D). Therefore, the magnitude of tachycardic response to methyl-atropine was greater (P = 0,0018) in SH than in control mice (ΔHR; 85 ± 39 vs 27 ± 15 beats. min^{-1}, data not shown).

The sympathetic tone to the vascular resistance with prazosin in mice from control (n = 8) and SH groups (n = 7) was also indirectly evaluated. The baseline MAP evaluated before the injection of prazosin (117 ± 4 vs 113 ± 3 mmHg, P = 0,7788, Fig. 5E) and the MAP after prazosin (76 ± 8 vs 70 ± 8 mmHg, P = 0,1991, Fig. 5E) were similar between the groups (SH vs Control). In both SH (76 ± 8 vs 117 ± 4 mmHg, P < 0,0001, Fig. 5E) and control group (70 ± 8 vs 113 ± 3 mmHg, P = , Fig. 5E) the MAP after prazosin was significantly reduced in comparison with the baseline values, in a way that controls and SH mice presented similar changes in MAP (ΔMAP; −40 ± 5 vs −43 ± 9 mmHg, P = 0,5962, data not shown) to the blockade of vasomotor sympathetic tone with prazosin.

4. Discussion

Specific neural networks in the brainstem are in charge of the generation and modulation of the autonomic and respiratory functions in order to preserve optimal blood flow supply for all tissues under normal or challenging conditions (Machado, 2001; Costa et al., 2014). Fall in the pressure of oxygen in the arterial blood (PaO_2) activates the peripheral chemoreceptors located mainly in the carotid bodies triggering autonomic and respiratory responses to restore the PaO_2 (Machado, 2001; Barros et al., 2002).

In a recent study, we documented that mice submitted to SH presented changes in the autonomic and respiratory patterns different from those observed in rats submitted to the same protocol of hypoxia. SH in mice produced a significant reduction in the baseline HR and a significant increase in the magnitude of bradycardic responses to chemoreflex activation, indicating an enhancement of parasympathetic drive to the heart. SH mice also presented a significant increase in respiratory frequency and ventilation (Rodrigues et al., 2021). Taking these findings into account and using the *in situ* working heart–brainstem (WHBP) preparation to evaluate the autonomic and respiratory coupling in mice

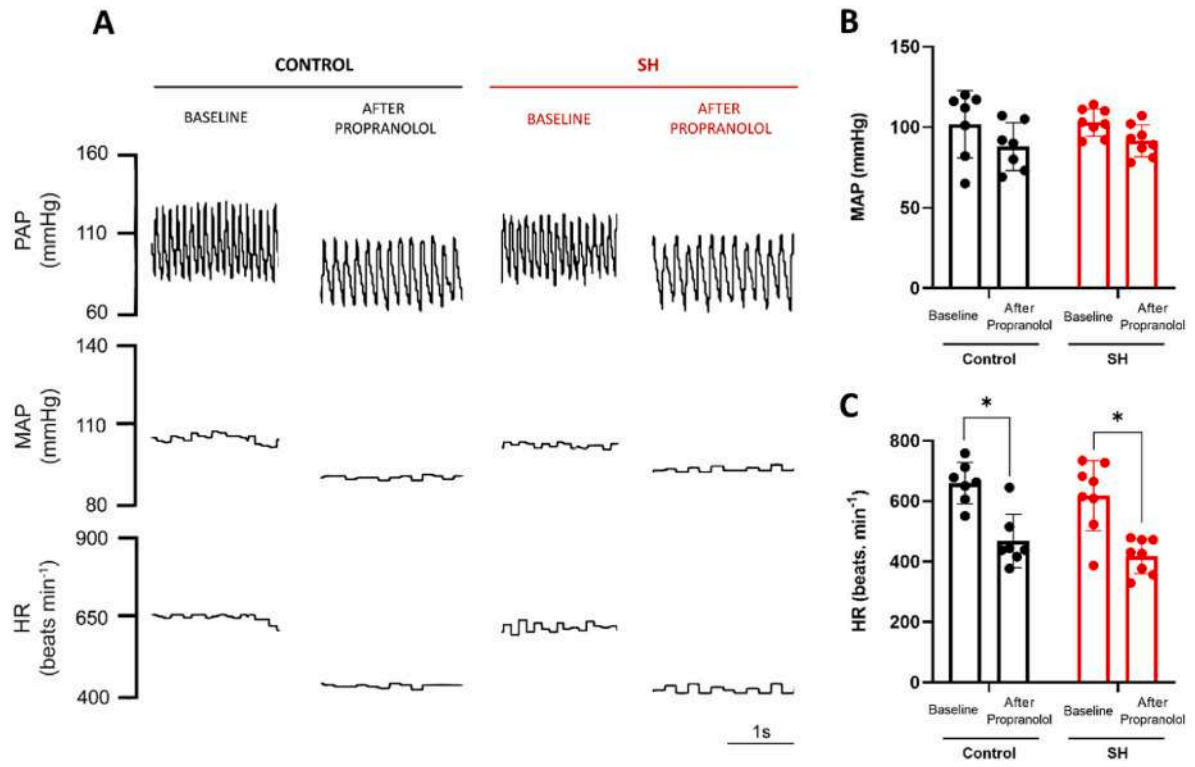

Fig. 3. Changes in the cardiovascular parameters in response to the antagonism of the sympathetic drive to the heart in mice from control (n = 7) and SH (n = 8) groups. Representative traces of changes in pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in response to the antagonism of sympathetic tone to the heart with propranolol of a control and SH mice (Panel A). Average values of MAP (Panel B) and HR (Panel C) before and after the injection of propranolol in control and SH groups. * Different within the same group ($P < 0.05$), one-way ANOVA.

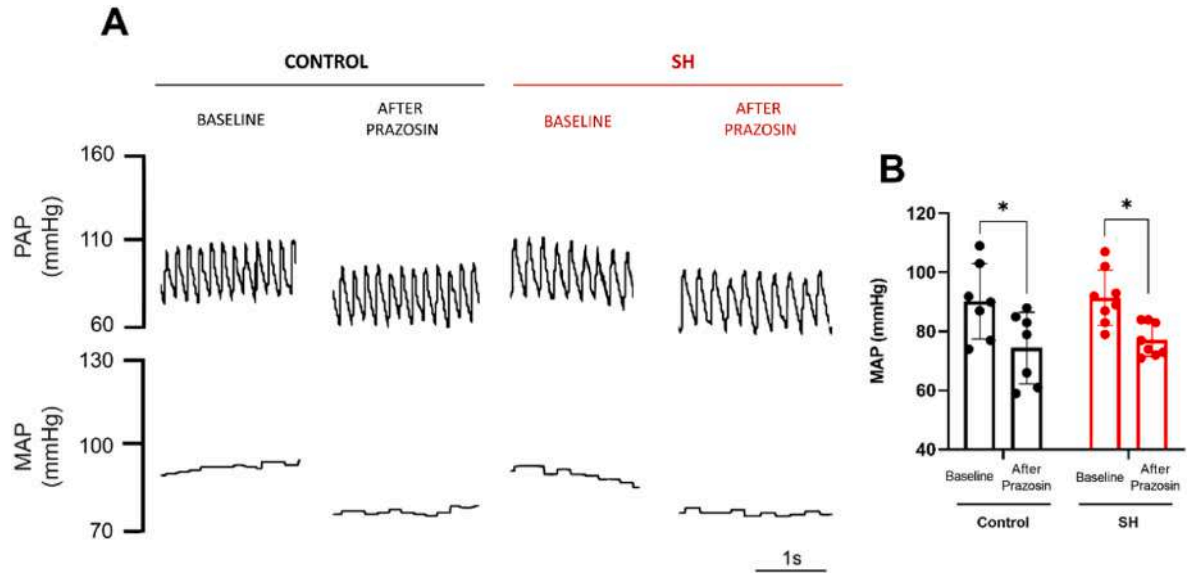

Fig. 4. Changes in mean arterial pressure (MAP) in response to the antagonism of the sympathetic vasomotor drive in mice from control (n = 7) and SH (n = 8) groups. Representative traces of fall in pulsatile arterial pressure (PAP) and mean arterial pressure (MAP) in response to the antagonism of sympathetic tone to the vessels with prazosin of a control and SH mice (Panel A). Average values of MAP (Panel B) before and after the injection of prazosin in control and SH groups. * Different within the same group ($P < 0.05$), one-way ANOVA.

submitted SH, we also demonstrated that the major changes in the respiratory pattern of SH mice were accompanied by a reduction in thoracic sympathetic nerve (tSN) activity during the final expiration (E2) and a large increase in cervical vagus (cVN) activity during the post-inspiration (Post-I) (Rodrigues et al., 2021). The augmented cVN activity during this phase of the respiratory cycle is an indicative of its overall activity mainly to the motor innervation of the upper airways muscles, which correlates with an increased parasympathetic drive to the smooth muscle to modulate the resistance of the upper airways in response to hypoxic challenges. Therefore, the observed increase in the cVN does not imply that the increased activity of this nerve is also linked to the parasympathetic branch to the heart. For this reason, the present

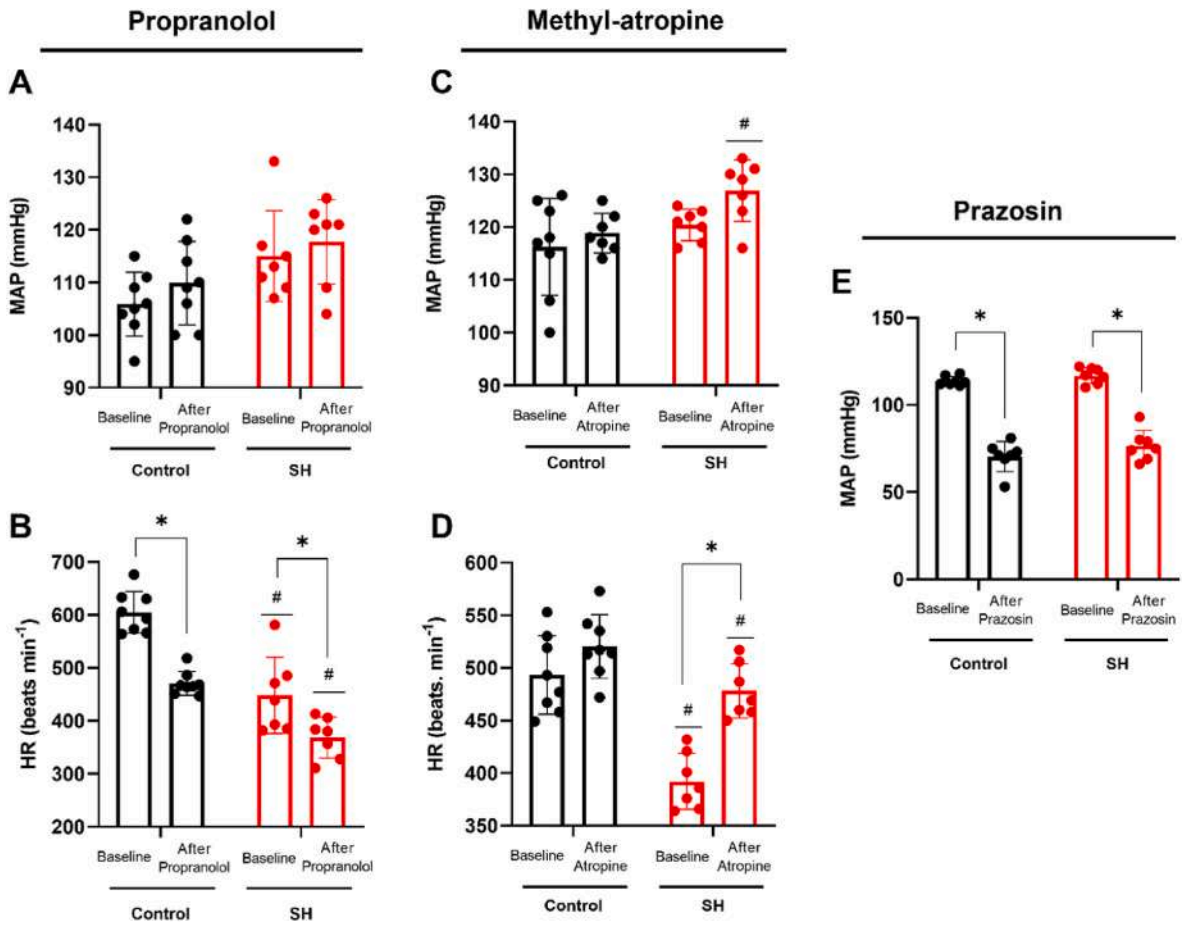

Fig. 5. Changes in the cardiovascular parameters in response to the sequential antagonism of the of the autonomic nervous system to the heart (sympathetic followed by parasympathetic) and to the antagonism of the sympathetic vasomotor drive in mice from control (n = 8) and SH (n = 7) groups. Average values of MAP (Panel A) and HR (Panel B) before and after the injection of propranolol in control and SH groups. Average values of MAP (Panel C) and HR (Panel D) before and after the injection of methyl-atropine in control and SH groups. Average values of MAP (Panel D) before and after the injection of prazosin in control and SH groups. * Different within the same group ($P < 0.05$), one-way ANOVA. #Different from control group ($P < 0.05$), two-way ANOVA.

study was performed to characterize the autonomic profile of conscious freely moving mice previously submitted to SH.

It is important to note that juvenile rats submitted to the same protocol of SH presented different autonomic changes in comparison to those that we are describing in mice (Moraes et al., 2014; Accorsi--Mendonça et al., 2015; Machado et al., 2017). Rats submitted to SH develop hypertension which was associated with changes in sympathetic-respiratory coupling accompanied by sympathetic overactivity (Moraes et al., 2014). The baseline heart rate of conscious freely moving rats submitted to SH was not different in relation to the control group, suggesting that the cardiac vagal tone to the heart was not altered. In addition, using the *in situ* WHBP of rats it was observed a significant reduction in the amplitude of the vagus nerve activity during the post-inspiration phase of the respiratory cycle after SH, while in mice submitted to SH the cVN activity was significantly increased (Rodrigues et al., 2021).

Our previous studies allowed us to suggest that SH in mice may induce an autonomic imbalance in favor of the parasympathetic component, probably due to major changes in the respiratory pattern. Those findings also allowed us to suggest that this autonomic imbalance in favor of the parasympathetic component contributes to preventing the development of hypertension in mice. However, the impact of the significant reduction in the baseline HR and the sympathetic drive to the vascular resistance in SH conscious freely moving mice were also not evaluated before.

The data of the present study obtained with the two sequences of autonomic antagonism to the heart (methyl-atropine followed by propranolol vs propranolol followed by methyl-atropine) are showing that after blocking the parasympathetic component to the heart, the magnitude of the HR increase was significantly greater in SH than in control mice, a finding that supports our concept that SH in mice produces an autonomic imbalance favoring the parasympathetic tone to the heart. In relation to the changes in MAP in response to methyl-atropine, in the protocol in which methyl-atropine was administrated first, we observed no significant change in the baseline MAP in both control and SH mice. The lack of major changes in baseline MAP after methyl-atropine is probably due to high baseline HR after the removal of the parasympathetic tone and the prevalence of sympathetic tone to the heart. These changes contribute to a shortening in diastole duration with a consequent reduction in the time of ventricular filling and stroke volume. This possibility is supported by a previous study by Janssen et al. (2002), in which they used miniaturized electromagnetic and transit-time flow probes and demonstrated that in mice the fluctuations in cardiac output are due to changes in stroke volume rather than in HR and also that the final diastolic volume progressively decreased with increasing HR in mice. In the second protocol (propranolol followed by methyl-atropine) we observed a significant increase in MAP after methyl-atropine in SH in comparison with control mice. This increase in MAP is probably related to the lack of the sympathetic drive to the heart after propranolol, preventing major changes in diastole duration and allowing an increase in cardiac output and consequently in MAP.

The data of the present study using both protocols are showing that

the tachycardia observed after the blockade of the parasympathetic tone to the heart produced no significant increase (ΔMAP) in the baseline MAP in SH mice, indicating that the lack of hypertension in mice submitted to SH was not due to the observed increase in the parasympathetic drive to the heart. In relation to the sympathetic tone to the heart, using the two sequences of autonomic antagonism to the heart (methyl-atropine followed by propranolol vs propranolol followed by methyl-atropine), we observed similar changes in HR in response to the blockade of the sympathetic tone to the heart with propranolol in both control and SH mice, indicating that SH produced no major changes in the sympathetic drive to the heart.

The intrinsic heart rate observed after the double antagonism to the heart (frequency discharge of the cardiac pacemaker without the inputs from the autonomic nervous system) in both experimental protocols (methyl-atropine followed by propranolol *vs* propranolol followed by methyl-atropine) in the two groups of control mice was lower than the baseline HR. This indicates that in control mice the high baseline HR (602 ± 37 beats. min^{-1}) is driven by the sympathetic tone. In mice submitted to SH the intrinsic heart rate accessed after the sequential antagonism with propranolol followed by methyl-atropine was in the same range of the baseline HR (448 ± 72 vs 478 ± 26 beats. min^{-1}), indicating that the cardiac pacemaker is under continuous influence of the parasympathetic tone.

To indirectly evaluate the possible changes in the sympathetic drive to the vessels regulating the vascular resistance in SH mice, we blocked alpha-1-adrenergic receptors with prazosin. The data are showing that the fall in MAP in both control and SH mice were similar indicating that the sympathetic vasomotor tone was not increased in SH mice, which corroborates with the possibility explored above that the lack of hypertension in SH mice is probably mainly due to the absence of an increase in the sympathetic activity rather than an increase in the parasympathetic drive to the heart. This possibility is supported by our previous study documenting that in the *in situ* preparation of mice the baseline sympathetic activity is reduced and also that the sympathetic-respiratory coupling during the late expiratory phase of the respiratory cycle is not observed in these rodents after SH (Rodrigues et al., 2021).

The main finding of this study indicates that mice submitted to SH present an autonomic imbalance in favor of the parasympathetic tone to the heart, which seems to be related to changes previously observed in the respiratory pattern of SH mice. The significant reduction in the baseline HR apparently is not the cause of the lack of hypertension in mice submitted to SH because the blockade of the parasympathetic tone to the heart produced no major changes in the baseline MAP. On the other hand, the blockade of the sympathetic drive to the resistance vessels produced similar changes in the baseline MAP in control and SH mice, indicating that the sympathetic activity is not increased in SH mice. These findings open interesting possibilities for new studies with the purpose of understanding why mice, different from rats, do not present a sympathetic-respiratory coupling and increase in the baseline sympathetic activity in response to the experimental model of sustained hypoxia. It is important to note that in this study we used only male mice, but further experiments in female mice are required to verify some possible sex differences in the changes in the autonomic balance to the heart in response to SH, which was previously observed in female rats submitted to chronic intermittent hypoxia (Souza et al., 2016).

CRediT authorship contribution statement

Juliana R. Souza: Designed the research, Performed the experiments, Analyzed the data, Wrote the manuscript. **Mauro de Oliveira:** Performed the experiments. **Benedito H. Machado:** Designed the research, Wrote the manuscript, All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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