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Stress-associated cardiovascular reaction masks heart rate dependence on physical load in mice

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HIGHLIGHTS

- Blood pressure and heart rate were recorded in behaviour and functional tests in mice.
- Manipulations induce acute stress reaction in mice that are not subject to habituation.
- Exercise-induced heart rate increase is masked with stress in the treadmill test.
- It is preferable to measure heart rate during wheel-running in home cage in mice.

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ABSTRACT

When tested on the treadmill mice do not display a graded increase of heart rate (HR), but rather a sharp shift of cardiovascular indices to high levels at the onset of locomotion. We hypothesized that under test conditions cardiovascular reaction to physical load in mice is masked with stress-associated HR increase.

To test this hypothesis we monitored mean arterial pressure (MAP) and heart rate in C57BL/6 mice after exposure to stressful stimuli, during spontaneous locomotion in the open-field test, treadmill running or running in a wheel installed in the home cage. Mice were treated with β_1-adrenoblocker atenolol (2 mg/kg ip, A), cholinolytic ipratropium bromide (2 mg/kg ip, I), combination of blockers (A + I), anxiolytic diazepam (5 mg/kg ip, D) or saline (control trials, SAL).

MAP and HR in mice increased sharply after handling, despite 3 weeks of habituation to the procedure. Under stressful conditions of open field test cardiovascular parameters in mice were elevated and did not depend on movement speed. HR values did not differ in I and SAL groups and were reduced with A or A + I. HR was lower at rest in D pretreated mice. In the treadmill test HR increase over speeds of 6, 12 and 18 m/min was roughly 1/7–1/10 of HR increase observed after placing the mice on the treadmill. HR could not be increased with cholinolytic (I), but was reduced after sympatholytic (A) or A + I treatment. Anxiolytic (D) reduced heart rate at lower speeds of movement and its overall effect was to unmask the dependency of HR on running speed. During voluntary running in non-stressful conditions of the home cage HR in mice linearly increased with increasing running speeds.

We conclude that in test situations cardiovascular reactions in mice are governed predominantly by stress-associated sympathetic activation, rendering efforts to evaluate HR and MAP reactions to workload unreliable.

1. Introduction

The mouse is one of the best-studied experimental animal species and is widely used for genetic and molecular studies. Moreover, genetic manipulations are now routinely used to investigate molecular mechanisms of various processes. Protocols for standardized evaluation of phenotype changes induced with a given mutation have been developed [1,2]. However, surprisingly few publications compared to the number of genetic models deal with the cardiovascular system, and when they do, the estimates are often limited to diurnal changes in blood pressure/heart rate [3–6].

One of the standard methods to estimate cardiovascular function is to record haemodynamic parameters during physical exercise. It is widely employed in humans and in a wide range of animal species.

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However, this approach is rarely used in mice, and a brief overview of the results obtained with this technique shows that mice do not display a graded increase of heart rate during exercise, but rather a sharp shift of cardiovascular indices to extremely high levels at the onset of locomotion [7,8]. This "yes or no" response is still more surprising when data for other systems responding to physical load are considered. For instance, oxygen consumption, the other classic correlate of increased metabolic demand during physical activity is responding to increase in running speed gradually and the resulting curves in mice are similar to those in any other species [9].

At the same time cardiovascular parameters along with body temperature are often recorded during behavioural tests in mice and rats as an estimate of stress-induced sympathetic activation. Mice respond to virtually any kind of stressor (cage rattling, cage switch, handling, open field test etc.) with a pronounced and long lasting increase in heart rate, blood pressure and body temperature [10,11]. Noticeably, heart rate values after exposure to stress are virtually the same as values during exercise.

We hypothesized that when standard test procedures are used in mice, cardiovascular reaction to physical load is masked with stress-associated reaction. To test this hypothesis we monitored cardiovascular parameters in C57BL/6 mice after exposure to stressful stimuli, during spontaneous locomotion in the open-field test, treadmill running and running in a wheel installed in the home cage. We used a miniature implantable telemetry probe to record blood pressure and heart rate and pharmacological interventions to analyse the mechanisms underlying cardiovascular responses.

2. Methods

2.1. Animals

Male C57BL/6 mice (Animal Breeding Facility — Branch of Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry) weighing 22–25 g were used in the experiments (n = 15). Throughout the experiments mice were housed individually under controlled environmental conditions (22 ± 1 °C, relative humidity 60 ± 20%, 12-h light cycle with light on at 9:00 a.m., food and water were supplied ad libitum). Individual housing was chosen to exclude possible interference from social interactions.

The design of the experiment was approved by the Ethical Committee of Institute for Biomedical Problems, Russian Academy of Science. The study conformed with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

2.2. Implantation of blood pressure transmitters

Arterial pressure was monitored using implantable probes and telemetry hardware (Data Science International, USA). Miniature probes (model PA-C10) weighing 1.4 g were implanted 3 weeks before the start of the experiments following the manufacturer's instructions. The catheter of the probe was implanted into the left carotid so that its tip just reached the aorta. The body of the transmitter was implanted subcutaneously on the flank.

Briefly, mice were anaesthetised with tiletamine/zolazepam 15 mg/kg each, ip, combined with xylazine, 3 mg/kg, ip. The depth of anaesthesia was assessed regularly by hind leg pinch and observation of respiration rate. In case the animal responded to pinch or an increase in respiration rate was recorded additional dose of the mixture (about 20% of the initial dose) was given intravenously. A midline incision was made on the neck. The carotid artery was separated and ligated 1–2 mm cranially to bifurcation. A second ligature was used to suspend the artery 4–5 mm proximal to the site of ligation. Through a small incision in the artery wall the catheter was advanced 6–7 mm towards the aorta and fixed in place with sutures and acrylic glue. A subcutaneous pocket on the flank of the animal was made with blunt scissors and the transmitter was inserted there with a small haemostat. The catheter and the transmitter were fixed in place with acrylic glue and the skin incision closed with absorbable sutures.

During the recovery period mice received ibuprofen 4 mg/ml and co-trimoxazole 4 mg/ml in drinking water. After 3 to 4 days mice with transmitters regained normal activity levels and the mixture was replaced with water. Body weight of operated mice was measured daily after surgery and they were considered fully recovered when weight returned to pre-surgery values (7–10 days after the intervention). Arterial pressure signal was checked and 10 mice with good BP signal (out of 12 operated) were chosen for the experiments.

2.3. Haemodynamic data handling

Blood pressure data were analysed to determine mean blood pressure and heart rate using custom software developed for Matlab. The procedure consisted of peak identification and beat-to-beat calculation of mean blood pressure and heart rate. After that mean blood pressure and heart rate for 1 s intervals were calculated. These 1 s averaged data were used as an input for further analysis.

2.4. Pharmacological treatments

The following chemicals were used: atenolol (Sigma), ipratropium bromide (Ipratropium STERI-NEB, IVAX Pharmaceuticals), diazepam (Relanium, Polfa Warszawa S.A.), tiletamine/zolazepam (Zoletil, Virbac), xylazine (Interchemie), ibuprofen (Nurofen, Reckitt Benckiser), and co-trixomazole (Bactrim, Roche).

Atenolol is a cardioselective beta-1 adrenergic blocker and was used to abolish sympathetic influence on the heart rate in the dose of 2 mg/kg [12]. In this dose the drug has little effect on mice behaviour [13]. Ipratropium bromide is a muscarinic antagonist not capable of crossing the blood brain barrier. It is effective in micromolar range of concentrations in vitro [14] and taking into account available pharmacokinetical data a dose of 2 mg/kg was chosen [15]. Diazepam is a benzodiazepine, a positive allosteric modulator of $GABA_A$ receptors by the mechanism of action. It was used to reduce anxiety in mice in the dose of 5 mg/kg [10]. All blockers were delivered intraperitoneally (ip) as an aqueous solution (injection volume — 10 ml/kg).

2.5. Preliminary experiment

In a separate preliminary experiment the effects of the pharmacological blockade of sympathetic and parasympathetic inputs to the heart or anxiolytic treatment were evaluated. All drugs were delivered intraperitoneally and the procedure of injection itself was the stressful stimulus. Effectiveness of the doses and time course of the drugs action were verified in this experiment.

After 30 min of recording cardiovascular parameters at rest, mice were injected intraperitoneally with saline, diazepam (2 mg/kg), atenolol (2 mg/kg), ipratropium bromide (2 mg/kg) or combination of atenolol and ipratropium. Following injection mice were promptly returned to their home cage and cardiovascular parameters were monitored for 90 min. Each of the mice received all treatments in a randomized manner, with 2 days between the tests for washout of the drugs.

2.6. Experiment 1: repeated handling

Repeated handling was chosen as a simple way to induce stress reaction in mice. Two ways of handling, each known to affect mice behaviour in the open-field test differently were chosen: handling by the base of the tail or in a paper tube (that was provided in home cage for environment enrichment). Handling sessions were repeated for 7 consecutive days. Blood pressure was monitored for 30 min in a quiet environment before handling to obtain values at rest. After that, taking care