Behavioural Brain Research 226 (2012) 404–410

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Role of lateral parabrachial opioid receptors in exercise-induced modulation of the hypotensive hemorrhage response in conscious male rats

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ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form 2 September 2011
Accepted 26 September 2011
Available online 1 October 2011

Keywords:
Voluntary exercise
Dorsolateral pons
Hemorrhage
Hypotension
Opioids
Exercise training
Dorsolateral pons

ABSTRACT

Some of the benefits of exercise appear to be mediated through modulation of neuronal excitability in central autonomic control circuits. Previously, we identified that six weeks of voluntary wheel running had a protective effect during hemorrhage (HEM), limiting both the hypotensive phase of HEM and enhancing recovery. The present study was undertaken to evaluate the role of opioid release in the lateral parabrachial nucleus (LPBN) on the response to severe HEM in chronically exercised (EX, voluntary) versus sedentary (SED) controls. Male Sprague Dawley rats were allowed either free access to running wheels (EX) or normal cage conditions (SED). After 6 weeks of "training" animals were instrumented with a bilateral cannula directed toward the dorsolateral pons and arterial catheters. After a recovery period, animals underwent central microinjection of either vehicle (VEH; $n = 3$/group) or the opioid receptor antagonist naloxone (NAL; $n = 6$/group) followed by withdrawal of 30% of their total estimated blood volume. Following VEH injection, the drop in MAP during and following HEM was significantly attenuated in the EX vs SED animals. Alternatively, NAL microinjection in the dorsolateral pons (20 μM, 200–500 nl) reversed the beneficial effect of EX on the HEM response. NAL microinjection in SED rats did not significantly alter the response to HEM. These data suggest chronic voluntary EX has a beneficial effect on the autonomic response to severe HEM which is mediated, in part, via EX-induced plasticity of the opioid system within the dorsolateral pons.

1. Introduction

Exercise has been shown to ameliorate a host of cardiovascular pathologies including hypertension [29,61], heart failure [5,18], and a number of other diseases in which there is marked autonomic dysregulation [24,59,64]. The peripheral effects of exercise are well documented [5,8,45] and constitute a large portion of the exercise literature; however recent work has begun identifying centrally mediated adaptations that also contribute to enhanced health outcomes associated with chronic exercise [4,40,52,53,67]. In particular, recent data suggest that some of the benefits of exercise may be mediated through a modulation of central sympathoexcitatory circuits [4,40,50,52,53,67]. Interestingly, some of these same central sites have also been identified to be involved in mediating autonomic changes during severe hemorrhage (HEM) [11,15,36,38,43,44,55,65]. Accordingly, in a recent study we identified that chronic voluntary exercise limits the hypotensive phase of HEM and enhances recovery from blood loss in conscious male rats [1]. This effect of exercise on the HEM response was coupled with indicators of both altered sympathovagal drive and neuronal activity (c-fos). Of the regions examined, the primary exercise-induced change in neuronal activity was localized to the lateral parabrachial nucleus (LPBN).

The LPBN is located in the dorsolateral pons and is interconnected with forebrain regions that regulate volume control and electrolyte balance (e.g., paraventricular nucleus of the hypothalamus, central nucleus of the amygdala and median preoptic nucleus [6,17,41,42]) as well as hindbrain regions that receive and relay baroreceptor and blood volume information (e.g., nucleus of the solitary tract [31]). Thus, the LPBN is ideally situated to act as an integration site for monitoring and influencing blood volume regulation. Previous studies have identified that blockade or lesion of the LPBN markedly alters the cardiovascular response to severe HEM [7,12,14,35,39].

The central release of opioids has been shown to play a critical role in the cardiovascular response to HEM in multiple regions of the brain [13,25,26,30]. Previous neuroatnaomical studies have identified that the LPBN contains large amounts of opioid receptors [16,49]. The impact of modulation of opioid receptors in the LPBN on the cardiovascular response to HEM, however, has not been previously evaluated in either sedentary or chronically exercised rats. Nonetheless, manipulation of these receptors within the LPBN has

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doi:10.1016/j.bbr.2011.09.036

been linked to increased salt intake [19], suggesting that one role of opioid release in the LPBN may be to modulate behavioral and possibly autonomic responses to blood volume perturbations. Thus, based the potential role for opioids in the LPBN in volume regulation and data suggesting that one impact of chronic exercise on central sensory processing may be mediated via an up-regulation of brainstem opioid levels [60], the present study was undertaken to test the hypothesis that the protective effect of exercise training during HEM is mediated by activation of opioid receptors in the LPBN.

2. Methods

2.1. General preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida. Male Sprague-Dawley rats (175–200 g, Harlan Industries, Minneapolis, IN) were randomly placed into one of two groups: exercise (EX) or sedentary (SED). All animals were pair-housed for six weeks in cages that did (EX) or did not (SED) contain a running wheel (Lafayette Instruments, IN). Animals were lightly handled and weighed weekly and were maintained in a 12 h lights on:12 h lights off, temperature controlled environment with food and water ad libitum. Following six weeks of "training" (EX or SED), animals underwent surgery for chronic instrumentation.

Both EX and SED animals underwent two recovery surgeries separated by 5–7 days. For the first surgery, animals were anesthetized (isofluorane anesthesia; 4% → 2–2.5%), secured into a stereotaxic head holder (Kopf Instruments, Tejunja, CA, USA) and then underwent stereotaxic implantation of bilateral stainless steel, 23-gauge guide cannulae (Plastics One, Roanoke, VA, USA) positioned into the brain using the following coordinates: 9.3 mm caudal to bregma, 2.1 mm lateral to midline. Each guide cannula had a 5.5 mm ventral projection such that the tip of each cannula was positioned approximately 1.5 mm dorsal to the LPBN. Cannulae were fixed to the cranium with dental acrylic resin and a jeweler's screw. A stainless steel, 30-gauge dummy cannula was then inserted into each guide (Plastics One). The skin incision was sutured closed and a topical antibiotic was applied. Following removal from the head holder, animals received subcutaneous injections of sterile saline (1 ml 0.09% NaCl, for rehydration) and analgesics (Buprenorphine, 0.1 ml/kg; Rimadyl, 0.1 ml/kg). Following recovery from surgery, animals were singly housed and allowed 5–7 days of recovery. EX animals were allowed access to a running wheel during this recovery time.

Two days prior to the experiment, animals were re-anesthetized (isofluorane, 4 → 2–2.5%) and surgically instrumented with bilateral femoral arterial catheters, given analgesics and allowed 2 days of recovery, including one day of acclimation to the recording chamber. For the EX animals, running wheels were locked the day before the experiment to eliminate any confounding short-term effects of exercise.

2.2. Experimental protocol

On the day of the experiment, each animal was brought to the lab, weighed, and both arterial catheters were connected to additional heparinized saline-filled tubing (10–50 IU/ml; PE-50). Each animal was then placed in the testing chamber (open bucket, ~12 in diameter) unrestrained. One of the arterial catheters was connected to a calibrated pressure transducer in-series with an amplifier (Stoelting, Wooddale, IL) for continuous monitoring of pulsatile arterial pressure (AP) before, during and after HEM. The other catheter was used for time blood loss.

Both pulsatile AP and mean AP (MAP) were recorded on-line at 100 Hz using a Cambridge Electronics Design computer interface and Spike2 data software. HR was derived on-line from the interval between peak systolic pressure waves in the AP trace. Animals were then randomly assigned to receive a 200–500 nl bilateral central microinjection of either the opioid receptor antagonist naloxone (NAL; 20 μM in 0.09% NaCl) or vehicle (VEH; 0.09% NaCl) into the LPBN just prior to HEM. Following 60 min of baseline recording of AP, MAP and HR in the experimental testing chamber, during which the animal was undisturbed in order to ensure a stable baseline measurement, bilateral injections into the LPBN were made using a 10 μl Hamilton syringe connected to the internal cannula (1.5 mm longer than the guide cannulas) with polyethylene tubing (50-PE → 10-PE) while the animals remained unrestrained in the recording chamber. Either NAL or VEH was first microinjected into one side of the LPBN over a 1 min period. The internal cannula remained in place for another 1 min following injection prior to removing the internal cannula and repositioning it on the contralateral side and repeating the process. The animal was then left undisturbed for 10 min. Next, all animals underwent the withdrawal of 30% of the estimated TBV over 15 min. As previously described, this volume of blood loss at this rate is sufficient to elicit both sympathoexcitatory and sympathoinhibitory phases of HEM [22,62] and results from our lab have previously shown that 6 weeks of EX significantly attenuates the sympathoinhibitory phase [1,2]. MAP, AP and HR were continuously recorded before during and for 45 min following the offset of HEM. Animals were then euthanized with a lethal dose of sodium pentobarbital (100–150 ml/kg). The same internal cannula was then used to inject a fluorescent marker (2% Fluorogold) into the brain for verification of cannula placement. Brains were then removed and allowed to sit in 4% paraformaldehyde for at least 24 h prior to being sectioned with a cryostat, slide-mounted, and evaluated for accuracy of injections.

2.3. Data analysis

Following recovery of the brain injection sites it was identified that the responses obtained by either bilateral or unilateral NAL injections ($n = 3$/group) in the LPBN were not significantly different for MAP ($p > 0.43$) or HR ($p > 0.41$), therefore, all data from experiments in which at least one NAL injection was correctly placed into the LPBN were grouped for statistical analyses. VEH injections included 3 successful unilateral injections in the LPBN of EX animals and 3 successful injections (1 bilateral and 2 unilateral) in the SED group. In the 6 additional instances, neither of the bilateral injections of NAL or VEH was identified to be within the boundaries of the LPBN (see Fig. 1B for example). Data from these animals were excluded from further analysis.

To evaluate the effects of microinjection MAP and HR were averaged over 1 min intervals prior to the first brain injection (PRE), 1 min following the second brain injection (POST, after removal of the internal cannula), and every 5 min from the onset of HEM (min 0) through the end of recovery (min 60). Within experimental groups, a one-way ANOVA was used to determine if there were any significant differences in baseline MAP, HR or body weight prior to the onset of the experiment. A two-way ANOVA with repeated measures was then used to identify the effects of experimental treatment (EX vs. SED or NAL vs. VEH) on MAP and HR over time (every minute: −1 through 30, and 60). When indicated, Student–Newman–Kuels post-hoc analysis or multiple paired t-tests with a Bonferonni adjustment for number of comparisons were used to isolate differences relative to baseline (POST) within treatment groups or between treatment groups at specific time points (delta MAP and HR). Differences were considered significant when $p < 0.05$ for all statistical analyses performed. All data were averaged and reported as the mean ± SEM.

3. Results

The average distance run per week per rat by the EX group progressed from 1.8 ± 0.08 km/day after week 1 to 6.2 ± 0.47 km/day at week 6 (similar to the results of our previous study [1]) and the level of running appeared to plateau between weeks 4 and 6. The average body weight of the SED animals ($n = 12$) at the time of experimentation was slightly higher than the age matched EX animals ($n = 12$) (346 ± 9 g vs 321 ± 6 g, respectively) and this difference was significant ($p = 0.03$). There was however no significant difference in baseline MAP (115 ± 2 mmHg vs 118 ± 3 mmHg, SED vs EX, respectively) or HR (353 ± 5 bpm vs 359 ± 7 bpm, SED vs EX, respectively) prior to central microinjection.

3.1. Effect of VEH microinjection in the dorsolateral pons on the cardiovascular response to HEM

Fig. 1A shows photomicrograph of a brain slice taken at the level of a recovered microinjection site and Fig. 1B demonstrates the reconstructed location of the best unilateral microinjection sites recovered from all SED (left side) and EX (right side) for both VEH and NAL injections. Six of the 24 injections were identified to be outside the boundaries of the LPBN and (asterisks in Fig. 1B) and data was not included in subsequent analysis.

Fig. 2A shows the averaged hemodynamic response to VEH microinjection in the LPBN before and during severe HEM in SED ($n = 3$) vs EX ($n = 3$) rats. Prior to HEM, VEH injections induced a significant increase in both MAP ($p < 0.01$) and HR ($p < 0.04$) independent of treatment group (EX vs SED; $p > 0.28$). The average increase in HR following central microinjection was 35 ± 11 bpm and the average increase in resting MAP was 10 ± 2 mmHg. For both EX and SED groups, at the onset of HEM, HR rose initially, followed by a precipitous fall between min 10 and 15 of HEM. The fall in HR was paralleled by a decrease in MAP that was less pronounced in the EX animals. Following the offset of HEM, both MAP and HR increased progressively, but recovery to baseline was more complete in the EX group. A two-way ANOVA with repeated measures identified a significant effect of time ($p < 0.001$) for both MAP and HR, a significant effect of treatment (EX vs SED; $p = 0.05$) and a