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ROLE OF LATERAL PARABRACHIAL OPIOID RECEPTORS IN EXERCISE-INDUCED MODULATION OF THE HYPOTENSIVE HEMORRHAGE RESPONSE IN CONSCIOUS MALE RATS

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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 (20µM, 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.

Keywords

voluntary exercise; exercise training; dorsolateral pons; hemorrhage; hypotension; opioids

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AUTHOR CONTRIBUTION STATEMENT

All experiments were performed in Dr. Hayward’s cardiovascular research lab at the University of Florida. Both Dr. Hayward and Dr. Ahlgren contributed equally to the conception and design of the experiments. Dr. Ahlgren was the primary individual involved in the collection and analysis of the data and both Drs. Hayward and Ahlgren were involved equally in the interpretation and writing of the manuscript. Both authors have read the current version submitted for publication and approve of this manuscript and its content.

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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 neuroanatomical 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 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.

METHODS

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 hour lights on: 12 hour 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: 9.3mm caudal to bregma, 2.1mm lateral to midline. Each guide cannula had a 5.5mm ventral projection such that the tip of each cannula was positioned approximately 1.5mm 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 (1ml 0.09% NaCl, for rehydration) and analgesics (Buprenorphine, 0.1ml/kg; Rimadyl, 0.1ml/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.

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 μ M 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 μ l Hamilton syringe connected to the internal cannula (1.5mm 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 (100–150ml/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 hours prior to being sectioned with a cryostat, slide-mounted, and evaluated for accuracy of injections.

Data Analysis

Following recovery of the brain injection sites it was identified that the responses obtained by either bilateral or unilateral NAL injections ($n=3/\text{group}$) 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 Bonferroni 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 $\pm$ SEM.

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 week 4–6. 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 vs 321 ± 6 gm, respectively) and this difference was significant ($p=0.03$). There was however no significant difference in baseline MAP (115 ± 2 vs 118 ± 3 mmHg, SED vs EX, respectively) or HR (353 ± 5 vs 359 ± 7 bpm, SED vs EX, respectively) prior to central microinjection.

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

Figure 1A shows photomicrograph of a brain slice taken at the level of a recovered microinjection site and Figure 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.

Figure 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 significant interaction between time and treatment for MAP following VEH injection ($p=0.01$). No significant difference in HR between EX and SED animals was identified ($p=0.25$). Subsequent analysis of the change in MAP during and following HEM relative to post-injection at selected time points (see Fig 2B) identified that the change in MAP between SED vs EX was significantly different at min 60 ($p=0.03$) and showed a strong trend towards significance at 15 min ($p=0.07$). No difference in the change in HR during or following HEM between SED vs EX however was identified at any time point ($p=0.82$).

Effect of NAL microinjection in the LPBN on the cardiovascular response to HEM

Figure 3 shows the averaged hemodynamic effects of VEH ($n=3$ /group) vs NAL ($n=6$ /group) microinjection in LPBN of EX (Fig 3A) and SED (Fig 3B) animals on the response to HEM. Similar to VEH, the process associated with NAL microinjection induced a significant increase in both MAP ($P<0.002$) and HR ($P<0.03$) independent of treatment group (EX vs SED; $P>0.4$). The average increase in resting HR following injection was 35 ± 14 bpm and the average increase in resting MAP was 12 ± 3 mmHg. Following central microinjection of NAL in EX animals (see Fig. 3A) the response to HEM was significantly altered compared to VEH microinjection, including a significant effect of time ($p<0.0001$), treatment ($p<0.02$) and an interaction between time and treatment ($p<0.02$) for MAP. Analysis of HR identified a significant effect of time ($p<0.0001$) and a strong trend for a treatment effect between VEH and NAL ($p=0.09$) in the EX group. In contrast, although a significant effect of time was identified ($p<0.01$), no significant treatment effect of VEH vs NAL microinjection in SED animals for either MAP ($p=0.7$) or HR ($p=0.8$) was identified (see Fig 3B).

Comparison of the change in MAP at min 15, 30 and 60 following central microinjection of VEH vs NAL in the EX animals identified a significant difference at min 15 ($p<0.0007$), min 30 ($p=0.02$) and a strong trend for a significant difference in the change in MAP at min 60 ($p=0.06$) following the onset of HEM (see Fig 4A). No significant difference however in the change in HR between VEH vs NAL treatment in the EX animals was identified ($p>0.2$; data not shown). A similar comparison of the change in MAP or HR at 15, 30 and 60 min following central microinjection of VEH vs NAL in SED animals did not identify any significant differences ($p>0.4$; data not shown). Finally, a comparison was made between the change in MAP in response to HEM following NAL microinjection in the LPBN of SED vs EX rats was made. As shown in Fig 4b, following NAL microinjection in the LPBN no significant differences between EX versus SED animals was identified ($p>0.3$; for comparison see Fig. 2B).

DISCUSSION

The major novel finding of the present set of experiments is that exercise-induced tolerance to severe HEM can be reversed by central blockade of opioid receptors in the dorsolateral pons. Interestingly, NAL microinjection in SED animals had no effect on the autonomic response to HEM. This observation supports numerous other studies which have demonstrated that in certain brain regions, local injection of NAL may induce little effect in SED animals but alters response characteristics in EX animals (28, 48, 58, 63). The results

of this study confirm previous observations that suggest that even modest daily activity may help attenuate cardiovascular consequences associated with hypovolemic shock (1, 10) and thus potentially improve survival outcome in trauma situations.

Effect of voluntary exercise on cardiovascular response to HEM

During HEM, the initial response or compensatory phase is primarily mediated by baroreflex adjustments in sympathetic and parasympathetic drive. Evidence from other studies has identified that exercise training can enhance baroreflex function (10), and this effect appears to be particularly significant for the baroreceptor unloading part of the baroreflex curve. In the present study (VEH treated EX animals) and in a previous study from our lab (1) we did not identify strong evidence that the compensatory phase of HEM was significantly altered by chronic voluntary EX. Indeed, in both studies, the offset of the compensatory phase of HEM appeared to occur at exactly the same time for both SED and EX animals. These results suggest that any effect of voluntary EX training on baroreflex function may only be modest at best in modulating the cardiovascular response to mild to moderate hypovolemia.

Following the compensatory phase, if blood loss continues, HEM is characterized by a second phase, the decompensatory or sympathoinhibitory phase. In both the present study (VEH treated animals) and our previous study (1), it was identified that EX training had a significant effect on this phase of HEM, including a significant attenuation of the drop in MAP. Finally, once blood loss was terminated, a recovery phase was initiated, including a progressive increase in both MAP and HR relative to nadir values. In the present study, EX training also had a significant effect on the recovery phase of HEM in VEH treated animals, and significantly minimized the drop in MAP from baseline at 15 and 45 min following the offset of HEM relative to SED animals. Thus, confirming our previous findings that voluntary EX training appears to attenuate both the sympathoinhibitory phase during HEM and augment the recovery phase following the offset of HEM.

Opioid blockade in the dorsolateral pons

Our results from NAL microinjections suggest that the selective effects of EX on HEM may be mediated in part by an increase in opioid receptor sensitivity or opioid release in the dorsolateral pons. Previous literature has shown that opioids are involved in the onset of HEM-induced sympatho-inhibition in multiple regions of the CNS. In the periaqueductal grey, for example, delta-opioid receptor antagonism prevents the sympathoinhibitory phase of HEM in conscious rabbits (46). Administration of μ -opioid (21) and kappa-opioid (20) receptor antagonists into the 4th ventricle also prevent the onset of hemorrhagic hypotension in conscious rabbits, suggesting the possibility that more than a single type of opioid receptor is involved in mediating the reflex bradycardia and hypotension during severe HEM. In rats, blockade of both delta- and μ -opioid receptors within the brainstem have been shown to modulate the sympathoinhibition response to HEM (3). Since chronically exercised individuals have been reported to exhibit decreased sensitivity to opioids (58), we had originally hypothesized that exercise-induced tolerance to severe HEM could, in part, be explained by altered opioid-triggered sympathoinhibition at one or multiple central loci. Our results support this hypothesis but suggest that the altered sensitivity is associated with enhanced opioidergic activation, which in the LPBN translates to increased sympathoexcitation.

Based on previous literature it was anticipated that central microinjection of NAL would have had little or no effect in SED compared to EX groups (28, 48, 58, 63). Indeed, NAL microinjection into the LPBN of SED rats induced no noticeable effects on HR or MAP in response to severe HEM. On the other hand, the training-induced tolerance to HEM was

completely blocked by NAL injected into the LPBN of EX rats. It should be noted that this effect of NAL on the HEM response in EX animals is unlikely to have been the result of drug leakage into the 4th ventricle, as intracisternal NAL injections have been shown to *postpone* or eliminate, not facilitate, the sympathoinhibitory phase of HEM in both conscious sheep (25, 26) and rabbits (20). Additionally, in the conscious rat, systemic administration of NAL is able to reverse HEM-induced hypotension (23) and intrathecal administration of NAL prior to the onset of HEM can totally block HEM-induced sympathoinhibition (3). In the present study we identified the opposite effect when NAL was locally microinjected into the dorsolateral pons of EX animals. Thus the results reported here could not have been due to leakage into the cerebrospinal fluid. In addition, the fact that NAL greatly impacted the EX rats but had no effect at all on SED rats contradicts the hypothesis that a decreased central opioid tone may underlie the tolerance to HEM seen following exercise training. Alternatively, there is some evidence to suggest that acute exercise may enhance central opioid release in the brainstem (9). Thus, one impact of chronic exercise may be to enhance central opioid release in response stress, alter sensory processing and modulate certain aspects of autonomic responses (32, 47) in a potentially beneficial way.

Although the volume of NAL microinjected into the dorsolateral pons was too large to pinpoint the effects to a specific nucleus, our previous results identified selective changes in response to HEM in EX vs SED animals to be localized to the LPBN. In addition to the well-established role of the LPBN in the relay of cardiovascular afferent information to more rostral brain sites (27, 31, 37, 42) and its ability to alter autonomic tone via efferent connections with both forebrain and brainstem nuclei (33, 56, 57), an opioidergic mechanism specifically within the LPBN has been identified to have a significant modulatory effect on the response to perturbations in blood volume. Iwasaki et al, for example, demonstrated that bilateral injection of NAL into the LPBN prior to simulated HEM prevented peripheral vasopressin release (34), suggesting that opioid receptor activation within the LPBN is critical for the humoral response to hypovolemia, as occurs with severe HEM. Although we did not measure circulating vasopressin in the present study, an increase in circulating vasopressin via enhanced opioidergic activity in the EX trained animals could explain our observations. Furthermore, since the majority of evidence suggests that opioid receptor activation in the LPBN (as well as other CNS sites) typically acts to inhibit neuronal activity (49, 66), and we had previously reported reduced c-fos in the LPBN of EX vs SED animals following severe HEM, it seems likely that opioid receptor activation within the LPBN could contribute to enhanced vasopressin release from forebrain regions such as the magnocellular paraventricular nucleus of the hypothalamus via inhibition of tonically inhibitory neurons involved in relaying osmo- or volume information. Additional studies, however, are needed to evaluate HEM induced changes in circulating AVP between the treatment groups (51).

Methodological Considerations—In the present study local microinjection into the dorsolateral pons of either VEH or NAL induced a significant increase in MAP and HR independent of the exercise state of the animal. The similarity in response between groups suggests that this effect was related to the experimental protocol which involved inserting and removing the injection cannula twice in the conscious unrestrained rat. Further studies evaluating molecular changes in opioid expression in the LPBN following EX and HEM and methods which don't stress the animals during local microinjection are needed to further examine the impact of EX on HEM.

Conclusions

The results of this study confirm our previous observation that six weeks of voluntary exercise leads to an enhanced ability to tolerate severe blood loss and we demonstrate for the first time that this EX-induced tolerance to HEM was reversed by chemical blockade of opioid receptors in dorsolateral pons but had no effect of opioids on modulating the HEM response in SED animals was identified. This demonstrates for the first time a centrally mediated exercise-induced tolerance to severe blood loss in the conscious rat that involves an alteration of an opiodergic mechanism within the LPBN. This opiodergic effect was identified by the use of NAL to antagonize opioid receptors. This drug preferentially binds to μ -opioid receptors; but, when used at higher concentrations—such as was utilized in the present study, NAL will also block kappa- and delta opioid receptors. Thus, further studies are needed to fully elucidate the exact group (or groups) of opioid receptors are altered in the dorsolateral pons following chronic exercise. More studies are warranted to elucidate the exact central mechanisms underlying the protection against hypovolemic shock afforded by modest amounts of physical exercise.

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Research Highlights

- LPBN opioid receptor blockade (NAL) reverses the protective effect of voluntary EX on the HEM response.
- NAL microinjection in SED rats did not significantly alter the HEM response.
- Evidence of EX-induced plasticity of the pontine opioid system

Figure 1. Microinjection sites

A. Photomicrograph images (1.25X) of a pontine brain slice showing typical pattern of internal cannula placement and bilateral injection sites. B. Schematic composite of injection sites used in group analyses (modified from Paxinos and Watson(54)). Injections for EX animals are displayed on the left and injections for SED animals are displayed on the right. For simplification, only one injection site per animal is displayed. Dashed lines indicate outline of parabrachial nucleus. Solid black symbols (pentagons) reflect location of naloxone injections localized to the parabrachial nucleus. Red circles identify site of vehicle injections. * indicates injection sites identified outside the parabrachial nucleus in the inferior colliculus not included in analysis. SCP = superior cerebellar peduncle.

Figure 2. Effect of vehicle (VEH) microinjection into the dorsolateral pons on the hemodynamic response to severe HEM in SED vs EX conscious rats

A. 5 min averages of HR and MAP before (pre-drug) and immediately following SHAM microinjection (arrow) and during and after HEM (grey box indicates time of blood withdrawal; min. 0–15). Dashed line indicates average post-injection baseline. B. Average change in HR and MAP at specified time points relative to post-injection baseline during and following HEM. * indicates significant difference between EX and SED responses ($p < 0.05$); # indicates trend for difference between EX and SED ($p = 0.07$).

A. EX

B. SED

Figure 3. Effect of naloxone (NAL) injection into the dorsolateral pons on the hemodynamic response to severe HEM in SED vs EX conscious rats

A. 5 min averages of HR and MAP before (pre-drug) and immediately following NAL microinjection (arrow) and during and after HEM (grey box indicates time of blood withdrawal; min. 0–15). Dashed line indicates average post-injection baseline. B. Average change in HR and MAP at specified time points relative to post-injection baseline during and following HEM. * indicates significant difference between EX and SED responses.

A. NAL vs VEH in EX animals

B. NAL in SED vs EX animals

Figure 4. A comparison of the effect of NAL microinjection into the dorsolateral pons on the hemodynamic response to severe HEM in EX and SED conscious rats

A. Average change in MAP at specified time points relative to post-injection baseline during and following HEM in VEH (n=3) and NAL (n=6) injected EX animal. B. The average change in MAP during and following HEM in NAL microinjection injected EX vs SED animals. * indicates significant difference between NAL and VEH responses ($p < 0.05$); # indicates trend for difference between NAL and VEH ($p = 0.07$).