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Variable role of carotid bodies in cardiovascular responses to exercise, hypoxia and hypercapnia in spontaneously hypertensive rats.

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Key words: peripheral chemoreceptor, selective ablation, blood pressure, exercise, baroreflex,

Key points summary:

Carotid bodies played a critical role in maintaining arterial pressure during hypoxia and this has important implications when considering resection therapy of the carotid body in disease states such as hypertension.

Key points

- Curbing hypertension in patients whether resting or under stress remains a major global health challenge.
- We demonstrated previously the benefits of removing carotid body afferent input into the brain for both alleviating sympathetic overdrive and reducing blood pressure in neurogenic hypertension.
- We describe a new approach in rats for selective ablation of the carotid bodies that spares the functional integrity of the carotid sinus baroreceptors, and demonstrate the importance of the carotid bodies in the haemodynamic response to forced exercise, hypoxia and hypercapnia in conditions of hypertension.
- Selective ablation reduced blood pressure in hypertensive rats and re-set baroreceptor reflex function accordingly; the rises in blood pressure seen during exercise, hypoxia and hypercapnia were unaffected, abolished and augmented, respectively after selective carotid body removal.
- The data suggest that carotid body ablation may trigger potential cardiovascular risks particularly during hypoxia and hypercapnia and that their activity suppression rather than obliteration may be a more effective and safer route to pursue.

Abstract

The carotid body has recently emerged as a promising therapeutic target for treating cardiovascular disease, however the potential impact of carotid bodies removal on the dynamic cardiovascular responses to acute stressors such as exercise, hypoxia and hypercapnia in hypertension is an important safety consideration that has not been studied. We first validated a novel surgical approach to selectively resect the carotid bodies bilaterally (CBR) sparing the carotid sinus baroreflex. Second, we evaluated the impact of CBR on the cardiovascular responses to exercise, hypoxia and hypercapnia in the conscious, chronically instrumented spontaneously hypertensive (SH) rats. Our results confirm that our CBR technique successfully and selectively abolished the chemoreflex, whilst preserving carotid baroreflex function. CBR produced a sustained fall in arterial pressure in the SH rat of ~20 mmHg that persisted across both dark and light phases ($P<0.001$), with baroreflex function curves resetting around lower arterial pressure levels. The cardiovascular and respiratory

responses to moderate forced exercise were similar between CBR and Sham. In contrast, CBR abolished the pressor response to hypoxia seen in Sham animals, although the increases in heart rate and respiration were similar between Sham and CBR groups. Both the pressor and respiratory responses to 7% hypercapnia were augmented after CBR ($P<0.05$) compared to sham. Our finding that the carotid bodies play a critical role in maintaining arterial pressure during hypoxia has important implications when considering resection therapy of the carotid body in disease states such as hypertension as well as heart failure with sleep apnoea.

Introduction

The carotid bodies have recently emerged as a promising therapeutic target for treating hypertension (Paton *et al.*, 2013; Ratcliffe *et al.*, 2014; Narkiewicz *et al.*, 2016; Pijacka *et al.*, 2016) and other cardiovascular diseases such as heart failure (Schultz & Marcus, 2012; Niewinski *et al.*, 2013; Andrade *et al.*, 2015; Niewinski *et al.*, 2017), where the peripheral chemoreceptors exhibit increases in both sensitivity and tonicity (Abdala *et al.*, 2012; McBryde *et al.*, 2013; Pijacka *et al.*, 2016). We, and others, suggest that abnormal chemoreflex activity drives a long-term increase in sympathetic over activity, thus resulting in a chronic, neurally-mediated hypertension (Sinski *et al.*, 2012; McBryde *et al.*, 2013; Moraes *et al.*, 2015; Pijacka *et al.*, 2016). However, the role that the carotid bodies play in mediating the dynamic cardiovascular response to acute stressors such as exercise, hypoxia and hypercapnia has never previously been studied under conditions of hypertension and may have important clinical implications especially if the carotid bodies are targeted surgically.

Exercise presents a major challenge to the cardiovascular system, where a pronounced functional hyperaemia in skeletal muscle vasculature, and subsequent fall in total peripheral resistance (TPR), must be countered by an opposing sympathetically mediated vasoconstriction to maintain or increase arterial pressure in order to avoid compromising organ perfusion (Mitchell *et al.*, 1983). The pressor response to exercise is mediated by activation of the sympathetic nervous system, and has been shown to be augmented in hypertension (Smith *et al.*, 2006; Delaney *et al.*, 2010). However, in hypertensive patients, exercise tolerance has been shown to be reduced by up to 30% vs. age-matched normotensive patients (Lim *et al.*, 1996); this may be a consequence of poor skeletal muscle blood flow due to intense vasoconstriction and/or reduced sympatholysis mediated by release of local metabolites. With the evidence that carotid bodies become more active in exercise (Linton & Band, 1985; Jacobi *et al.*, 1989; Ward, 1994; Paterson, 1996; Chu *et al.*, 2007), they may play an essential role for ensuring that arterial pressure is maintained. Given their tonicity and sensitisation in hypertension (see above) the carotid body reflex vasoconstrictor response may be exaggerated during exercise and offset sympatholysis. We have directly addressed this speculation.

The classical stimulant for the carotid bodies is hypoxia. However, hypoxia also reduces vascular smooth muscle tone, causing a lowering of TPR and reduction in arterial pressure (Kulandavelu *et al.*, 2015). The hypoxia induced reduction in TPR may be opposed by homeostatic-reflexes that increase sympathetic outflow to maintain arterial pressure and preserve organ blood flow; the peripheral chemoreceptors participate in such mediation (Marshall & Metcalfe, 1988; Itskovitz *et al.*, 1991; Stein *et al.*, 1999). Importantly, if the carotid bodies are to be a clinically viable treatment target for cardiovascular disease, an understanding of the ability of the cardiovascular system to cope with hypoxia is highly pertinent, especially given the high prevalence of sleep apnoea in patients with cardiovascular diseases.

Hypoxia alone rarely occurs without concomitant hypercapnia, which also stimulates peripheral chemoreceptors (Pepper *et al.*, 1996; Vidruk *et al.*, 2001). However, hypercapnia also stimulates central chemoreceptors to drive hyperventilation, increased blood pressure and elevated sympathetic activity (Kanbar *et al.*, 2010; Takakura & Moreira, 2011). To study the effect of hypercapnia on the carotid body without co-activation of central chemoreceptors, previous studies have either isolated the carotid body circulation from the cerebral circulation or assessed responses pre- and post- carotid body denervation. The evidence suggests that hypercapnic stimulation of the carotid bodies in rats triggers hyperpnoea (Fiamma *et al.*, 2013), but the sympathoexcitatory and pressor responses evoked by hypercapnia remained unchanged after denervation of carotid bodies in conscious rats suggesting they play little role in mediating these cardiovascular responses (Oikawa *et al.*, 2005; Sabino *et al.*, 2013). We have re-assessed this herein to ensure that appropriate cardiovascular adjustment can be made during hypercapnia after selective carotid body resection.

Based on the cited studies described above, we tested the hypothesis that selective carotid body resection in hypertensive rats would lead to an inability to maintain control of arterial pressure during the stressors of both exercise and hypoxia but not hypercapnia.

Materials and Methods

Ethical approval

All procedures were carried out in accordance to the UK Animals (Scientific Procedures) Act 1986, under licence to the Home Office. All investigators understand the ethical principles under which the *Journal of Physiology* operates, and their work complies with the animal ethics checklist described in Grundy (2015). Experiments were conducted on 16-20 weeks old male spontaneously hypertensive (SH) rats bred within the University of Bristol Animal Services Unit and housed with a 12/12h light (7.00-18.00) /dark (19.00-6.00) period and *ad libitum* access to food and water. In total 29 rats were used.

Validation of selective carotid body resection in the terminally anaesthetised SH rat

These experiments were performed to establish and validate our selective carotid body removal procedure. Rats (n=11) were anaesthetised with intramuscular injections of ketamine (60mg/kg; Vetalar, Zoetis, London, UK) and Medetomidine hydrochloride (250 μg/kg; Domitor, Elanco Animal Health, Hampshire, UK). Anaesthetic depth was assessed by the withdrawal reflex following a pinch to the tail or a hind paw and an additional 1/6 of an initial dose of ketamine/Medetomidine hydrochloride given as needed. The femoral vein was catheterized to allow venous access for drug infusions, and arterial pressure was measured via radio-telemetry (see below). Through a midline neck incision, the common carotid arteries were cleared, and 3.0 silk suture used to allow easy retraction and improve access to the carotid bifurcation. In all animals the aortic depressor nerves were identified, as described previously (Pickering *et al.*, 2008) and sectioned bilaterally (ADNX). Following a 30-minute recovery period to record a stable baseline, the chemoreflex was tested with an i.v. bolus infusion of sodium cyanide (100ul; 0.04%), and baroreflex function assessed using i.v. infusions of vasoactive drugs phenylephrine (0.1 $mg.ml^{-1}$, i.v.) and sodium nitroprusside (0.1 $mg.ml^{-1}$, i.v.) (Sigma-Aldrich Co., Poole, UK) to produce ramp changes in arterial pressure, as described previously (Abdala *et al.*, 2012; McBryde *et al.*, 2013; Lincevicius *et al.*, 2015). Animals were then randomly divided into two groups – Time Control and Carotid Body Resection (CBR). In the CBR group, the carotid body on each side was visualised using a modular routine stereo microscope Leica M80 and surgically removed under x25 magnification, using fine surgical forceps, with care taken to preserve fine branches of the carotid sinus nerve. Chemo- and baro-reflex testing was repeated to verify that CBR was

successful as reflected by an elimination of the chemoreflex evoked pressor response and preserving of the carotid baroreflex response. Finally, we cut the carotid sinus nerves bilaterally (CSNX) to complete a full sino-aortic denervation. Baroreflex testing was then repeated, in order to verify that the prior ADNX procedure had been performed successfully. In the Time Control Group, only ADNX was performed followed by baro- and chemo- receptor reflex testing at time intervals matching those of the study protocols.

Recovery surgical protocols

Rats were implanted with radio-telemetry devices (PA-C40; DSI, USA) to record arterial pressure, and a chronic femoral vein catheter as described previously (Waki *et al.*, 2006; McBryde *et al.*, 2013; Pijacka *et al.*, 2016). Briefly, under ketamine/medetomidine anaesthesia as described above, the arterial pressure catheter tip was inserted into the abdominal aorta below the level of the renal arteries, and secured in place. Non-steroidal anti-inflammatory pain relief was administered pre- and post-operatively (0.004ml/100g of Metacam, Boehringer Ingelheim, Germany). Animals were given at least 7 days recovery before recording baseline. Femoral venous catheters were flushed with 0.9% saline/100U Heparin every second day to maintain their patency.

CBR was carried out under anaesthesia as described above. Like the non-recovery protocol, the common carotid arteries were accessed through a midline neck incision. A 3.0 silk suture was used to retract the common carotid artery to improve visualisation and access to the bifurcation of the common carotid artery. Each common carotid artery was separated from the sternohyoid muscle and the carotid artery bifurcation gently pulled away from the superior cervical ganglion; this allowed visualisation of the CB and the CSN. Using fine surgical forceps and under x25 magnification the CB was surgically removed with care taken to preserve fine branches of the carotid sinus nerve.

Experimental protocol for conscious SH rat experimentation

After recovery from implantation surgery, blood pressure was recorded continuously (Spike2 version 8, CED, Cambridge, UK) for one week before (Baseline) and two weeks after both SHAM (n=9) and CBR (n=9) surgeries. Data represent a weekly average for light (7.00-18.00) and dark (19.00-6.00) phases. On separate days, the cardiovascular responses to baroreflex

and chemoreflex activation, moderate exercise (10m/min), hypoxia (10% O_2, N_2) and hypercapnia (7% CO_2, 93% O_2) were tested before and 2 weeks after SHAM or CBR.

Baroreflex and chemoreflex tests

Bradycardic and tachycardic reflex responses produced by ramp changes in arterial pressure as described above. A 4-parameter sigmoidal regression function was fitted to produce baroreflex function curves, using purpose-written scripts in Spike2. The chemoreflex was tested as above.

Spontaneous baroreflex sensitivity and spectral analysis

Spontaneous baroreflex sensitivity (BRS) and spectral analysis parameters settings were used as described previously (Waki *et al.*, 2006), and applied using open access software CardioSeries v2.4 (www.danielpenteado.com). For BRS sequences of at least 4 consecutive beats in which increases/decreases in systolic arterial pressure were followed by response in pulse interval were used to fit linear regression curves; $r^2 > 0.8$. The spontaneous BRS is presented as the slope (ms/mmHg) of the linear regression analysis between systolic blood pressure and pulse interval. For heart rate and systolic blood pressure spectral analysis, beat-by-beat series of pulse intervals and systolic blood pressure were converted to evenly spaced series using cubic spline interpolation (10 Hz) and divided into half-overlapping sequential sets of 512 data points (Welch periodogram). Segments with transients that could affect the calculation of power spectral density were excluded. A Hanning window was used to attenuate side effects and the spectrum of each stationary segment was calculated using a fast Fourier Transform (FFT) algorithm for discrete time series. The spectra of pulse intervals were integrated in low-frequency (LF; 0.2–0.75 Hz) and high-frequency (HF; 0.75–3 Hz) bands and the results are expressed as normalized units (nu) as described before (Burr, 2007). The spectra of systolic arterial pressure were integrated only in low-frequency (LF; 0.2–0.75 Hz) and the results are expressed in absolute ($mmHg^2$) units. The LF/HF ratio was calculated to assess sympatho-vagal balance.

Exercise test

The cardiovascular responses to forced moderate exercise were assessed in a purpose-built motorised wheel. Rats were exercised for a total of 10 min, in a pattern consistent with

voluntary exercise patterns: 40s run/20s break, at a speed of 10m/min (Leasure & Jones, 2008) during their active phase, 19.00-21.00. Because it has been previously reported that chronic exercise training decreases resting blood pressure in the SH rat (Burger *et al.*, 1998; Graham & Rush, 2004; Gu *et al.*, 2015), we decided not to train our experimental animals. Rats were thus not previously exposed to the exercise wheel, therefore the exercise most likely includes an element of stress.

Hypoxia and hypercapnia tests

Hypoxia and hypercapnia experiments were carried out in the afternoon between 12.00 and 16.00. Before experiments began, each rat was given at least one hour to acclimatize to the chamber. The responses to hypoxia (10% oxygen, balance nitrogen, BOC) and hypercapnia (7% CO_2, 93% O_2, BOC) were tested in a normobaric chamber on the same animals on separate days. Baseline blood pressure, heart rate and respiratory rate were recorded during the delivery of humidified atmospheric air (21% O_2/N_2) followed by 15 min exposure to either hypoxia or hypercapnia at a rate of 8L/min. Note, as shown herein and reported previously, hyperoxia fails to attenuate the response of the carotid bodies to hypercapnia in a variety of species including rat (Carroll & Bureau, 1988; Pepper *et al.*, 1995; Rodman *et al.*, 2001); hence, 7% CO_2 was mixed with 93% oxygen.

Histology

On completion of the experimental protocol, animals were terminally anaesthetised with an overdose of sodium pentobarbital (100 mg/kg) and the carotid bifurcations removed and fixed (4% paraformaldehyde for 24h, then stored in 30% sucrose/0.05% sodium azide). Using a cryostat, 10-µm thickness sections were cut and mounted on Superfrost Plus slides, then stained with haematoxylin and eosin. Briefly, slides were stained with Ehrlich's haematoxylin for 3 minutes, washed, de-stained in 1% acid alcohol for 20 seconds washed, then counterstained with eosin for 10 seconds. Slides were then progressively dehydrated (70%, 90% and 100% ethanol), immersed in xylene (3 x 5 minutes each) and covered with coverslips using a mounting medium. Images were obtained using a light microscope and ImageJ software.

Statistical analysis

Statistical analysis was conducted using SPSS (IBM SPSS version 23) and GraphPad v 6.0, baroreflex curve and sigmoidal regression performed in Spike2 software (version 8, CED, Cambridge, UK) using purpose-written scripts provided by CED. Responses during exercise, hypoxia and hypercapnia were analysed by two-way ANOVA with repeated measures on two factors (time and intervention, before and after surgery). Post-hoc tests used are reported in the corresponding figure legends. The factor analysed by post-hoc test is 'Intervention' and the data are compared at each time point before and after surgery within either Sham or CBR group. Exercise, hypoxia and hypercapnia responses were also analysed by the area under the curve (AUC) method, which compared the area under the curve between before and after surgery. The statistical test performed is indicated in figure legend. Data are presented as mean ± SEM, with a significance level of $p<0.05$.

Results

Successful removal of the carotid bodies was confirmed using histochemistry on the carotid bifurcations removed from CBR rats; data were compared with sham rats (Fig 1). Fig 1 shows an absence of glomus cells after CBR. An absence of the carotid body was found in all 15 rats that underwent CBR surgery. Carotid bodies were always found in sham rats (n=14).

Physiological validation of selective carotid body resection – Anesthetised Rats

Eleven (5 Time Control; 6 CBR) anaesthetised male SH rats were used in order to confirm selective carotid body resection (CBR). The SBP response to chemoreflex activation was present, but significantly lower under anaesthesia compared to those obtained in the same rat when conscious (ΔSBP, Anesthetized: 8±2mmHg vs Conscious: 86±8mmHg; $P<0.001$); however, a similar degree of bradycardia was observed (ΔHR, Anesthetized: -99±13bpm vs Conscious: -132±12bpm; $P>0.05$).

Chemoreflex testing was performed during the baseline period after resection of the aortic depressor nerves (ADNX) and repeated after carotid body resection (CBR) or a sham procedure in the Time Control Group. CBR resulted in the abolishment of the chemoreflex response seen as a loss of the increase in SBP; $P<0.05$; (Figure 2, B) and an absence of bradycardia, $P<0.05$; (Figure 2, A). The Time Control group showed an increase in the SBP response over time ($P<0.05$), which may reflect an increased sensitivity to repeated chemoreflex stimulation. However, the HR response was similar.

Baroreceptor reflex gain was preserved after combined ADNX and CBR, (Figure 2, C, D; NS). At the end of the experiment, rats in the CBR group underwent bilateral carotid sinus nerve denervation (CNSX), after which the heart rate baroreceptor reflex were completely abolished, confirming complete sino-aortic denervation (Figure 2, E).

Baseline changes in blood pressure after selective resection of the carotid bodies in conscious SH rats

Eighteen (9 Sham; 9 CBR) male spontaneously hypertensive rats were used in order to study the effect of the selective CBR on cardiovascular and respiratory parameters in conscious freely moving animals. Data are presented in Figure 3 for both dark and light phases at

baseline - week 0 (W0), one week (W1) and two weeks (W2) after CBR. A significant reduction in SBP was observed in the CBR group relative to baseline during both light and dark phases ($P<0.001$; Fig 3). Reductions in DBP ($P<0.001$), heart rate ($P<0.001$) and respiratory rate ($P<0.001$) also occurred in both light and dark phases (Fig 3). Sham operated rats show increases, when compared to baseline, in SBP ($P<0.05$) and DBP ($P<0.05$) recorded in the light phase (only) and an increase in RR in both phases ($P<0.05$, Figure 3).

Chemoreflex and baroreflex responses before and after selective carotid body resection in conscious rats

The arterial chemoreflex mediated pressor/bradycardia response, tested 2 weeks post-surgery, was abolished after selective CBR (ΔSBP: before 79±10mmHg vs. after 9±17mmHg; ΔHR: before -130±17bpm vs. after -4±4bpm, $P<0.001$; whereas the responses in the Sham operated animals remained (ΔSBP: before 92±13mmHg vs. after 84±12mmHg, ΔHR: before -134±20bpm vs. after -130±17bpm, NS; Figure 4 A).

Spontaneous baroreflex gain (sBRG) did not change after CBR (0.9±0.09ms/mmHg vs. 1.2±0.29ms/mmHg; NS) or in sham rats (1.1±0.17ms/mmHg vs. 1.1±0.1ms/mmHg; NS; Fig 4B). Sigmoidal baroreflex function curves showed no significant difference after CBR, but a leftwards resetting of the operating points to the lower level of arterial pressure (Figure 4B, right graph).

Spectral analysis of pulse interval and SBP after CBR in conscious rats

Spectral analysis of the pulse interval (Figure 5) showed that CBR was associated with a reduction in LF power (30.4±1nu vs. 23.2±2nu; $P<0.05$), and an increase in HF power (69.6±1nu vs. 76.8±2nu; $P<0.05$) resulting in a marked reduction in the LF/HF ratio (0.48±0.03 vs. 0.33±0.03; $P<0.05$). SHAM did not elicit significant changes in LF power (25.5±2nu vs. 30.2±4nu; NS), HF power (from 74.5±2nu to 70.3±3nu; NS) and LF/HF ratio (0.38±0.04 vs. 0.5±0.08nu; NS). These suggests that CBR leads to an improvement in cardiac sympatho-vagal balance. Regarding SBP spectral analysis, we observed a significant reduction in the LF component of SBP in the CBR group (5.3±0.9 $mmHg^2$ vs. 1.9±0.6 $mmHg^2$; $P<0.05$) but not in SHAM group (2.3±0.5 $mmHg^2$ vs. 3.2±1.3 $mmHg^2$; NS) suggesting a reduction in sympathetic vasomotor tone after CBR.

Exercise before and after selective resection of the carotid bodies

Sham and CBR rats did not show any differences in the ability to exercise; all groups showed an increase in blood pressure, heart rate and respiration, ($P<0.001$, Figure 6 A, B). Our exercise challenge produced similar increases in blood pressure, heart rate and respiratory rates in sham and CBR rats (NS; Figure 6 A, B). AUC analyses similarly showed that HR and RR responses to exercise were not different between sham and CBR rats in (NS; Figure 6 A, B) but the pressor response in the Sham group increased after surgery (SBP, $P<0.05$, Figure 6 A).

Hypoxic challenge before and after selective resection of the carotid bodies

Two-way Anova show that exposure to hypoxia (10% oxygen) produced a pressor response in sham animals, and in the CBR group before resection of carotid bodies, accompanied by an increase in heart rate and respiration (Figure 7 A, B $P<0.001$). In contrast, CBR abolished the pressor response (SBP; $P<0.05$) whereas responses in heart rate and respiratory rate were similar to before CBR animals (HR, RR; NS).

Following the sham surgery, animals show an increase in the pressor response to hypoxia (SBP; $P<0.05$) but responses in heart rate and respiratory rate were similar to before Sham (NS).

The statistical analyses on the AUCs, confirmed these results. Interestingly, AUC analyses also showed that the RR decreased after CBR; ($P<0.001$).

Hypercapnia challenge before and after selective resection of the carotid bodies

Exposure to 7%CO_2/93%O_2 resulted in increase in SBP, HR and RR in all groups, ($P<0.001$, Figure 8 A, B). The SBP response to hypercapnia was augmented after CBR ($P<0.01$, Figure 8 B). The responses in the Sham group did not differ before and after surgery (NS, Figure 8A). Analyses performed on AUC confirmed these responses. Additionally, it identified that the RR increased after CBR; ($P<0.01$).

Discussion

We demonstrate for the first time that the carotid bodies can be surgically resected while preserving carotid sinus baroreflex function in the SH rat for at least 2 weeks. We have carefully validated our surgical approach to confirm both that: (i) the carotid bodies were removed (ii) carotid sinus baroreceptor function was preserved and not dissimilar to sham animals, and (iii) carotid chemoreceptor reflex function was abolished. Chronic blood pressure recordings indicated that selective CBR produced a sustained and significant reduction in arterial pressure in the SH rat across both light *and* dark phases; the magnitude of this reduction was consistent with our previously reported findings where the carotid sinus nerves were denervated bilaterally (Franchini & Krieger, 1992; Abdala *et al.*, 2012; McBryde *et al.*, 2013), and involved reductions in sympathetic drive to both the heart and vasculature, as measured indirectly with spectral analysis. In these hypertensive rats, we also revealed an essential role of the carotid chemoreflex in mediating the pressor response to hypoxia. Notably, the pronounced pressor response to hypoxia was absent post CBR. In contrast, we found that the cardiovascular response to exercise was unchanged after CBR, suggesting either that the carotid bodies do not play a critical role in mediating this response, or that compensation by alternate pathways occurred. Finally, the pressor response to hypercapnia was augmented post CBR.

Our ability to selectively remove the carotid bodies is an important advance, as previous techniques by ourselves and others (Abdala *et al.*, 2012; Del Rio *et al.*, 2013; McBryde *et al.*, 2013; Marcus *et al.*, 2014; Iturriaga *et al.*, 2015; Pijacka *et al.*, 2016) have relied on stripping the carotid sinus of all nerves, thus removing baro-receptive as well as chemo-receptive afferents. Impressively, despite the bilateral denervation of the carotid sinus baroreceptors in our previously published studies, a functional baroreflex was observed to be maintained in these animals, presumably via compensation from the aortic depressor baroreceptor pathway (Abdala *et al.*, 2012; McBryde *et al.*, 2013). Our current results extend this previous work, showing for the first time that following specific CBR when a fully functional baroreflex is maintained (i.e. carotid sinus and aortic), with a leftward shift resetting around the lower level of arterial pressure, a substantial anti-hypertensive response persists.

Given the evidence of raised carotid body activity during exercise (Jacobi *et al.*, 1989; Ward, 1994) and improved exercise tolerance post CBR in humans (Niewinski *et al.*, 2017), we were surprised to find no difference in the cardiovascular response after CBR. Although the cardiovascular responses were not reported, Lugliani *et al* (1971) showed that the respiratory response to moderate steady-state exercise were not affected by CBR, which is consistent with our finding that the cardiovascular responses to exercise are not reliant on input from the carotid bodies, at least in the SH rat. However, we acknowledge that the exercise protocol we used was not without environmental stress as the animals were forced to run in an enclosed motorized running wheel. Further, we chose not to condition the animals to the running wheel, as carotid body sensitivity is reduced with exercise training (Burger *et al.*, 1998; Graham & Rush, 2004; Gu *et al.*, 2015). Thus, the absence of an effect of CBR on the blood pressure and heart rate responses during exercise in our study may include a stress component. Therefore, the effect of CBR on blood pressure control during exercise in SH rats remains equivocal.

Our observation that CBR blunts the ventilatory and reverses the pressor response to hypoxia is consistent with recent studies in human patients with heart failure, who underwent bilateral CBR (Niewinski *et al.*, 2014). In keeping with our current results, Niewinski *et al* showed that CBR reduced the respiratory and arterial pressure responses to hypoxia, whilst the heart rate response was unchanged. Similarly, early human studies where bilateral CBR was performed to treat bronchial asthma, found that the respiratory response to hypoxia was absent (Lugliani *et al.*, 1971). Our data in SH rats and that in humans may have important implications when evaluating the carotid body as a potential therapeutic target in cardiovascular disease, as subjects lacking carotid bodies may be less able to cope with situations where oxygen availability is decreased. This is borne out by the recent observation of worsening blood oxygen saturations at night in heart failure patients after bilateral CBR (Niewinski *et al.*, 2017). This supports our contention that carotid body therapy should modulate, not abolish, its function (Pijacka *et al.*, 2016).

We performed bilateral CBR as unilateral carotid sinus denervation was ineffective in lowering blood pressure in SH rats (McBryde *et al.*, 2013). In contrast, unilateral carotid body denervation in drug resistant hypertensive patients was effective in ~60% of patients tested

suggesting a possible species difference. In our study (Narkiewicz *et al.*, 2016) and that of others (Limberg *et al.*, 2015), unilateral carotid body ablation lowered arterial pressure in some patients, which was well maintained at 3 and 6 months follow up with some showing a relapse by 12 months; the latter may reflect compensation from the contralateral carotid body. Nevertheless, preservation of the contralateral carotid body may be necessary to preserve protection against hypoxia in these patients, especially during sleep. This view is supported by a recent case report examining various sympatho-excitatory reflex tests in a patient with (prior) unilateral CB resection for paraganglioma (Larson *et al.*, 2017). The authors reported that hypoxic ventilatory responses were normal, but that the sympatho-excitatory responses to static exercise appeared to be blunted (Larson *et al.*, 2017).

Exposing rats without carotid bodies to hyperoxic hypercapnia produced an exaggerated pressor response compared to sham controls. We propose that this is due to a greater plasma level of CO_2 that results from a reduced ventilatory response; this is borne out by the reduced breathing frequency response to hypercapnia after CBR. We presume the plasma contains an elevated level of CO_2 that provides a greater stimulus to the central chemoreceptors. We recognise that this will need to be confirmed using blood sampling which was not tenable in the present study. We acknowledge that the use of hyperoxia might have: (i) suppressed basal discharge of the carotid bodies in the sham control group and (ii) caused a confounding vasoconstrictive effect. However, this would be expected to be the same in the sham and CBR groups making their comparison relative. Also, hyperoxia does not attenuate the response of the carotid bodies to hypercapnia in the rat (Carroll & Bureau, 1988; Pepper *et al.*, 1995; Rodman *et al.*, 2001) so this is not problematic. All told, the exaggerated rise in blood pressure to hypercapnia after CBR is potentially worrisome and could pose problems clinically in terms of inducing stroke.

Translational Perspective

The present study raises potential clinically relevant problems with bilateral carotid body resection. Although there are positive effects on blood pressure control in conditions of hypertension, the SH rat was not able to control blood pressure after CBR when exposed to hypoxia and exhibited excessive rises in blood pressure to hypercapnia. These could trigger end organ damage and may be particularly pertinent to human patients with sleep apnoea.

Through extension, it might be expected that in other situations where the carotid bodies would normally be engaged, the homeostatic control of blood pressure and ventilation may become jeopardised. Given that the carotid body has multiple other functions e.g. blood glucose control (Limberg *et al.*, 2014; Sacramento *et al.*, 2017), multiple levels of organ and systems failure could occur under different states of health and disease without carotid bodies. We surmise that blunt resection is not optimal and efforts now are needed to find pharmacological approaches that can normalise carotid body function by abolishing hyperreflexia and tonicity without destroying physiological function; the purinergic P2X3 receptors is one such example that we have proposed (Pijacka *et al.*, 2016) but others have also been suggested including anti-oxidant therapy (Iturriaga et al. 2015) and caffeine, which is known to block adenosine receptors and decrease carotid body sensitisation following chronic intermittent hypoxia (Sacramento et al. 2015). The relevance of the latter is that habitual coffee drinking was found to lower blood pressure especially in women (Geleijnse 2008).

Competing interests

The authors declare that they have no competing interests.

Author contributions

JFRP was responsible for acquisition of funding, administrative support, study conception, design of the experiments and drafting the manuscript. WP designed the experiments, collected, analysed and interpreted the data. FDM analysed and interpreted the BRG data. Both WP and FDM wrote the manuscript. PLK performed and interpreted the spectral analsyis. GSL and PLK performed immunohistochemistry. HCS and RRC contributed to the to the editing of the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Figure 1

Histological confirmation of carotid body resection.

A) Sham surgery with intact carotid body; B) carotid body resection (CBR) with absence of the peripheral chemoreceptor. Eosin and haematoxylin staining of representative images. CC-common carotid artery, IC-internal carotid artery, EC-external carotid artery, CB-carotid body

Figure 2

Assessment of chemoreflex and baroreflex sensitivity before and after progressive and selective chemo- and baro-reflex denervation in anesthetized SH rats.

A) Chemoreflex-induced changes in heart rate (HR) and B) - in systolic blood pressure (SBP) by i.v. bolus infusion of sodium cyanide (0.04% NaCN) were abolished after carotid body resection (CBR); $P < 0.05$. C) The cardiac baroreceptor reflex was preserved after CBR in the absence of aortic depressor nerves (ADNX); D), E) representative images illustrating the individual response to phenylephrine (PH) and sodium nitroprusside (SNP) in the time control and CBR group. CSNX resulted in abolishment of the baroreceptor reflex in CBR group. Data were analysed by two-way ANOVA with Sidak *post-hoc* test. Sham n=5, CBR=6, $*P < 0.05$. Data are presented as mean ± SEM.

Figure 3

Blood pressure and heart rate change after selective carotid body resection in conscious rats

Cardiovascular responses to CBR in conscious SH rats. Temporal responses in systolic blood pressure (SBP), diastolic blood pressure (DBP), respiratory rate (RR) and heart rate (HR) are presented during light and dark phase. Data represent maximal response to the CBR or sham surgery recorded within first (W1) and second (W2) week post treatment. W0 represent baseline. Data were analysed by two-way ANOVA with Tukey *post hoc* test; n=9; $*P < 0.05$, $**P < 0.01$, $*** P < 0.001$. Data are presented as the mean ± SEM.

Figure 4

Chemo- and baro-reflex function after selective carotid body resection (CBR) in the conscious SH rat.

A) Chemoreflex testing in the Sham and CBR groups. Surgical, selective, bilateral resection of the carotid bodies (CBR) abolished the chemoreflex response to 0.04% NaCN (SBP-systolic blood pressure, HR-heart rate; n=7; P<0.001)). B) Sham (left graph) and CBR (right graph) group baroreflex test. The cardiac baroreflex function curve was shifted leftwards over lower pressure ranges after CBR in SH rats (right graph, P<0.05). Data were analysed by two-way ANOVA with Sidak *post-hoc* test (panel A) and paired t-test; n=6 (panel B); ∗P < 0.05. Data are presented as the mean ± SEM.

Figure 5

Effect of carotid body resection (CBR) on cardiac sympatho-vagal balance in conscious SH rats.

Sympatho-vagal balance was unaffected in the Sham group, NS. The CBR decreased LF(nu), increased HF(nu) and it decreased the LF/HF ratio. CBR also reduced the LF spectra of SBP suggesting sympathoinhibition. SBP, P<0.05. Data were analysed by two-way ANOVA with Sidak *post-hoc* test; n=6; ∗P < 0.05, ∗∗P < 0.01. Data are presented as the mean ± SEM.

Figure 6

Exercise challenges before and after selective carotid body resection (CBR) or sham surgery

Exercise produced similar increases in systolic blood pressure (SBP), heart rate (HR) and respiratory rates (RR) in Sham A) and CBR B) rats over the time, P<0.001. Neither Sham nor CBR influenced the rat ability to exercise, NS. Data were analysed by two-way ANOVA with Sidak *post hoc* test comparing before vs. after surgery at each time point; n=7. Additionally, the AUC (top right corner of each graph) showed that the pressor response in the Sham group increased after surgery *P<0.05. Data are presented as the mean ± SEM.

Figure 7

Hypoxia challenge before and after selective carotid body resection (CBR) or sham surgery

Exposure to 10% oxygen increased systolic blood pressure (SBP), heart rate (HR) and respiratory rate (RR) in A) Sham group and B) CBR group before surgery (P<0.001). However, CBR abolished (P<0.05) and Sham surgery further exacerbated (P<0.05) the pressor response. CBR did not alter the response to hypoxia either in HR or in RR (NS). Data were analysed by two-way ANOVA with Sidak *post-hoc* test comparing before vs. after surgery at each time

point ∗P<0.05; n=7. Moreover, the AUC (top right corner of each graph) confirmed the SBP responses and identified that the RR decreased after CBR; **P<0.01 and ***P<0.001. Data are presented as the mean ± SEM.

Figure 8

Hypercapnia challenge before and after selective carotid body resection (CBR) or sham surgery

Exposure to 7% CO_2/93% O_2 produced a pressor response in all animals, accompanied by an increase in heart rate and respiration, (P<0.001). After CBR the pressor response was augmented relative to the before CBR group (P<0.01). CBR did not alter the response in heart rate (HR) to hypercapnia and the respiratory rates (RR; NS). Data were analysed by two-way ANOVA with Sidak *post-hoc* test comparing before vs. after surgery at each time point ∗P<0.05; n=7. Moreover, the analysis on AUC (top right corner of each graph) confirmed CBR effect on SBP. Additionally, it showed that RR increased after CBR; **P<0.01 and ***P<0.001. Data are presented as the mean ± SEM.

Figure 1

Histological confirmation of carotid body resection.

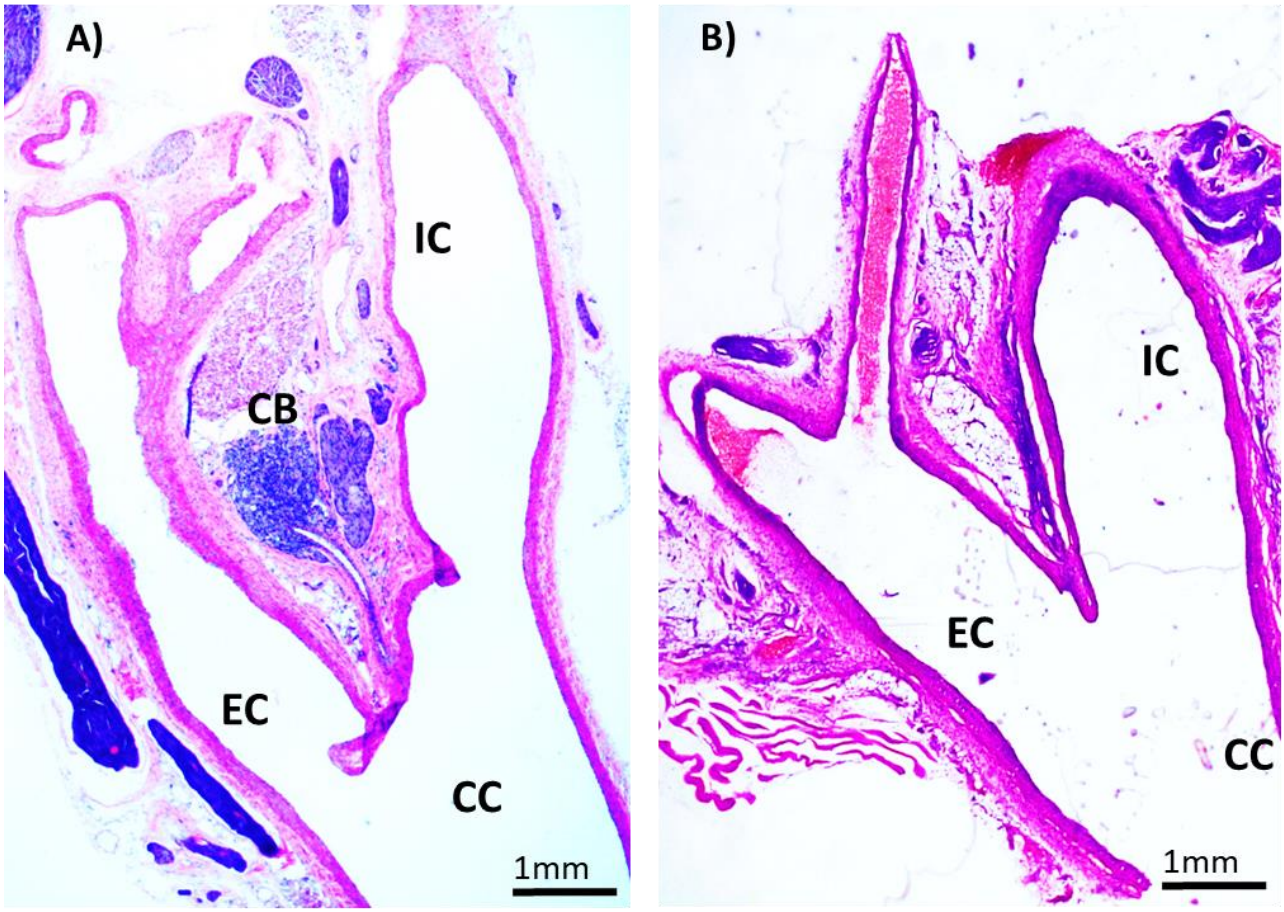
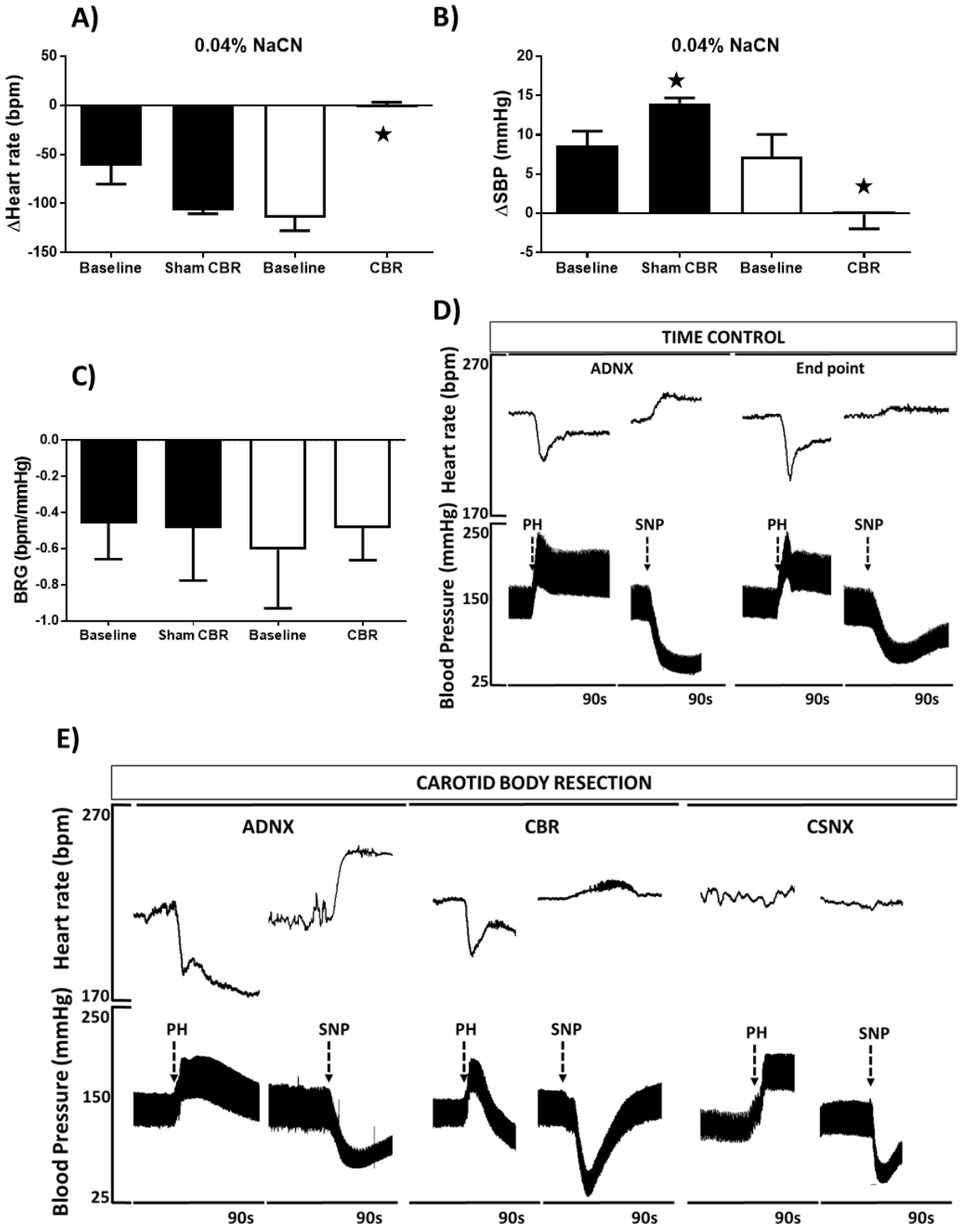


Figure 2

Assessment of chemoreflex and baroreflex sensitivity before and after progressive and selective chemo- and baro-reflex denervation in anesthetized SH rats



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694 **Figure 3**

Blood pressure and heart rate change after selective carotid body resection (CBR) in conscious rats

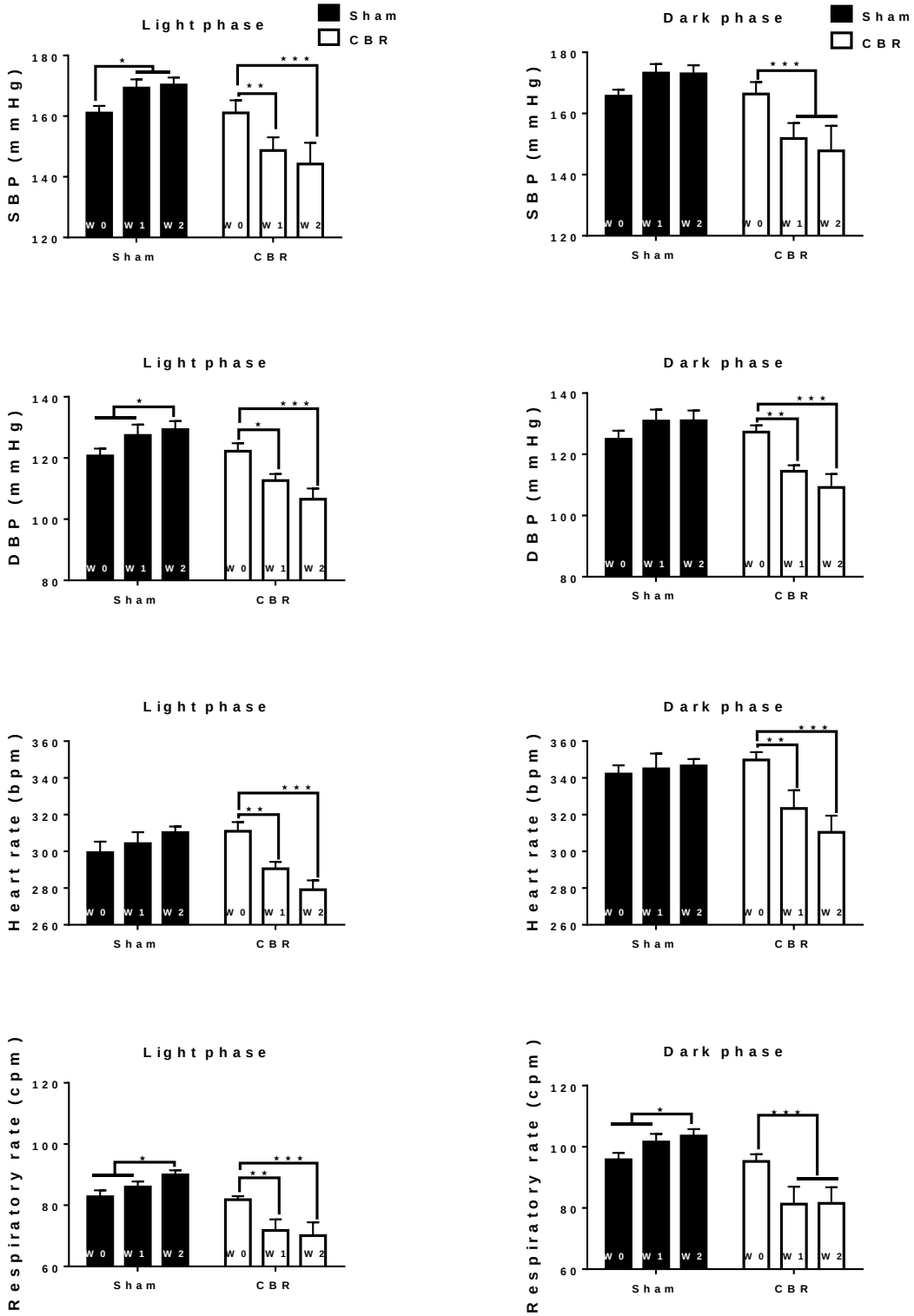


Figure 4

Chemo- and baro-reflex function after selective carotid body resection (CBR) in the conscious SH rat.

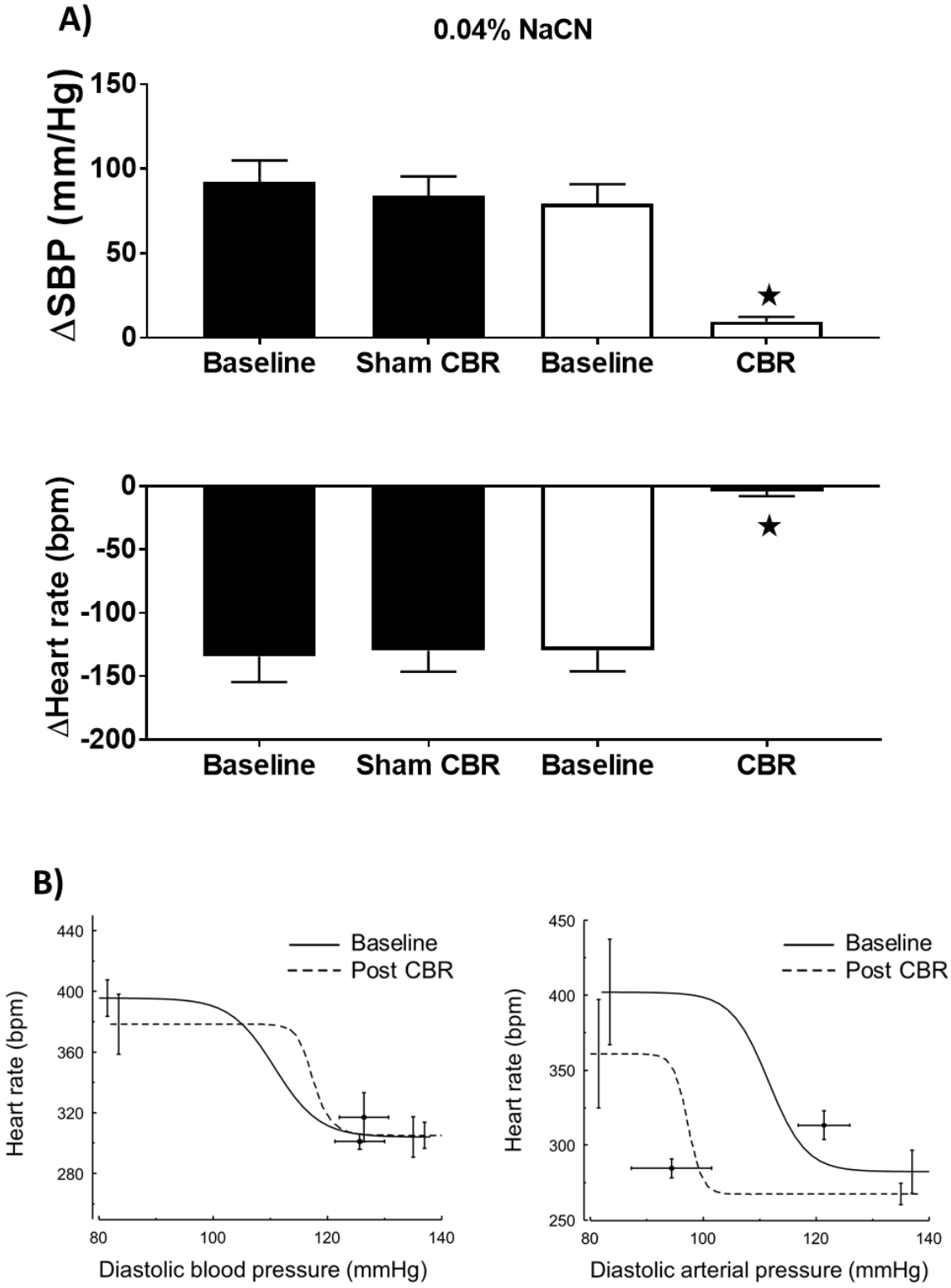


Figure 5

Effect of carotid body resection (CBR) on sympatho-vagal balance in conscious SH rats.

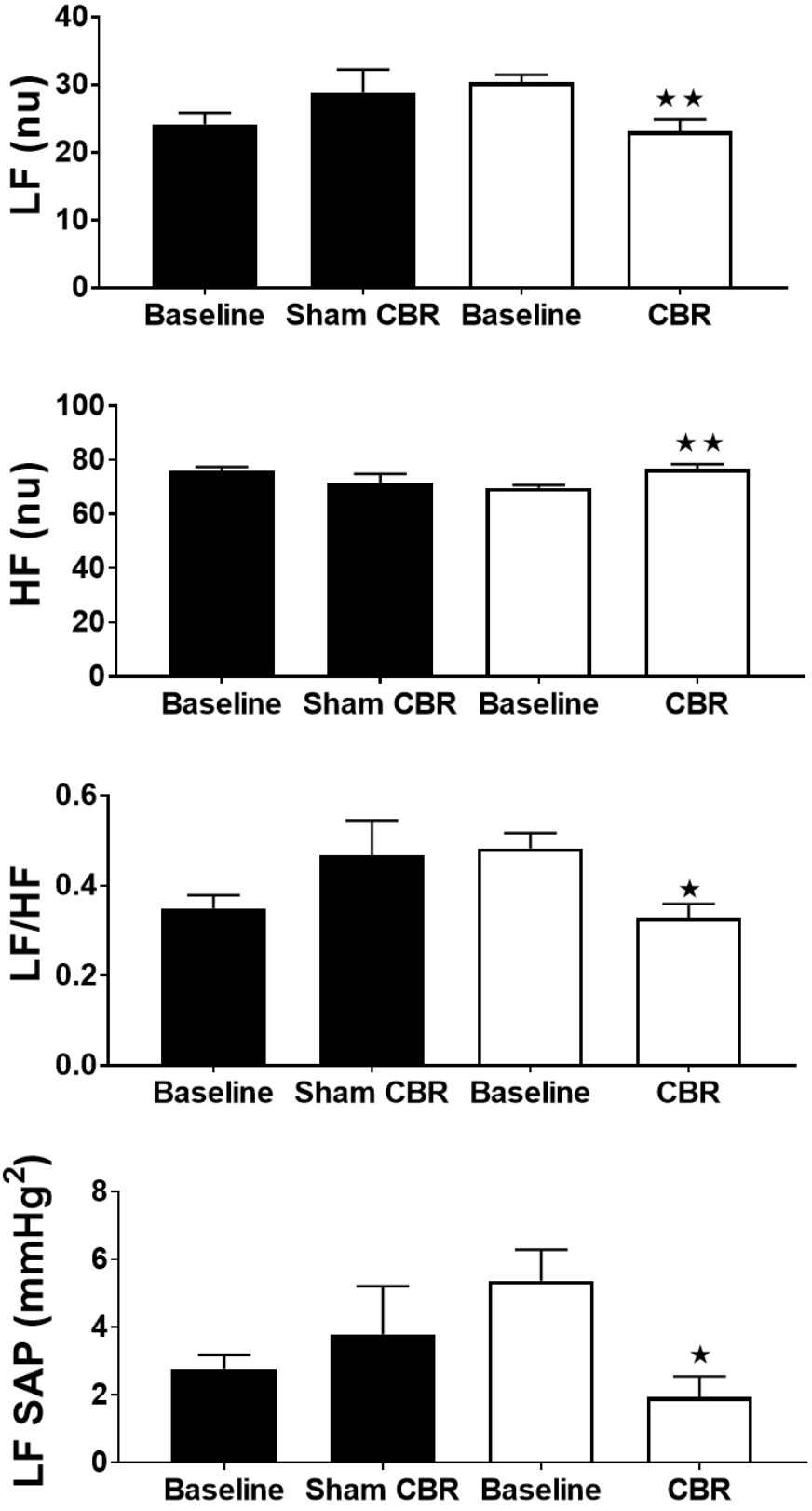


Figure 6

Exercise challenges before and after selective carotid body resection or sham surgery

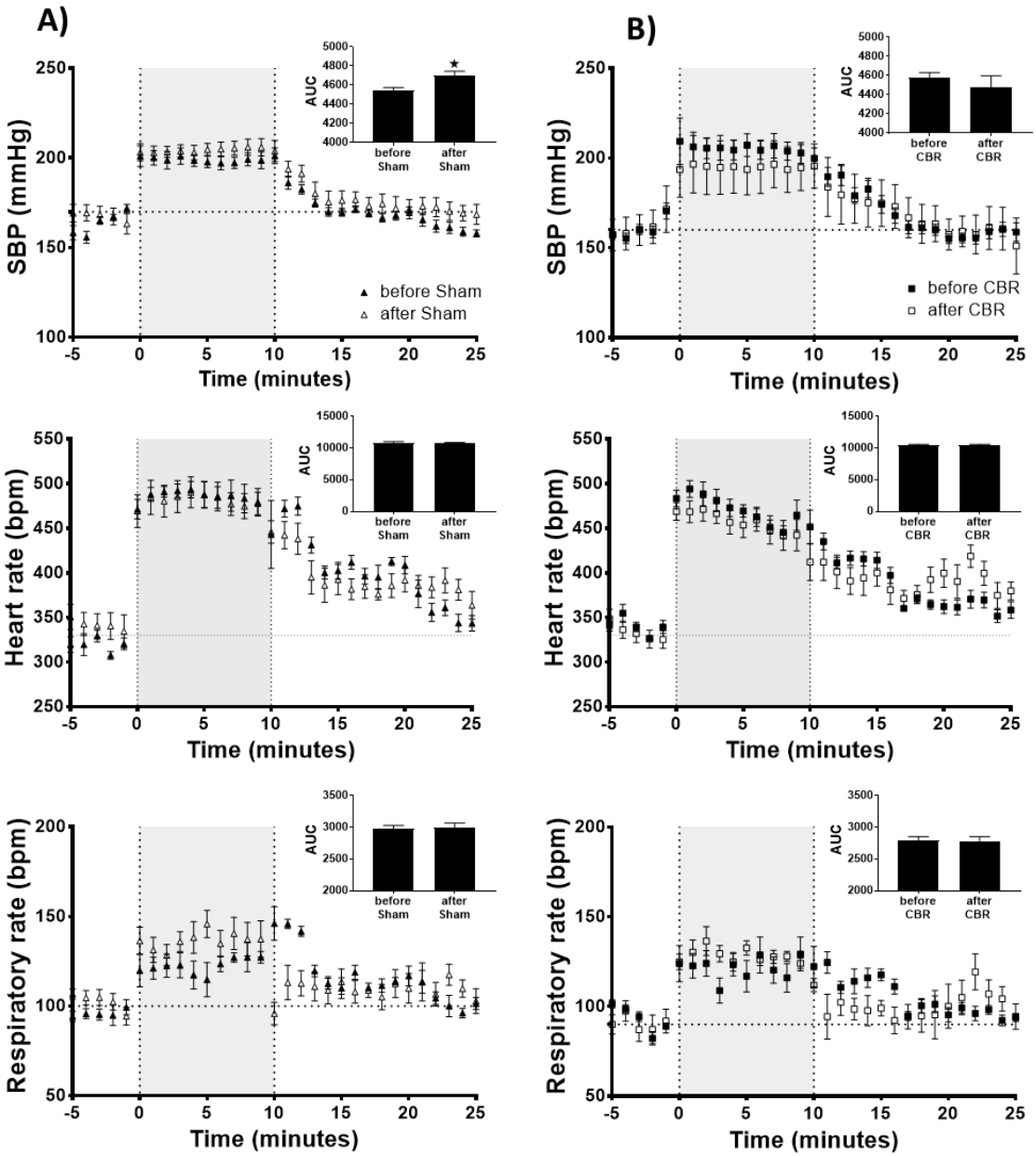


Figure 7

Hypoxia challenge before and after selective carotid body resection (CBR) or sham surgery

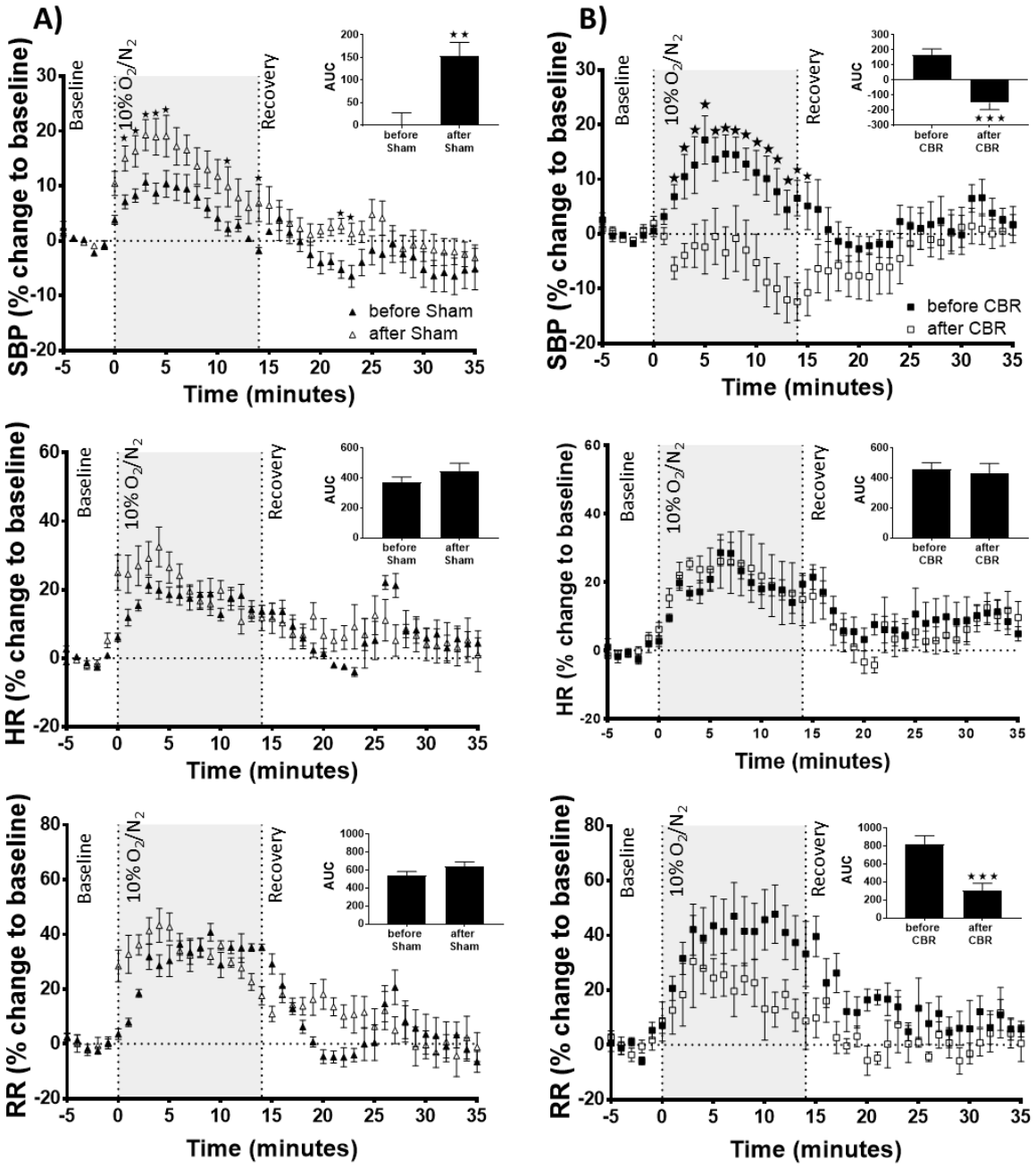
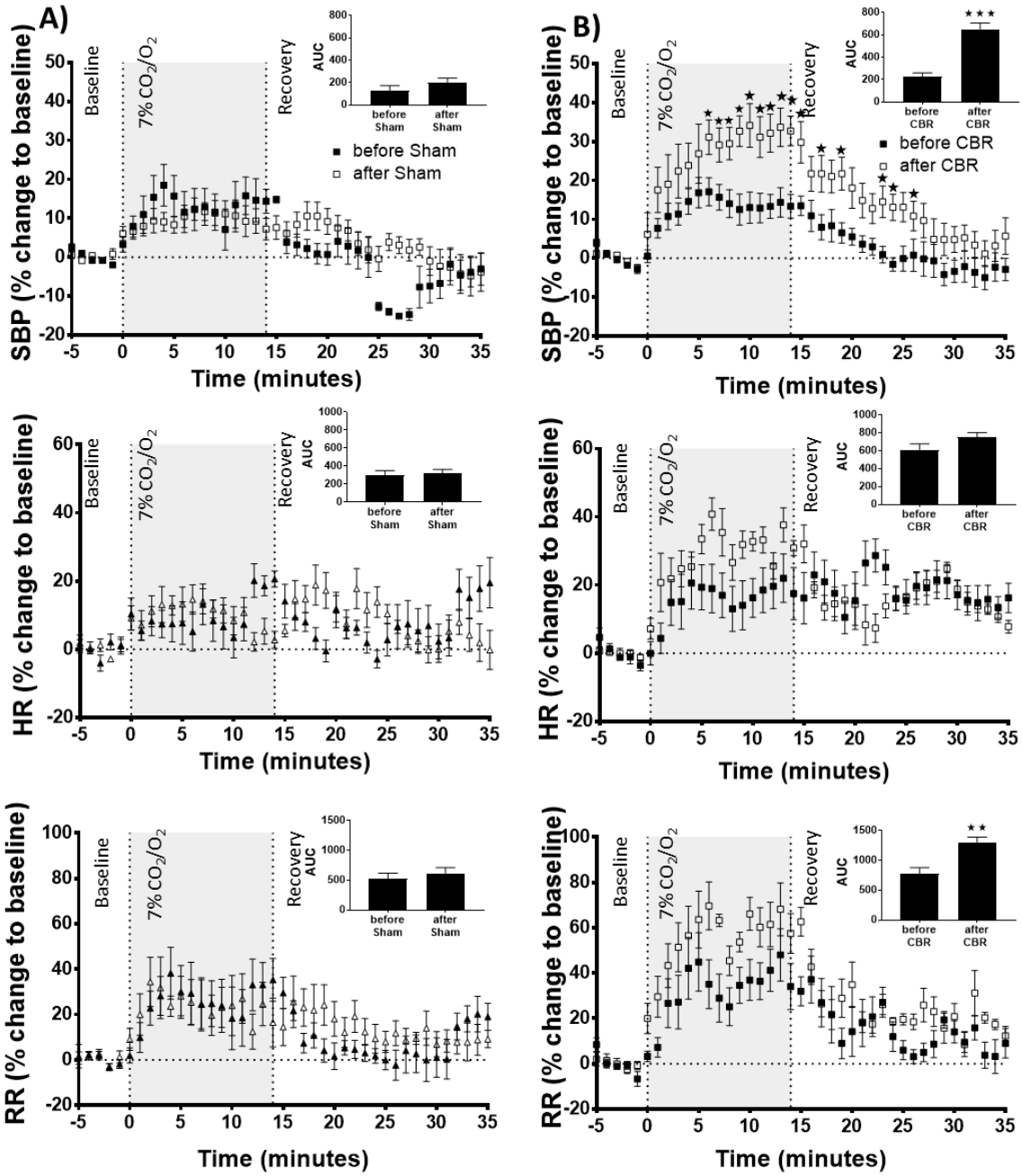


Figure 8

Hypercapnia challenge before and after selective carotid body resection (CBR) or sham surgery



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