Field stimulation of the carotid baroreceptor complex does not compromise baroreceptor function in spontaneously hypertensive rats

Zahra Kouchaki, Dimitrios Georgakopoulos, Mark Butlin, and Alberto P Avolio

Abstract— **Field stimulation of the carotid baroreceptors has been successfully used to induce a long-term reduction in blood pressure. However, baroreceptor stimulation may interfere with or compromise the beneficial short-term blood pressure regulation function of the baroreceptors. This study aims to quantify the baroreceptor function before and during acute, unilateral field stimulation of the carotid baroreceptors. Spontaneously Hypertensive Rats (n=7) were anaesthetised and instrumented to measure heart rate and mean arterial pressure (MAP), aortic pulse wave velocity (a surrogate measure of arterial stiffness), abdominal aortic flow and renal artery flow. A custom made field stimulation device was fitted to the left common carotid artery. Baroreceptor function was measured by quantifying heart rate response to MAP change induced by bolus injection of phenylephrine. Field stimulation of the baroreceptors reduced heart rate by 20 bpm (p=0.003) with MAP reduction of 18 mmHg (p=0.008). Maximal baroreceptor gain without stimulation was -1.20±0.41 bpm/mmHg and during stimulation -1.41±0.52 bpm/mmHg (p=0.59). The MAP at which maximal gain occurred also did not change (152±11, 160±9 mmHg respectively, p=0.22). This study indicates that unilateral field stimulation of the carotid baroreceptor complex, while causing a sustained reduction of arterial pressure, does not alter acute baroreceptor function peak gain.**

I. INTRODUCTION

Baroreceptor stimulation can acutely lower systemic arterial blood pressure. Past studies have not been able to maintain long term baroreceptor stimulation due to the contact between the electrode and the nerve deteriorating with time. Electrical field stimulation of the baroreceptor provides a solution to this problem, and field stimulation devices have been used for sustained, long term reductions in blood pressure in the order of 30 mmHg brachial artery systolic and 20 mmHg diastolic [1] with the effect being sustained through long-term down regulation of sympathetic activity [2]. The technique is therefore a potentially useful therapy for conditions such as resistant hypertension [3].

As baroreceptor stimulation is driving one (unilateral) or two (bilateral) carotid baroreceptor complexes, it is plausible that baroreceptor function may be changed during stimulation. However, baroreceptors are located in both carotid arteries and in the aortic arch. Baroreflex function may be preserved by signals from the aortic arch and carotid baroreceptors that are not stimulated. This study tests the hypothesis that baroreceptor function is changed during stimulation by measuring baroreceptor function with and without acute, unilateral field stimulation of the carotid baroreceptor complex in hypertensive rats.

This work was supported in part by grants from the Australian Research Council (ZK, ARC Linkage LP120100463; MB, ARC Discovery DP110101134) and by Macquarie University Research Postgraduate Scholarship (ZK). Barostimulation electrodes were supplied by CVRx Inc.

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II. METHODS

A. Animals

Male, spontaneously hypertensive rats (SHR, Animal Resource Center, Perth, n=5), 15 to 19 weeks of age were fed on standard rat chow and water *ad-libitum* and housed in a temperature controlled, 12/12 hour light/dark cycle until the day of the experiment.

Macquarie University Animal Ethics Committee approved all experiments, which were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals, as endorsed by the National Health and Medical Research Council of Australia.

B. Cardiovascular measurements

Rats were anaesthetised (urethane, induced at 1.3 g/kg i.p. and maintained i.v. as necessary) and fitted with limb leads for recording the electrocardiogram (ECG) and a cannula introduced in the femoral vein for administration of anaesthetic and vasoactive substances. Two high fidelity, solid state pressure sensors (Scisense, 1.6F catheter diameter) were introduced via peripheral arteries and positioned in the descending aorta approximately at the level of the upper thoracic and upper abdominal aorta. The catheter diameter of 1.6F had little impact on aortic hemodynamics, providing a 7% reduction in the cross sectional area for an average aortic diameter of 2 mm. Aortic and renal flow was measured using perivascular Doppler ultrasound probes (Transonic) positioned using a ventral approach.

Aortic and renal resistance was calculated beat-to-beat as the ratio of mean pressure in the abdominal aorta and mean flow (aortic and renal). Vessel stiffness was quantified by the pulse wave velocity (PWV) between the two pressure sensors. The transit time was measured from the diastolic foot of the proximal waveform to the diastolic foot of the distal waveform, where the foot of the waveform was located by the peak of the second time derivative of pressure [4].

C. Baroreceptor field stimulation

The left carotid artery was exposed and a single wire placed around the common carotid artery immediately proximal to the bifurcation. This positive electrode was on the

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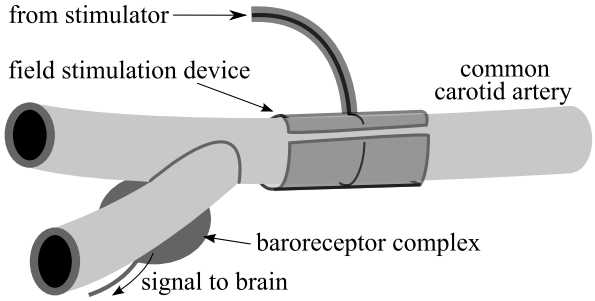

Fig. 1. A schematic of the baroreceptor field stimulation device in place around the common carotid artery. There is no direct contact with the baroreceptor complex or nerve, with stimulation being achieved through an electrical field.

inner surface of a silastic tube to hold the apparatus in place upon the common carotid artery (Fig. 1). A large negative electrode was placed nearby and closer to the body surface. Stimulation was driven at a frequency of 100 Hz, with a 0.53 ms pulse width. Signal amplitude was adjusted to between 3 and 5 V to obtain a maximal mean arterial pressure drop. This pressure drop was maintained for approximately 30 minutes.

D. Baroreceptor function measurement

A bolus of phenylephrine (15 μg/ml, 0.1 ml) was delivered through the venous line to induce a change in blood pressure and the response in heart rate was measured (Fig. 2). As field stimulation sometimes interfered with the ECG signal, heart rate was calculated from the interval between the peaks of the second derivative of pressure, corresponding to the diastolic foot of the waveform. A phenylephrine bolus was delivered during baseline (no stimulation) conditions and during carotid baroreceptor stimulation.

E. Data analysis and statistics

The baroreceptor function curve relating heart rate to mean arterial pressure (Fig. 2) was fitted with a 5-parameter function (b_1, b_2, k_1, k_2, k_3, Equation 1-3 that describes the non-symmetrical baroreceptor function well [5] using the 'drc' (Analysis of Dose-Response Curves) package [6] within the statistical package R (version 3.0.2). Baroreceptor gain was defined as the absolute change in heart rate with respect to change in mean blood pressure and the peak change in heart rate with respect to pressure was defined as maximal gain. The MAP at which the maximal gain occurred was also calculated.

$$heart\ rate = k_1 + \frac{k_2 - k_1}{1 + f \cdot e^{b_1 \cdot g} + (1-f) \cdot e^{b_2 \cdot g}} \quad (1)$$

$$f = \frac{1}{1 + e^{(2 \cdot b_1 \cdot b_2 / |b_1 + b_2|) \cdot g}} \quad (2)$$

$$g = \ln(MAP) - \ln(k_3) \quad (3)$$

Comparison of hemodynamic parameters from baseline (no stimulation) and during carotid baroreceptor stimulation was made by paired t-test. Baroreceptor function parameters were compared between control and stimulation using paired t-tests and Bland-Altman analysis [7].

TABLE I

MEASURED HEMODYNAMIC PARAMETERS BEFORE (BASELINE) AND DURING FIELD STIMULATION OF THE LEFT CAROTID BARORECEPTOR COMPLEX

	baseline	stimulation	p
heart rate (bpm)	373±9	353±10	0.003
systolic pressure (mmHg)	131±8	112±4	0.014
diastolic pressure (mmHg)	76±6	58±3	0.009
mean pressure (mmHg)	99±7	81±4	0.008
pulse pressure (mmHg)	55±4	54±3	0.60
mean aortic flow (ml/min)	41±2	34±1	0.011
mean renal flow (ml/min)	7.4±1.2	6.6±1.1	0.004
aortic resistance (ml/min/mmHg)	2.4±0.2	2.4±0.2	0.69
renal resistance (ml/min/mmHg)	14.9±2.1	13.6±2.0	0.074
aortic PWV (m/s)	4.4±0.5	4.1±0.5	0.058

Aortic flow measured in the abdominal, supra-renal section.

TABLE II

MAXIMUM GAIN AND THE MAP AT WHICH THAT OCCURRED DURING BASELINE (NO STIMULATION) AND FIELD STIMULATION.

	maximum gain (bpm/mmHg)	MAP (mmHg)
baseline	-1.20±0.41	152±11
stimulation	-1.41±0.52	160±9
p	0.59	0.22

Maximum gain is the maximum change in heart rate per mmHg change in MAP.

III. RESULTS

An example of the acute effect of unilateral field stimulation of the carotid baroreceptor complex is provided in Fig. 3. Confirmation of effective stimulation was provided through a concomitant reduction in heart rate and MAP indicative of vagal activation and sympathetic inhibition.

Field stimulation of the left carotid baroreceptor complex resulted in a reduction in heart rate from 373±9 bpm to 353±10 bpm (p=0.003) accompanied by a mean arterial pressure drop from 99±7 mmHg to 81±4 mmHg (p=0.008). The arterial pressure reduction was able to be maintained for 30 minutes in all cases with longer reductions plausible but not attempted in this experiment. Both abdominal aortic blood flow and renal blood flow were reduced with baroreceptor stimulation (Table I). Abdominal aortic resistance remained unchanged and renal resistance showed a modest but not statistically significant reduction (Table I).

Field stimulation of the carotid baroreceptor did not affect the baroreceptor peak gain, with the peak gain at baseline (no stimulation) conditions being -1.20±0.41 bpm/mmHg and during stimulation, -1.41±0.52 bpm/mmHg (p=0.59). Nor did stimulation affect the MAP at which maximal gain occurred (Table II). This was an average peak gain difference of 0.21±0.95 bpm/mmHg with a difference in the pressure at which that occurred of 7±14 mmHg (Fig. 4).

IV. DISCUSSION

This is the first study to examine baroreflex function during field stimulation of baroreceptors using an actively

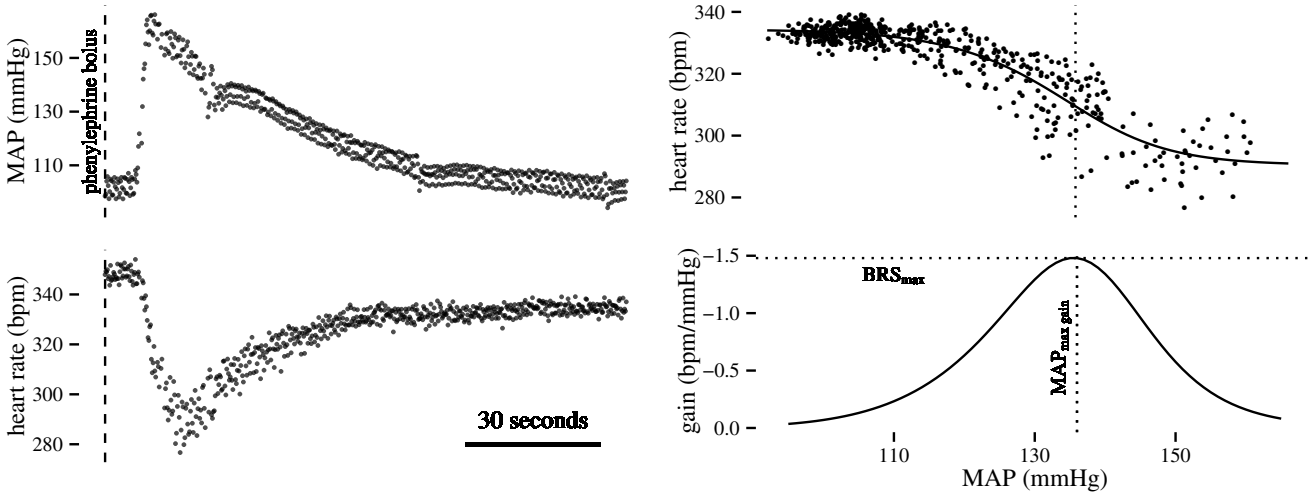

Fig. 2. Left: Example data showing the effect of an intravenous bolus of phenylephrine (PE) on mean arterial pressure (MAP) and the resulting reduction in heart rate due to the baroreceptor response. Each point represents a single pulse. Right: A typical baroreceptor response to a blood pressure fall following the pressure rise induced by a bolus injection of phenylephrine. The baroreceptor feedback loop causes a reduction in heart rate with increasing pressure (or increase in heart rate with decrease in pressure). The maximum slope, or gain (reversed ordinate axis to indicate gain magnitude) that occurs at the inflection point is indicated by the dashed line. The MAP and heart rate at which this occurs is indicated by the dotted lines. Baroreceptor gain (lower panel) is expressed as the absolute change in heart rate relative to change in MAP.

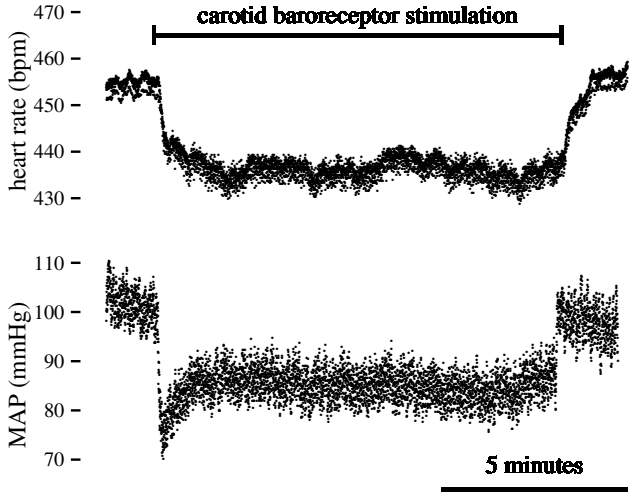

Fig. 3. An example of field stimulation of the carotid baroreceptors for approximately a 10 minute period. Heart rate (HR) and mean arterial pressure (MAP) show a rapid change upon activation and de-activation of stimulation (dashed lines) with MAP being relatively stable during the stimulation period. Each point indicates an individual beat, with the range of variation reflecting normal respiratory activity. Stimulation was at a frequency of 100 Hz, with a 0.53 ms pulse width. Pulse amplitude in this case was 3.2 V.

driven change in blood pressure. This strong and reliable baroreceptor challenge before and during stimulation of the carotid baroreceptor complex showed that the baroreceptor function remained unchanged with stimulation. The baroreflex function during baroreceptor stimulation has previously been quantified in humans, but only using spontaneous changes in blood pressure [8]. This sequence technique of measuring baroreflex sensitivity also demonstrated no change in the baroreflex function with stimulation. The sequence technique was not used in this study within rats, with a blood pressure intervention approach being used as a strong driver to quantify baroreceptor function. However, future work may investigate baroreceptor function in terms of the sequence technique.

Hemodynamic variables including heart rate, arterial blood pressure variables, and aortic and renal flow showed significant changes with carotid baroreceptor stimulation, despite a relatively small sample size in this study (n=7) in part due to the strength of a pairwise experimental protocol. Although significant changes were seen in blood pressure and blood flow, no significant differences were detected in baroreceptor function peak gain not the MAP at which that occurred.

Whilst field stimulation of the baroreceptors did not affect baroreceptor function peak gain, further study is required to investigate the effect on individual physiological regions that have sympathetic inputs. For example, renal flow reduced by 10.3% while abdominal aortic flow reduced by 23% with baroreceptor stimulation. The data suggest that stimulation may reduce renal resistance, though this trend was not significant in the current data.

SHR have a dampened baroreflex compared to their control strain, the Wistar-Kyoto rat [9]. This appears to be due to the increased stiffness of vessels and therefore reduced stretch of the baroreceptors, and not due to the conduction pathway, as demonstrated by preserved baroreceptor function when the aortic depressor nerve is directly stimulated [10].

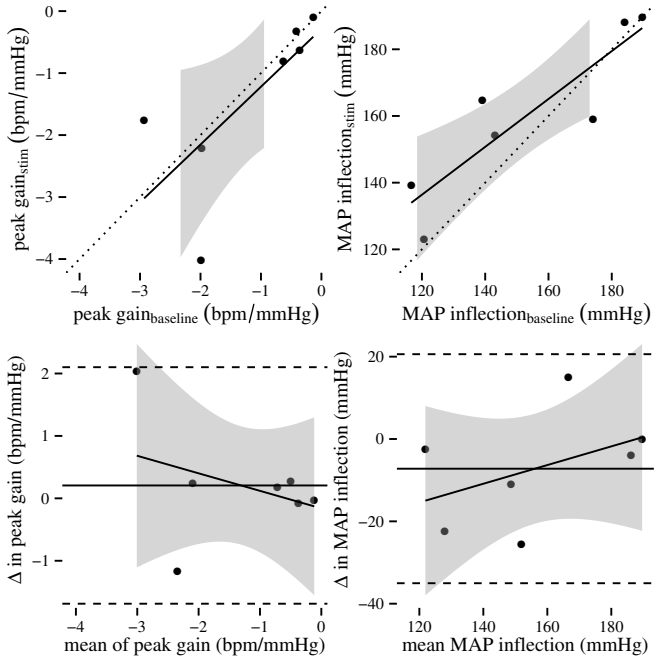

Fig. 4. Bland-Altman representation of the result. The regression between stimulation (stim) and baseline is shown by the solid line, the standard deviation of the difference by the dotted line, and the ideal zero difference between stimulation and baseline indicated by the dashed line.

Further study is required to ascertain whether, in cases where the baroreceptor function is not dampened, the baroreceptor function is also maintained during field stimulation of the baroreceptor complex. A similar study of normotensive animals would provide such information. Also worthy of assessment are any differences between anesthetised rats, as used in this study, and baroreceptor function in conscious hypertensive rats [11], [12].

A stimulation frequency of 100 Hz, with a 0.53 ms pulse width was used at a voltage amplitude that returned a maximal drop in blood pressure. Below this voltage, there appeared to be a voltage dependent blood pressure response. That is, lower voltages resulted in a lower blood pressure drop. However, this study was not designed to detect this voltage dependent response. Future studies might quantify this response, and also the effect of different stimulation frequency and pulse width on the stability of heart rate and blood pressure.

Field stimulation of baroreceptors in this study was acute, and conducted under anaesthesia. Analysis throughout this study compares baseline conditions before carotid baroreceptor stimulation and conditions during stimulation. Upon withdrawal of stimulation, all cardiovascular variables returned to baseline with one to two minutes, with blood pressure and heart rate returning to baseline in ten to twenty seconds. The study by Heusser *et al.* also analysed baroreceptor function under conditions of acute barostimulation [8]. However, field stimulation of the baroreceptors is intended as a chronic therapy. Further studies are required to quantify baroreceptor function under long term blood pressure modulation where the method of action may depend not only on reduced sympathetic activity but also fluid balance as maintained by the kidneys [13].

V. CONCLUSIONS

This is the first study to demonstrate preserved baroreceptor function peak gain during field stimulation of the carotid baroreceptor complex using induced changes in blood pressure. This provides support for the use of field stimulation of baroreceptors as a means of blood pressure lowering therapy, whereby the acute and transient control of blood pressure in daily life is maintained.

REFERENCES

[1] I. J. M. Scheffers, A. A. Kroon, J. Schmidli, J. Jordan, J. J. M. Tordoir, M. G. Mohaupt, F. C. Luft, H. Haller, J. Menne, S. Engeli, J. Ceral, S. Eckert, A. Erglis, K. Narkiewicz, T. Philipp, and P. W. de Leeuw, "Novel baroreflex activation therapy in resistant hypertension: results of a european multi-center feasibility study." *J Am Coll Cardiol*, vol. 56, no. 15, pp. 1254–1258, Oct. 2010.

[2] T. E. Lohmeier, R. Iliescu, T. M. Dwyer, E. D. Irwin, A. W. Cates, and M. A. Rossing, "Sustained suppression of sympathetic activity and arterial pressure during chronic activation of the carotid baroreflex." *Am J Physiol Heart Circ Physiol*, vol. 299, no. 2, pp. H402–H409, Aug. 2010.

[3] G. Mancia, G. Parati, and A. Zanchetti, "Electrical carotid baroreceptor stimulation in resistant hypertension." *Hypertension*, vol. 55, no. 3, pp. 607–609, Mar. 2010.

[4] M. Butlin, "Structural and functional effects on large artery stiffness: An in-vivo experimental investigation." Doctorate of philosophy thesis, University of New South Wales, 2007.

[5] J. H. Ricketts and G. A. Head, "A five-parameter logistic equation for investigating asymmetry of curvature in baroreflex studies." *Am J Physiol*, vol. 277, no. 2 Pt 2, pp. R441–54, Aug 1999.

[6] C. Ritz and J. Strebig, *Package drc Version 2.3-96*, Dec 2013.

[7] J. M. Bland and D. G. Altman, "Statistical methods for assessing agreement between two methods of clinical measurement." *Lancet*, vol. 1, no. 8476, pp. 307–10, Feb. 1986.

[8] K. Heusser, J. Tank, S. Engeli, A. Diedrich, J. Menne, S. Eckert, T. Peters, F. C. G. J. Sweep, H. Haller, A. M. Pichlmaier, F. C. Luft, and J. Jordan, "Carotid baroreceptor stimulation, sympathetic activity, baroreflex function, and blood pressure in hypertensive patients." *Hypertension*, vol. 55, no. 3, pp. 619–26, Mar. 2010.

[9] N. Minami, Y. Imai, M. Munakata, S. Sasaki, H. Sekino, K. Abe, and K. Yoshinaga, "Age-related changes in blood pressure, heart rate and baroreflex sensitivity in shr." *Clin Exp Pharmacol Physiol Suppl*, vol. 15, pp. 85–87, 1989.

[10] H. C. Salgado, A. R. Barale, J. A. Castania, B. H. Machado, M. W. Chapleau, and R. Fazan, Jr, "Baroreflex responses to electrical stimulation of aortic depressor nerve in conscious shr." *Am J Physiol Heart Circ Physiol*, vol. 292, no. 1, pp. H593–H600, Jan. 2007.

[11] G. A. Head and M. A. Adams, "Time course of changes in baroreceptor reflex control of heart rate in conscious shr and wky: contribution of the cardiac vagus and sympathetic nerves." *Clin Exp Pharmacol Physiol*, vol. 15, no. 4, pp. 289–292, Apr. 1988.

[12] N. Minami and G. A. Head, "Relationship between cardiovascular hypertrophy and cardiac baroreflex function in spontaneously hypertensive and stroke-prone rats." *J Hypertens*, vol. 11, no. 5, pp. 523–533, May 1993.

[13] T. E. Lohmeier and R. Iliescu, "Chronic lowering of blood pressure by carotid baroreflex activation: mechanisms and potential for hypertension therapy." *Hypertension*, vol. 57, no. 5, pp. 880–886, May 2011.