Role of Chemoreceptor Activation in Hemodynamic Responses to Electrical Stimulation of the Carotid Sinus in Conscious Rats

Pedro L. Katayama, Jaci A. Castania, Daniel P.M. Dias, Kaushik P. Patel, Rubens Fazan Jr, Helio C. Salgado

Abstract—Electric carotid baroreflex activation has been used to treat patients with resistant hypertension. It is hypothesized that, in conscious rats, combined activation of carotid baro- and chemoreceptors afferences attenuates the reflex hypotension. Rats were divided into 4 groups: (1) control group, with unilateral denervation of the right carotid chemoreceptors; (2) chemoreceptor denervation group, with bilateral ligation of the carotid body artery; (3) baroreceptor denervation group, with unilateral denervation of the left carotid baroreceptors and right carotid chemoreceptors; and (4) carotid bifurcation denervation group, with denervation of the left carotid baroreceptors and chemoreceptors, plus denervation of the right carotid chemoreceptors. Animals were subjected to 4 rounds of electric stimulation (5 V, 1 ms), with 15, 30, 60, and 90 Hz applied randomly for 20 s. Electric stimulation caused greater hypertensive responses in the chemoreceptor denervation group than in the control group, at 60 Hz (−37 versus −19 mm Hg) and 90 Hz (−33 versus −19 mm Hg). The baroreceptor denervation group showed hypertensive responses at all frequencies of stimulation. In contrast, the carotid sinus denervation group showed no hemodynamic responses. The control group presented no changes in heart rate, whereas the chemoreceptor denervation group and the baroreceptor denervation group showed bradycardic responses. These data demonstrate that carotid chemoreceptor activation attenuates the reflex hypotension caused by combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. These findings may provide useful insight for clinical studies using baroreflex activation therapy in resistant hypertension and heart failure. (Hypertension. 2015;66:598-603. DOI: 10.1161/HYPERTENSIONAHA.115.05316.) ● Online Data Supplement

Key Words: baroreflex ■ hemodynamics ■ hypertension ■ pressoreceptors ■ sympathetic nervous system

Electric stimulation of the carotid sinus has been used recently in clinical trials to treat hypertensive patients resistant to pharmacological therapy.1,2 The rationale for supporting this approach is that electric activation of the carotid baroreflex leads to activation of the cardiac parasympathetic drive and inhibition of sympathetic activity to the heart and peripheral vessels.3 More recently, electric stimulation of the carotid sinus has emerged as a therapeutic tool in the management of heart failure as well.4,6

During electric stimulation of the carotid sinus in patients with resistant hypertension1,2 or with heart failure,4,6 the anatomic position of the carotid body may allow undesirable activation of the carotid chemoreceptors, a possibility raised by Zucker et al7 and based on studies in dogs with heart failure. It is well known that activation of chemoreceptors produces an increase in arterial pressure (sympathetic activation) and bradycardia (parasympathetic activation), as well as conspicuous ventilatory responses.5,8

Recently, an important role for carotid body chemoreceptors in the pathophysiology of cardiovascular disease has been suggested.10,11 Studies of carotid body deafferentation in rabbits12 and spontaneously hypertensive rats10 have demonstrated that removal of the carotid bodies is an effective means for sustained sympathoinhibition10,12 and may be considered as a therapeutic option, possibly transferable to humans for reduction of sympathetic drive, disordered breathing patterns, and incidence of arrhythmia in heart failure.12 Therefore, it can be hypothesized that the cardiocirculatory effects triggered by attendant chemoreflex activation during baroreflex activation therapy (BAT)13,14 could potentially interfere with the classical hemodynamic response (hypotension and bradycardia) caused by the activation of the baroreflex.15 Of note, the recent study from Alnima et al,16 using short-term electric activation settings of 2 and 4 minutes, provided remarkable information for BAT using the Rheos system. These authors observed that this electroceutical therapy in patients with...
resistant hypertension did not stimulate respiration at several electric device activation parameters suggesting that there is no attendant activation of carotid body chemoreceptors during device therapy. 1, 16

In our laboratory, studies on short-term electric stimulation of the aortic depressor nerve in conscious normotensive 17 or hypertensive 18 rats have provided important information on the autonomic regulation of arterial pressure and heart rate (HR) by the aortic baroreflex. In the current study, the methodological procedure for stimulating the aortic depressor nerve in conscious rats was adapted to stimulate the carotid sinus. Thus, simultaneous electric stimulation of the carotid sinus and carotid sinus nerve in conscious rats was combined with surgical procedures for the selective denervation of the carotid chemo 8, 19 and baroreceptors (unpublished data) to investigate the role played by the peripheral (carotid body) chemoreflex during combined electric activation of the carotid sinus and carotid sinus nerve.

The aim of the current study was to investigate whether the attendant activation of carotid chemoreceptor afferents attenuates the reflex hypotension caused by combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. To accomplish this, an experimental model with selective denervation of carotid chemo 8, 19 and baroreceptors (unpublished data) in rats was used. By means of this experimental approach, it was possible to examine the relative contribution of both baroreceptors and chemoreceptors on the hemodynamic parameters of arterial pressure and HR in response to combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. It is possible that these finding will provide useful insight for the clinical use of electric stimulation of the carotid sinus as a therapeutic tool in patients with resistant hypertension and heart failure.

Methods

All procedures used in this study were reviewed and approved by the Committee of Ethics in Animal Research of the Medical School of Ribeirão Preto, University of São Paulo (Protocol No. 143/2013).

Surgical Procedures and Experimental Groups

The surgical procedures were performed under ketamine/xylazine anesthesia. All rats were implanted with polyethylene catheters into the left femoral artery and vein for arterial pressure recording and drug administration and underwent right side carotid chemoreceptor denervation as described elsewhere. 5 Before the implantation of a bipolar stainless steel electrode around the left carotid sinus and the carotid sinus nerve (Figure 1), 27 rats were assigned into 4 experimental groups:

1. Control group (CONT; n=7): Left carotid bifurcation was maintained intact.
2. Chemoreceptor denervation group (CHEMO-X; n=7): Left carotid body denervation was performed by cutting off the carotid body artery. 6
3. Baroreceptor denervation group (BARO-X; n=6): Left carotid baroreceptor denervation was performed by carefully cutting off the baroreceptor afferents from the carotid sinus.
4. Carotid bifurcation denervation group (TOTAL-X; n=7): Left carotid chemoreceptor and baroreceptor denervation were performed according to the procedures described in items 2 and 3 above.

Detailed surgical procedures are provided in the online-only Data Supplement.

Experimental Protocol

Twenty-four hours after surgical procedures, conscious freely moving rats had the pulsatile arterial pressure recorded during 20 minutes under baseline conditions, followed by successive 20 s long sessions of combined electric stimulation of the carotid sinus and the carotid sinus nerve (voltage: 5 V; pulse width: 1 ms; frequency: 15, 30, 60, and 90 Hz, applied in a random order). At least a 5-minute interval was observed for each period of stimulation. Baseline values of mean arterial pressure (MAP) and HR were collected from a 20-s period which preceded the beginning of each frequency of electric stimulation of the carotid sinus. The maximum responses in MAP and HR to electric stimulations were recorded.

Expanded Methods section is provided in the online-only Data Supplement.

Results

Basal Hemodynamics

Baseline values of systolic arterial pressure, diastolic arterial pressure, MAP, and HR from all groups are shown in the Table. The hemodynamic parameters were not statistically different among all groups.

Hemodynamic Responses to Combined Electric Stimulation of the Left Carotid Sinus and the Carotid Sinus Nerve

Typical traces of the responses of pulsatile arterial pressure, MAP (white line; top), and HR (bottom) to 20-s combined

Figure 1. Schematic drawing of the position of the bipolar electrodes on left carotid sinus of the rat. APA indicates ascending pharyngeal artery; BA, baroreceptor afferents; CA, chemoreceptor afferents; CB, carotid body; CBA, carotid body artery; CC, carotid canal; CCA, common carotid artery; CS, carotid sinus; CSN, carotid sinus nerve; ECA, external carotid artery; GPN, glossopharyngeal nerve; ICA, internal carotid artery; LA, lingual artery; OA, occipital artery; PA, pterygopalatine artery; PLF, posterior lacerated foramen; and SCG, superior cervical ganglion. Adapted from Sapru and Krieger 20 with permission of the publisher. Copyright © 1977, The American Physiological Society. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
The major new finding obtained from conscious rats was the clear-cut demonstration that chemoreceptors, as well as baro-receptors, were transiently activated during combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. The results have shown that when the carotid bifurcation was intact (ie, in the CONT group), combined electric stimulation of the carotid sinus and the carotid sinus nerve elicited a significant hypotensive response. This finding is in line with results obtained in dogs and drug-resistant hypertensive patients. Nevertheless, unlike the results seen in dogs and drug-resistant hypertensive patients, HR did not significantly decrease in intact conscious rats (the CONT group).

It is of interest to note that bilateral carotid body denervation (as in the CHEMO-X group) hampered the hemodynamic influences of the carotid chemoreceptors during combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. This procedure led to an augmented hypotensive response, at frequencies of 60 and 90 Hz, indicating that carotid chemoreceptor activation attenuated the hypotensive response elicited by the carotid baroreceptors in intact rats (ie, the CONT group). In addition, carotid baroreceptor denervation alone (ie, the BARO-X group) led to a significant hypertensive response, combined with marked bradycardia, after electric stimulation of the carotid sinus. This finding provides support for the hypothesis that carotid chemoreceptors were also activated during combined electric stimulation of the carotid sinus and the carotid sinus nerve. When the carotid sinus was electrically stimulated in the absence of carotid chemoreceptors and carotid baroreceptors (ie, the TOTAL-X group), no changes in hemodynamic

### Discussion

The major new finding obtained from conscious rats was the clear-cut demonstration that chemoreceptors, as well as baro-receptors, were transiently activated during combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. The results have shown that when the carotid bifurcation was intact (ie, in the CONT group), combined electric stimulation of the carotid sinus and the carotid sinus nerve elicited a significant hypotensive response. This finding is in line with results obtained in dogs and drug-resistant hypertensive patients. Nevertheless, unlike the results seen in dogs and drug-resistant hypertensive patients, HR did not significantly decrease in intact conscious rats (the CONT group).

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### Table

<table>
<thead>
<tr>
<th>Variables</th>
<th>CONT (n=7)</th>
<th>CHEMO-X (n=7)</th>
<th>BARO-X (n=6)</th>
<th>TOTAL-X (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>117±3</td>
<td>122±5</td>
<td>128±3</td>
<td>116±4</td>
</tr>
<tr>
<td>DAP</td>
<td>89±2</td>
<td>86±3</td>
<td>90±2</td>
<td>84±2</td>
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<tr>
<td>MAP</td>
<td>103±2</td>
<td>102±3</td>
<td>107±3</td>
<td>99±2</td>
</tr>
<tr>
<td>HR</td>
<td>369±10</td>
<td>352±15</td>
<td>343±9</td>
<td>347±8</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. BARO-X indicates denervated carotid baroreceptor; CHEMO-X, denervated carotid body; CONT, control; DAP, diastolic arterial pressure; HR, heart rate; MAP, mean arterial pressure; SAP, systolic arterial pressure; and TOTAL-X, denervated carotid body plus denervated carotid baroreceptor.

### Figure 2

A to D, Representative traces showing pulsatile arterial pressure (PAP), mean arterial pressure (white line; top), and heart rate (HR; bottom) responses to combined electric stimulation (5 V; 1 ms; 90 Hz) of the carotid sinus and carotid sinus nerve in conscious rats during 20 s. BARO-X indicates denervated carotid baroreceptor; CHEMO-X, denervated carotid body; CONT, control; ES, electric stimulation; and TOTAL-X, denervated carotid body plus denervated carotid baroreceptor.
(MAP and HR) responses were observed. This finding provides additional support for the role played by the afferents from chemo- and baroreceptors when activated during combined electric stimulation of the carotid sinus and the carotid sinus nerve.

About the reflex bradycardia, the CONT group showed no bradycardic response to combined electric stimulation of the carotid sinus and the carotid sinus nerve, whereas the chemoreceptor-denervated rats (the CHEMO-X group) showed consistent bradycardia. It is well known that baroreflex activation leads to vagally mediated bradycardia.15 In addition, as previously indicated, peripheral chemoreflex activation also leads to bradycardia involving the parasympathetic drive.8,19 Nevertheless, the lack of conspicuous bradycardia after combined electric stimulation of the carotid sinus and the carotid sinus nerve in rats from the CONT group is consistent with data from a previous report by Murata et al.24 These authors demonstrated that carotid chemoreceptor activation through intracarotid injection of sodium cyanide inhibited the baroreflex vagal bradycardia elicited by electric stimulation of the aortic depressor nerve in anesthetized rats. They concluded that a mechanism dependent on afferent...
inputs from the carotid chemoreceptors blunted the baroreceptor function.\textsuperscript{24}

It is interesting that the absence of carotid baroreceptors (ie, in the BARO-X group) seems to enable full electric activation of the carotid chemoreceptors, because under these conditions, in addition to the already mentioned hypertensive responses, a significant bradycardia was observed at frequencies of 30, 60, and 90 Hz. The interplay of baro- and chemoreceptor control of autonomic function has been investigated under both experimental\textsuperscript{25–27} and clinical\textsuperscript{9,28,29} research conditions. Somerset al\textsuperscript{9} provided direct evidence in humans of the unique ability of the chemoreflex to concurrently activate the parasympathetic drive to the heart and the sympathetic discharge to resistance vessels. Therefore, the remarkable bradycardia elicited by the activation of the chemoreflex in the absence of the baroreflex in conscious rats corroborates the hypothesis of a convergence of baro-

Of note, the current study did not investigate the respiratory function during the combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats. Nevertheless, Alnima et al\textsuperscript{16} demonstrated that a short-term (2–4 minutes) protocol for carotid BAT in patients with resistant hypertension caused no change in end-tidal carbon dioxide, partial pressure of carbon dioxide, breath duration, and breathing frequency, combined with highly significant decrease in mean arterial pressure during electric activation. These authors concluded that carotid BAT using the Rheos system involved no appreciable attend-

In conclusion, combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats produced a significant hypotensive response, which was potentiated by carotid body denervation. These findings demonstrated that concomitant carotid chemoreceptor activation blunts carotid baroreflex–mediated hypotension. Moreover, combined electric stimulation of the carotid sinus and the carotid sinus nerve in the absence of the carotid baroreceptors, but with intact carotid chemoreceptors, caused hypertension and bradycardia; this observation provides support for the hypothesis that carotid chemoreceptors are concomitantly activated by combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats.

Perspectives

Combined electric stimulation of the carotid sinus and the carotid sinus nerve in conscious rats is a useful experimental model that, from the translational point of view, may bring important insights into the mechanisms underlying BAT. Using this experimental model, the present study shows that combined electric stimulation of the carotid sinus and the carotid sinus nerve concomitantly activates both carotid baroreceptors and chemoreceptors, indicat-

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Disclosures

None.

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Perspectives

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3

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The role of chemoreceptor activation in hemodynamic responses to electrical stimulation of the carotid sinus in conscious rats

Pedro L. Katayama¹, Jaci A. Castania¹, Daniel P. M. Dias¹, Kaushik P. Patel², Rubens Fazan Jr.¹, Helio C. Salgado¹

¹ Department of Physiology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
² Department of Cellular & Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA

Corresponding author:
Helio C. Salgado, MD, PhD, Department of Physiology, Medical School of Ribeirão Preto, University of São Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirão Preto, São Paulo, Brazil; e-mail: hcsalgado@fmrp.usp.br; telephone: +55 16 3315 3201; fax: +55 16 3315 0221
Data Supplement

Methods

Animals
The experiments were conducted on male Wistar rats (270–310 g) from the Central Animal Facility of the Medical School of Ribeirão Preto, University of São Paulo. Rats were individually housed with unrestricted access to food and tap water and maintained in a light controlled (12:12 h light–dark cycle) environment. All procedures used in this study were reviewed and approved by the Committee of Ethics in Animal Research of the Medical School of Ribeirão Preto, University of São Paulo (Protocol N. 143/2013).

Surgical procedures
Rats were anesthetized with a mixture of ketamine/xylazine (50 mg/kg and 10 mg/kg, respectively) given intraperitoneally and then were subjected to a single surgical procedure. Polyethylene catheters (PE-50 attached to PE-10, Becton Dickinson, Sparks, MD, USA) were implanted in the left femoral artery and vein. Catheters were tunneled subcutaneously and exteriorized on the back of the nape. Then, the animals underwent a midline ventral neck incision, followed by exposure of the right common carotid artery and right carotid bifurcation. The right carotid body artery was carefully isolated under microscope magnification, tied, and cut distally to the ligation, according to the carotid chemoreceptor denervation procedures previously described by Franchini and Krieger.\(^1\) Next, the left common carotid artery and left carotid bifurcation were exposed, and different procedures were carried out depending on the experimental protocol. CHEMO-X rats were subjected to the left carotid chemoreceptor denervation. BARO-X rats were subjected to the left carotid baroreceptor denervation by carefully cutting barorreceptor afferents in the carotid sinus region via a modification of the surgical technique described by Krieger.\(^2\) TOTAL-X rats underwent both procedures described above.

Finally, all rats were implanted with a bipolar stainless steel electrode (0.008 inch bare, 0.011 inch Teflon coated; A-M Systems, Sequim, WA, USA) placed around the left carotid sinus including carotid sinus nerve, after the superior cervical ganglion was slightly shifted to expose the carotid sinus (Figure 1). Electrode leads consisted of 2 mm-long hooks separated by 2 mm. Next, the leads were carefully covered with silicone elastomer (Kwik-Sil; World Precision Instruments, Sarasota, FL, USA) to insure proper insulation. A 30 s period was allowed for complete polymerization of the silicone elastomer. The open ends of the wires were exteriorized on the back of the nape and soldered to a small plug (GF-6, Microtech, Boothwyn, PA, USA) and the ventral neck skin incision was sutured.

Experimental protocol
Twenty-four hours after surgical procedures, conscious, freely moving rats had the arterial catheter connected to a pressure transducer (MLT844; ADInstruments, Sydney, Australia), coupled to an analogical-to-digital interface (DI-220 Dataq Instruments, Akron, OH, USA). The pulsatile arterial pressure was continuously sampled (2 kHz) by an IBM PC equipped with a LabChart (ADInstruments, Sydney, Australia) that processed the signal by applying an algorithm to detect cycle-to-cycle inflection points and generated beat-by-beat time series of mean arterial pressure (MAP) and HR. An electrical stimulator (S48 Square Pulse Stimulator; Grass Products/Natus Neurology Incorporated, Middleton, WI, USA) was used to stimulate the carotid sinus. Rats that showed any sign of distress during the electrical stimulation were excluded from the study. Pulsatile arterial pressure was recorded during 20 min under baseline conditions, followed by successive 20 s long sessions of electrical stimulation of the carotid sinus (pulse width: 1 ms; voltage: 5 V at 15 Hz, 30 Hz, 60 Hz,
and 90 Hz, applied in a random order. At least a 5 min interval was observed for each period of stimulation. Baseline values of MAP and HR were collected from a 20 s period which preceded the beginning of each frequency of electrical stimulation of the carotid sinus. The maximum responses in MAP and HR to electrical stimulations were recorded.

**Test for efficacy of chemoreceptor and baroreceptor denervation**
Following the completion of all electrical stimulation protocols, rats from all groups were subjected to KCN (40 µg; IV) administration in order to test the efficacy of carotid body denervation surgery in CHEMO-X and TOTAL-X rats. Carotid chemoreceptors denervation was confirmed by the absence of hemodynamic responses to intravenous KCN administration. Additionally, to test the effectiveness of the selective left carotid baroreceptor denervation in BARO-X and TOTAL-X rats, a technique described by Sato and colleagues was adapted and carried out. Briefly, under ketamine/xylazine anesthesia, BARO-X and TOTAL-X rats were subjected to a midline ventral neck incision to identify and expose the left external and internal carotid, occipital, and carotid body arteries. The left common carotid artery was ligated proximally, and the distal portion was clamped with a bulldog spring clamp. Then, the left common carotid artery was immersed in a warm saline bath. In the present study, instead of using ball bearings for embolization of internal carotid and pterygopalatine arteries at the entrance at the skull, nylon monofilaments sutures were tied around both vessels. Polyethylene tubing (PE-50, Becton Dickinson) filled with saline and connected to a fluid-filled dome pressure transducer (MLT844, ADInstruments, Sydney, Australia) was inserted into the common carotid artery through an incision in the vascular wall made between the clamped and the ligated portions of the vessel. Next, when the polyethylene tubing reached the suture level, the threads were tied, and the bulldog spring clamp was released, allowing changes (increase and decrease) in the left carotid sinus pressure. The test consisted of changes in the left carotid sinus pressure between 60 mmHg and 180 mmHg, in 20 mmHg steps with 1 min duration. During this procedure, the pulsatile arterial pressure was continuously recorded. The absence of the left carotid baroreceptors in both groups was confirmed by a lack of hemodynamic responses to progressive changes in the left carotid sinus pressure.

**Statistical analysis**
Results are presented as mean ± standard error of the mean. The Kolmogorov–Smirnov test was applied to assess the normality of data distribution. Comparison between the mean values of MAP and HR before and after each session of electrical stimulation of the carotid sinus was performed by means of the paired t-test. MAP and HR responses to electrical stimulation of the carotid sinus, at each stimulation frequency, were compared among groups by one-way ANOVA followed by the post-hoc Newman–Keuls test. The significance level was set at P<0.05. Statistical analysis was performed using SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA).

**Results**

**Efficacy of the denervation of the carotid bodies and/or carotid baroreceptors**
Intravenous administration of KCN caused no hemodynamic responses (ΔMAP and ΔHR) in CHEMO-X and TOTAL-X groups; this observation confirmed that the chemoreceptors had been denervated. The CONT and BARO-X groups exhibited hypertensive (20±6 mmHg and 28±8 mmHg, respectively) and bradycardic (~54±13 bpm and ~54±13 bpm, respectively) responses following KCN administration. The absence of the left carotid baroreceptors in both the BARO-X and TOTAL-X groups was confirmed under anesthesia by the lack of hemodynamic (ΔMAP and ΔHR) responses to progressive increases in left carotid sinus pressure.
References

Figure S1. Frequencies of electrical stimulation of the carotid sinus and the carotid sinus nerve versus mean arterial pressure (MAP) response. Groups: control, CONT; denervated carotid body, CHEMO-X; denervated carotid baroreceptor, BARO-X; denervated carotid body plus denervated carotid baroreceptor, TOTAL-X. Data are expressed as mean ± standard error of the mean; *P<0.05 compared to CONT group; †P<0.05 compared to CHEMO-X group; ‡P<0.05 compared to BARO-X group.
Figure S2. Individual hemodynamic responses to combined electrical stimulation of the carotid sinus and the carotid sinus nerve with different frequencies (Hz). Groups: control (CONT, n=7); chemoreceptor denervated (CHEMO-X, n=7); baroreceptor denervated (BARO-X, n=6); total denervated (TOTAL-X, n=7). 15 Hz: panel A; 30 Hz: panel B; 60 Hz: panel C; 90 Hz: panel D. ΔMAP: change in mean arterial pressure; ΔHR: change in heart rate.