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Interaction between baroreflex and chemoreflex in the cardiorespiratory responses to stimulation of the carotid sinus/nerve in conscious rats

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ABSTRACT

Electrical stimulation of the carotid baroreflex has been thoroughly investigated for treating drug-resistant hypertension in humans. However, a previous study from our laboratory, performed in conscious rats, has demonstrated that electrical stimulation of the carotid sinus/nerve (CS) activated both the carotid baroreflex as well as the carotid chemoreflex, resulting in hypotension. Additionally, we also demonstrated that the carotid chemoreceptor deactivation potentiated this hypotensive response. Therefore, to further investigate this carotid baroreflex/chemoreflex interaction, besides the hemodynamic responses, we evaluated the respiratory responses to the electrical stimulation of the CS in both intact (CONT) and carotid chemoreceptors deactivated (CHEMO-X) conscious rats. CONT rats showed increased ventilation in response to electrical stimulation of the CS as measured by the respiratory frequency (fR), tidal volume (V_T) and minute ventilation (V_E), suggesting a carotid chemoreflex activation. The carotid chemoreceptor deactivation abolished all respiratory responses to the electrical stimulation of the CS. Regarding the hemodynamic responses, the electrical stimulation of the CS caused hypotensive responses in CONT rats, which were potentiated by the carotid chemoreceptors deactivation. Heart rate (HR) responses did not differ between groups. In conclusion, the present study showed that the electrical stimulation of the CS, in conscious rats, activates both the carotid baroreflex and the carotid chemoreflex driving an increase in ventilation and a decrease in AP. These findings further contribute to our understanding of the electrical stimulation of CS.

1. Introduction

Hypertension is a major risk factor for cardiovascular diseases, including coronary heart disease, heart failure and stroke (Benjamin et al., 2018; Whelton et al., 2018). Because cardiovascular diseases remain the leading cause of death worldwide (Benjamin et al., 2018; Roth et al., 2017), the concern with the arterial pressure (AP) of population is a current challenge. However, some factors such as unawareness of the condition and poor adhesion to treatment difficult the blood pressure control (Burnier, 2017; Chow, 2013). In addition, a considerable number of hypertensive patients do not respond to the pharmacological treatment (Sheppard et al., 2017). This condition is known as drug-resistant hypertension and is defined as uncontrolled AP (> 140/90 mm Hg) even under the treatment with three or more antihypertensive agents, from different classes, including a diuretic (Calhoun et al., 2008; Sheppard et al., 2017).

In this context, the electrical activation of the carotid baroreflex has emerged as a new treatment for drug-resistant hypertension; and more recently for heart failure as well (Doumas et al., 2012; Jordan et al., 2012). Activation of the carotid sinus baroreflex inhibits the sympathetic activity besides increasing the parasympathetic drive to the heart (Heusser et al., 2010; Kansal et al., 2016), determining the lowering of the AP levels. Clinical studies employing the electrical stimulation of the carotid baroreflex in drug-resistant hypertensive patients have shown promising results, reducing the AP acutely and chronically (De Leeuw et al., 2017; Illig et al., 2006; Tordoir et al., 2007). However, as first pointed out by Zucker et al. (2007), besides baroreflex activation, the electrical stimulation of the carotid sinus region in dogs could, concomitantly, activates the carotid chemoreflex. Activation of the carotid chemoreflex leads to increase in sympathetic activity, AP, and ventilation, while decreases HR (Franchini and Krieger, 1993; Freet et al., 2013). Thus, a potential carotid chemoreflex activation is certainly undesirable, mainly because it could counteract the baroreflex activation-mediated sympathetic inhibition, aimed by the electrical stimulation of the carotid sinus. In this context, Alnima et al. (2015) carried out a study to test whether the electrical stimulation of the

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carotid sinus, in drug-resistant hypertensive patients, would simultaneously activate the baroreflex and the chemoreflex. Their data suggested that the electrical stimulation of the carotid sinus, in humans, appears to activate only the carotid baroreflex; because it was observed a hypotensive response without any relevant respiratory change.

On the other hand, we have demonstrated that, in conscious rats, the electrical stimulation of the carotid sinus/nerve (CS) decreased the AP (Katayama et al., 2015), reproducing the findings of studies carried out in dogs (Lohmeier et al., 2004) and humans (Bisognano et al., 2011; Illig et al., 2006). In addition, we found that the carotid chemoreceptor deactivation potentiated the hypotensive response to the electrical stimulation of the CS, whereas the carotid baroreceptor deactivation caused a hypertensive response (Katayama et al., 2015). Taken together, our previous findings suggested that the electrical stimulation of the CS, in conscious rats, simultaneously activated the carotid baroreflex as well as the carotid chemoreflex (Katayama et al., 2015). However, the respiratory responses to the electrical stimulation of the CS, which would further confirm this hypothesis, were not evaluated at that time. Therefore, to address this issue, we investigated in the present study the respiratory responses to the electrical stimulation of the CS, in conscious rats, and the interaction between the carotid baro- and chemoreceptors on these responses.

2. Materials and methods

2.1. Ethical approval

All experimental procedures were approved by the Ethical Committee on Animal Experimentation of the Ribeirão Preto Medical School, University of São Paulo (Protocol # 143/2013). The authors are in agreement with the *Autonomic Neuroscience: Basic and Clinical* ethical principles and guidelines and, hence, the present study was performed in accordance with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines.

2.2. Animals

The study was carried out on male Wistar rats (270–310 g) from the Central Animal Facility of the Ribeirão Preto Medical School, University of São Paulo. Rats were individually housed in plastic cages, provided with access to food and water *ad libitum* and kept in a controlled 12:12 h light–dark cycle environment.

2.3. Surgical procedures

All rats were prepared for AP monitoring, intravenous drug infusions and left-side electrical stimulation of the carotid sinus as described in a previous study from our laboratory (Katayama et al., 2015). Animals from the control group (CONT; $n = 7$) were implanted with a bipolar stainless-steel electrode around the intact left carotid sinus encompassing the carotid sinus nerve. Animals from the carotid chemoreceptor deactivated group (CHEMO-X; $n = 7$), before the electrode implantation, were first subjected to the ligation and sectioning of the left carotid body artery for chemoreceptor deactivation. In addition, animals from both CHEMO-X and CONT were subjected to the right carotid chemoreceptor deactivation. This procedure was needed to allow the confirmation of the left carotid chemoreceptor deactivation in CHEMO-X rats or the integrity of the left carotid chemoreceptor in CONT rats, at the end of the experiments, by an intravenous injection of KCN (40 μg per rat).

The surgeries were performed in a single-session procedure under general anesthesia with a cocktail of ketamine (50 mg kg^{-1}, I.P.; União Química Farmacêutica Nacional S/A, Embu-Guaçu, SP, Brazil) and xylazine (10 mg kg^{-1}, I.P.; Hertape Calier Saúde animal S/A, Juatuba, MG, Brazil). First, polyethylene catheters (PE-50 attached to PE-10, Becton Dickinson, Sparks, MD, USA) were inserted into the left femoral artery and vein for AP monitoring and drug administration, respectively. The catheters were tunnelled subcutaneously and exteriorized in the interscapular region on the nape of the rats. Next, through a midline ventral neck incision, the right carotid bifurcation was exposed and under microscope magnification (MC-M902MFZ; D.F.V. Com. Ind. Ltda, São Paulo, SP, Brazil) the right carotid body artery was isolated, tied, and cut distally to the ligation; in accordance with the carotid chemoreceptor denervation method described by Franchini and Krieger (1993). CHEMO-X rats were additionally subjected to the left-side carotid chemoreceptor denervation. Finally, for the electrical stimulation of the carotid sinus in conscious rats, a previously reported approach developed by our laboratory was used (Katayama et al., 2015). Briefly, the left carotid bifurcation was isolated and the superior cervical ganglion was carefully shifted to allow the visualization of the carotid sinus. A bipolar stainless-steel electrode (0.008 in. bare, 0.011 in. Teflon coated; A-M Systems, Sequim, WA, USA) was implanted around the left carotid sinus encompassing the carotid sinus nerve. The electrode was carefully insulated with silicon elastomer (Kwik-Sil; World Precision Instruments, Sarasota, FL, USA). The open ends of the wires were exteriorized in the interscapular region in the back and welded to a small plug (GF-6; Microtech, Boothwyn, PA, USA). Postoperatively, the rats received a single dose of flunixin meglumine (2.5 mg kg^{-1}, I.M.; Schering-Plough, Cotia, SP, Brazil).

2.4. Data acquisition and experimental protocol

Following surgical procedures, the rats were individually housed and allowed to adapt to the experimental room (12:12 h light–dark cycle, 25 °C) overnight. Twenty-four hours after surgery, each rat was individually placed inside a plethysmography chamber (5-L volume) and allowed to adapt for 60 min before beginning the experimental protocol. The experimental protocol was conducted on conscious, freely moving rats.

For respiratory measurements, the whole-body plethysmography method described by Bartlett and Tenney (1970) was used. Briefly, the pressure oscillations within the plethysmography chamber caused by the rat's respiration were detected and amplified by a differential pressure transducer (ML141 Spirometer; ADInstruments, Bella Vista, NSW, Australia) connected to an analog-digital interface (PowerLab system; ADInstruments, Bella Vista, NSW, Australia). To calibrate the differential pressure transducer, a known volume of air (1 ml) was injected into the plethysmography chamber. The temperature at the experimental room and within the chamber was constantly monitored. For AP recordings the arterial catheter was connected to a pressure transducer (MLT844; ADInstruments, Sydney, NSW, Australia), the AP signal was amplified (ML224; ADInstruments, Bella Vista, NSW, Australia) and sampled at 2 kHz by the PowerLab system. For the electrical stimulation of the CS, the small plug exteriorized at the back of the rats was connected to an electrical stimulator (S48 Square Pulse Stimulator; Grass Products/Natus Neurology Incorporated, Middleton, WI, USA). The experimental protocol consisted of respiratory and AP signals recordings during baseline conditions and in response to continuous electrical stimulation of the CS (pulse width: 1 ms; voltage: 3 V; duration: 20 s) at 4 different frequencies of stimulation (15, 30, 60 and 90 Hz) applied in a random order with at least 5 min intervals between each session of stimulation. During intervals, the plethysmography chamber was opened to avoid CO_2 accumulation and a new volume calibration was performed at each subsequent measurement. At the end of the experimental protocol, the rats were subjected to the administration of KCN (40 μg; IV) in order to test the effectiveness of the left carotid chemoreceptor denervation in CHEMO-X rats or the integrity of left carotid chemoreceptor in CONT rats. Carotid chemoreceptor deactivation was confirmed by the lack of respiratory and cardiovascular responses to KCN, whereas its integrity was confirmed by increases in respiration and AP accompanied by bradycardia (Franchini and

Krieger, 1993).

2.5. Data analysis

The respiratory parameters evaluated in the present study were respiratory frequency (fR), tidal volume (V_T) and minute ventilation (V_E). V_T was calculated using the formula described by Malan (1973) and V_E was calculated as the product between fR and V_T. Values of mean arterial pressure (MAP) and HR were obtained from the recorded pulsatile AP signals. Baseline values of fR, V_T, V_E, MAP and HR were averaged from 1 minute-period recordings before any electrical stimulation of the carotid sinus. In addition, for each studied frequency of electrical stimulation of the CS, a respective baseline was calculated using the average value from the 20 s period that preceded the stimulus. Regarding the responses to electrical stimulation of the CS, the whole 20 s period of each stimulation session was taken into account and the maximum responses (1 s) of fR, V_T, V_E, MAP and HR were assessed. For the time course analysis, the changes in V_T, MAP and HR were averaged every second during the onset of stimulation.

2.6. Statistical analysis

Results are expressed as mean ± SD and comparisons between CONT and CHEMO-X were performed using Student's unpaired *t*-test. For time course analysis, a two-way ANOVA with Tukey's multiple comparisons post-test was used. 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).

3. Results

3.1. Baseline respiratory and cardiovascular parameters in CONT and CHEMO-X rats

As shown in Fig. 1 the baseline values of respiratory and cardiovascular parameters were similar in CONT and CHEMO-X rats: fR (86 ± 18 *versus* 93 ± 18 breaths/min; P = 0.475; Fig. 1A), V_T (9.8 ± 2 *versus* 8.8 ± 1 ml/kg; P = 0.235; Fig. 1B), V_E (859 ± 303 *versus* 815 ± 158 ml/kg/min; P = 0.740; Fig. 1C), MAP (104 ± 7 *versus* 103 ± 7 mm Hg; P = 0.816; Fig. 1D) and HR (348 ± 30 *versus* 356 ± 28 beats/min; P = 0.627; Fig. 1E).

3.2. Respiratory and cardiovascular responses to electrical stimulation of the CS in CONT and CHEMO-X rats

As illustrated in Fig. 2A the electrical stimulation of the CS increased ventilation, decreased AP and did not change HR in CONT rats. Nevertheless, the carotid chemoreceptor deactivation abolished the increase in ventilation, decreased AP and elicited a small bradycardic response as illustrated in Fig. 2B.

The increase in ventilation observed in CONT rats was abolished in CHEMO-X rats at all frequencies of electrical stimulation of the CS (Fig. 3). The fR responses to electrical stimulation of the CS at 15, 30, 60 and 90 Hz were significantly greater in CONT than in CHEMO-X rats, respectively: 27 ± 21 *versus* −1 ± 15 breaths/min (P = 0.015; Fig. 3A), 40 ± 21 *versus* 3 ± 11 breaths/min (P = 0.001; Fig. 3B), 30 ± 18 *versus* 4 ± 8 breaths/min (P = 0.004; Fig. 3C) and 31 ± 17 *versus* 3 ± 19 breaths/min (P = 0.012; Fig. 3D). Similarly, V_T responses to electrical stimulation of the CS were greater in CONT when compared to CHEMO-X rats at 15, 30, 60 and 90 Hz, respectively: 2.6 ± 2 *versus* 0.4 ± 0.5 ml/kg (P = 0.015; Fig. 3E), 3.5 ± 2 *versus* 0.5 ± 0.9 ml/kg (P = 0.006; Fig. 3F), 4.8 ± 2.1 *versus* 0.9 ± 1.6 ml/kg (P = 0.002; Fig. 3G) and 4.2 ± 2.5 *versus* 0.6 ± 1.5 ml/kg (P = 0.008; Fig. 3H). Accordingly, changes in V_E in response to the electrical stimulation of the CS were larger in CONT than in CHEMO-X rats at 15, 30, 60 and 90 Hz, respectively: 596 ± 417 *versus* 39 ± 172 ml/kg/min (P = 0.007; Fig. 3I), 820 ± 465 *versus* 80 ± 191 ml/kg/min (P = 0.002; Fig. 3J), 672 ± 363 *versus* 100 ± 217 ml/kg/min (P = 0.004; Fig. 3K) and 665 ± 348 *versus* 60 ± 256 ml/kg/min (P = 0.003; Fig. 3L).

Regarding the cardiovascular responses to electrical stimulation of the CS, CONT rats showed less pronounced hypotension than the CHEMO-X rats at 60 and 90 Hz, respectively: −24 ± 7 *versus* −39 ± 16 mm Hg (P = 0.045; Fig. 4C) and −21 ± 7 *versus* −40 ± 14 mm Hg (P = 0.009; Fig. 4D). The hypotensive responses to electrical stimulation of the CS did not differ between CONT and CHEMO-X rats at either 15 Hz (−13 ± 4 *versus* −22 ± 12 mm Hg, respectively P = 0.095; Fig. 4A) or 30 Hz (−18 ± 7 *versus* −25 ± 9 mm Hg, respectively; P = 0.138; Fig. 4B). Heart rate changes during electrical stimulation of the CS were not different between CONT and CHEMO-X rats at 15, 30, 60 and 90 Hz, respectively: −10 ± 29 *versus* −25 ± 22 beats/min; P = 0.301; Fig. 4E, −12 ± 23 *versus* −45 ± 43 beats/min; P = 0.101; Fig. 4F, −13 ± 27 *versus* −35 ± 33 beats/min; P = 0.192; Fig. 4G and −24 ± 17 *versus* −46 ± 37 beats/min; P = 0.179; Fig. 4H.

The time course of respiratory and cardiovascular changes during

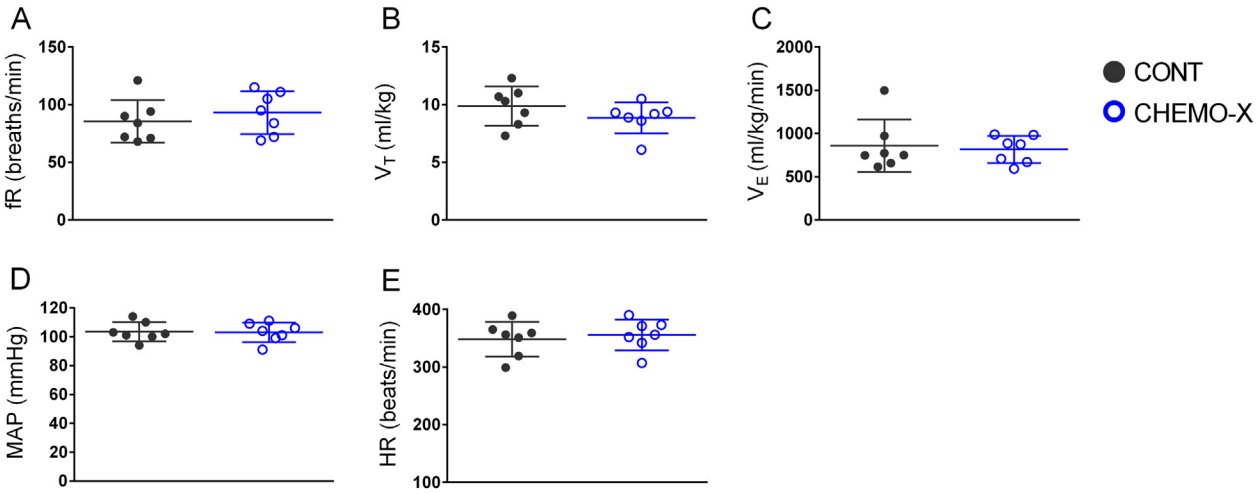

Fig. 1. Baseline respiratory and cardiovascular parameters in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats.
Respiratory frequency (fR; panel A), tidal volume (V_T; panel B), minute ventilation (V_E; panel C), mean arterial pressure (MAP; panel D) and heart rate (HR; panel E) from CONT and CHEMO-X rats during baseline conditions.

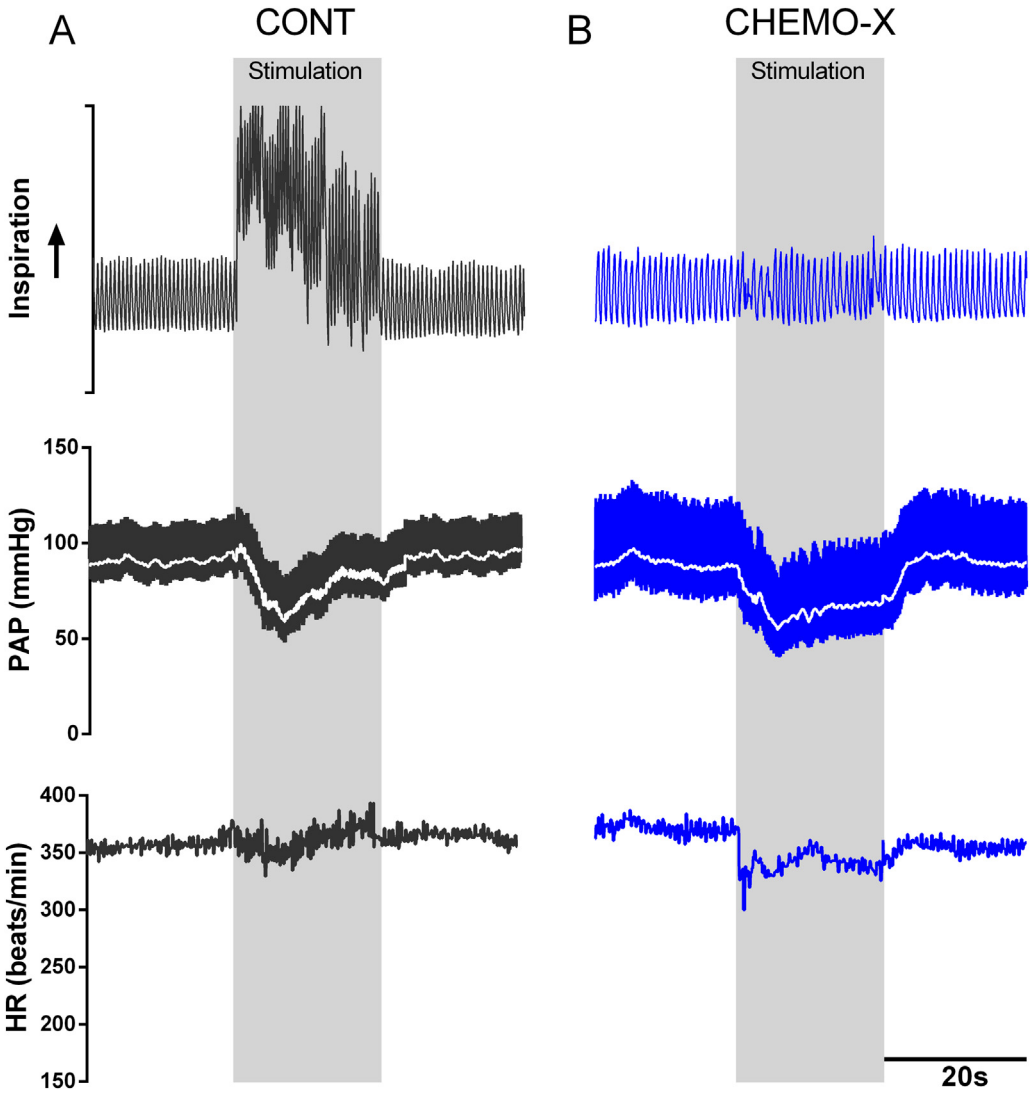

Fig. 2. Typical respiratory and cardiovascular responses to electrical stimulation of the carotid sinus/nerve (CS) in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats.
Representative tracings from CONT (A) and CHEMO-X rats (B). Upper panel shows the plethysmographic recording of respiration; Middle panel shows the pulsatile (PAP) and mean arterial pressure (white line); Lower panel shows the heart rate (HR) before, during (grey bar) and after the electrical stimulation of the CS (60 Hz, 20 s duration).

the onset of electrical stimulation of the CS is illustrated in Fig. 5. CONT rats showed greater changes in V_T throughout the whole period of stimulation as compared to CHEMO-X rats ($P < 0.05$, Fig. 5A). On the other hand, the hypotensive responses were significantly greater in CHEMO-X than in CONT rats from 4 s to the end of stimulation ($P < 0.05$, Fig. 5B), except at 7 s. In addition, the peak hypotensive response was reached faster in CHEMO-X rats (5 s) than in CONT rats (7 s). The changes in HR over time did not differ between CONT and CHEMO-X (Fig. 5C).

3.3. Integrity of the carotid chemoreceptors in CONT and effectiveness of carotid chemoreceptors deactivation in CHEMO-X rats

Intravenous administration of KCN elicited increases in ventilation and MAP combined with bradycardia in CONT rats, while it did not affect neither the ventilation nor the cardiovascular parameters in CHEMO-X rats (Fig. 6). The magnitude of the responses to KCN was significantly greater in CONT as compared to CHEMO-X rats, respectively: fR (23 ± 14 *versus* 5 ± 6 breaths/min; P = 0.008; Fig. 6A), V_T (3.1 ± 1.3 *versus* 0.1 ± 1 ml/kg; $P < 0.001$; Fig. 6B), V_E (556 ± 205 *versus* 27 ± 110 ml/kg/min; $P < 0.001$; Fig. 6C), MAP (25 ± 12 *versus* −4 ± 5 mm Hg; $P < 0.001$; Fig. 6D) and HR (−90 ± 84 *versus* 2 ± 16 beats/min; P = 0.016; Fig. 6E).

4. Discussion

To the best of our knowledge, this is the first study to investigate both respiratory and cardiovascular responses to electrical stimulation of the CS in conscious animals, as well as the interaction between the carotid baro- and chemoreceptors in these responses. The present study strengthens the notion that the electrical stimulation of the CS in conscious rats concomitantly activates the carotid baroreceptors as well as the carotid chemoreceptors. First, the electrical stimulation of the CS in CONT rats caused hypotensive effects combined with increased ventilation. Second, the increase in ventilation triggered by electrical stimulation of the CS in CONT rats was absent in CHEMO-X rats. In addition, as previously demonstrated by our laboratory (Katayama et al., 2015), the carotid chemoreceptor deactivation caused a more pronounced hypotensive response to electrical stimulation of the CS.

Of note, only recently, studies carried out in both experimental

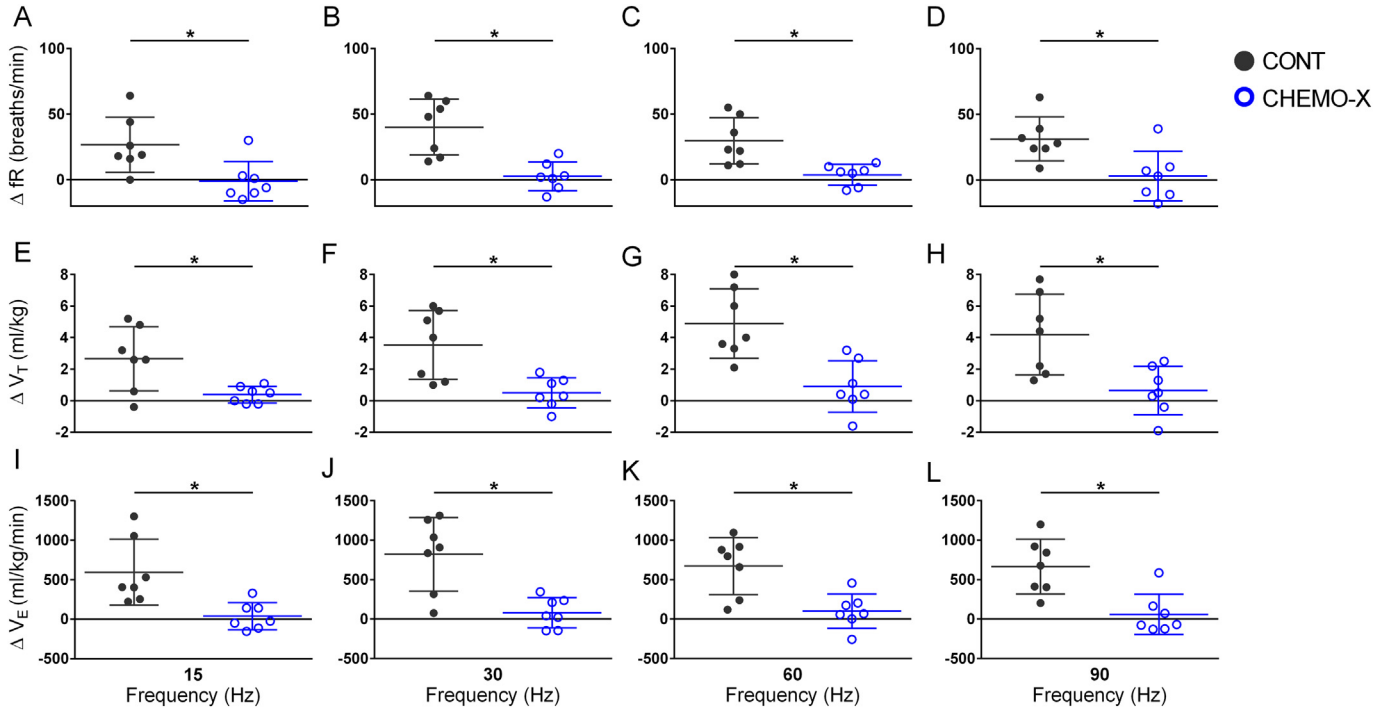

Fig. 3. Respiratory responses to electrical stimulation of the carotid sinus/nerve (CS) in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats. Changes in respiratory frequency (ΔfR; panels A, B, C and D), tidal volume (ΔV_T; panels E, F, G and H) and minute ventilation (ΔV_E; panels I, J, K and L) in response to electrical stimulation of the CS (15, 30, 60 and 90 Hz) from control (CONT) and chemoreceptors deactivated (CHEMO-X) rats. *P < 0.05.

animals (Katayama et al., 2015) and humans (Alnima et al., 2015) have been designed to test the hypothesis that electrical stimulation of the carotid sinus baroreflex could simultaneously activate the carotid baro- and chemoreceptors. A previous study from our laboratory (Katayama et al., 2015) showed that electrical stimulation of the CS in conscious rats caused a hypotensive response, which was potentiated by carotid chemoreceptor deactivation; this finding indicated that the carotid chemoreflex was counteracting the carotid baroreflex-mediated hypotension in control rats. In addition, rats subjected to selective carotid baroreceptor denervation presented an increase in AP in response to electrical stimulation of the CS, indicating a single chemoreflex activation (Katayama et al., 2015). However, our previous study did not evaluate the respiratory responses to electrical stimulation of the CS. In order to address this issue, we evaluated in the present study the respiratory responses to electrical stimulation of the CS in conscious rats. Of note, at all frequencies of electrical stimulation of the CS it was observed a higher response of fR, V_T and V_E in CONT as compared to CHEMO-X rats. These findings further strengthened the notion that electrical stimulation of the CS in conscious rats elicits simultaneous carotid baroreflex/chemoreflex activation, determining an interaction between these important reflexes. On the other hand, Alnima et al. (2015) found that in humans, the electrical activation of the carotid sinus baroreflex lowered AP without any relevant change in ventilation. In their study, drug-resistant hypertensive patients previously implanted with the Rheos system for electrical activation of the carotid sinus baroreflex (Bisognano et al., 2011; Tordoir et al., 2007) were evaluated. These patients were subjected to several sets of stimulation, with frequencies varying from 20 to 90 Hz and intensities of 3 and 6 V;

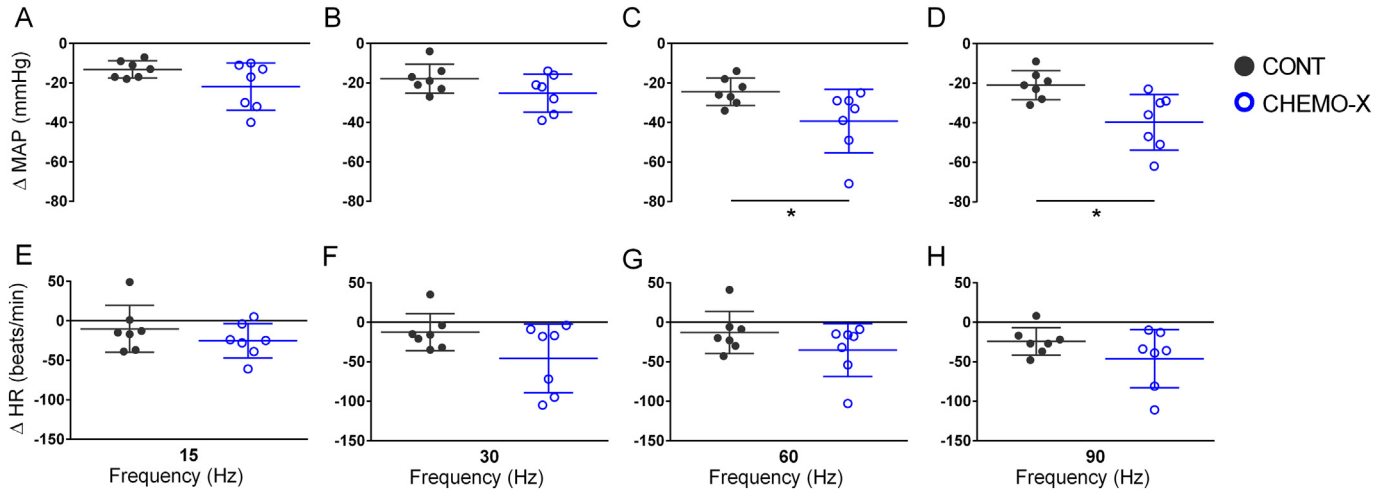

Fig. 4. Cardiovascular responses to electrical stimulation of the carotid sinus/nerve (CS) in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats. Changes in mean arterial pressure (ΔMAP; panels A, B, C and D) and heart rate (ΔHR; panels E, F, G and H) in response to electrical stimulation of the CS (15, 30, 60 and 90 Hz) in CONT and CHEMO-X rats. *P < 0.05.

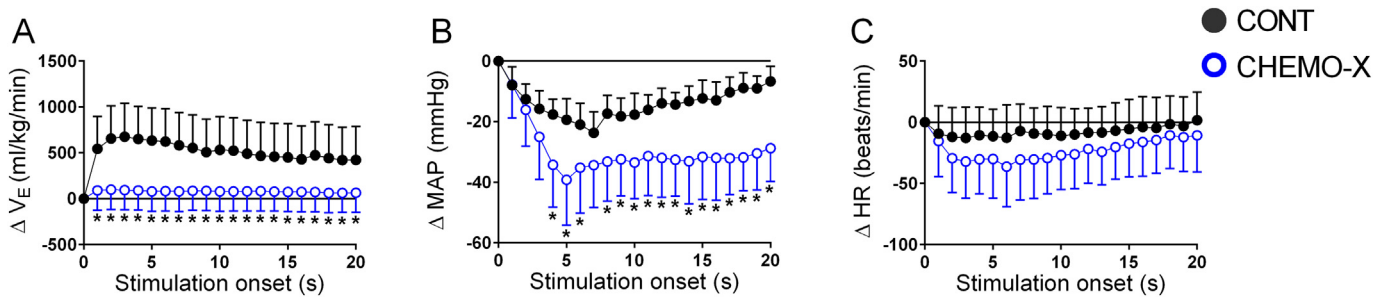

Fig. 5. Typical time course of respiratory and cardiovascular responses to electrical stimulation of the carotid sinus/nerve (CS) in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats.
Changes over time in minute ventilation (ΔV_E, panel A), mean arterial pressure (ΔMAP, panel B) and heart rate (ΔHR, panel C) during the onset of electrical stimulation of the CS (60 Hz) in CONT and CHEMO-X rats. *P < 0.05 *vs* CONT.

while the AP, HR and respiratory variables were conspicuously monitored (Alnima et al., 2015). These authors found that the electrical activation of the carotid sinus baroreflex caused a significant decrease in AP but did not affect fR, expiratory tidal volume, V_E, end-tidal CO_2 and arterial partial pressure CO_2. Therefore, they reached the conclusion that the electrical stimulation of the carotid sinus, in humans, activates only the carotid baroreceptors, without activating the carotid chemoreceptors.

One possible reason for the divergence between the present study and that from Alnima et al. (2015), concerning the respiratory responses to electrical stimulation of the carotid sinus, is related to the position of the electrodes in the carotid sinus. In the current study we have used an approach developed in our laboratory in which the electrode bared tips are implanted around the carotid sinus encompassing the carotid sinus nerve (For a schematic illustration see Katayama et al., 2015). Because the carotid sinus nerve carries both carotid baro- and chemoreceptor afferents (McDonald, 1983; Porzionato et al., 2018), the simultaneous carotid baroreflex/chemoreflex activation, observed in the present study, is very plausible. In fact, the results found in the present study are in line with previous studies that investigated the physiological effects of electrical stimulation of the isolated carotid sinus nerve. Eldridge (1972) reported that the electrical stimulation of the carotid sinus nerve in anesthetized cats increased ventilation. However, Eldridge (1972) observed that the respiratory responses were dependent on the timing of the applied stimulus. This author found that when intermittent electrical stimuli to the carotid sinus nerve was applied during the inspiration, or when electrical stimuli to the carotid sinus nerve were applied continuously throughout the respiratory cycles, a clear increase in V_T and fR was observed. However, when the intermittent stimuli were applied during the expiratory phases, no obvious effect on fR or V_T was observed, but only a prolonged expiration. Levy and Zieske (1976) found that electrical stimulation of the carotid sinus nerve in anesthetized dogs increased both the respiratory depth and frequency, while the continuous (steady) stimulation was more effective than the intermittent stimulation for evoking these responses. It is important to highlight that, in the present study, a continuous pattern of stimulation (20 s duration) was applied. In contrast with the abovementioned investigations, the study of Alnima et al. (2015) used a “glove-like” electrode implanted at the surface of the carotid sinus wall (For an illustration, see Tordoir et al., 2007). It could be that the electrode used in the study of Alnima et al. (2015) elicits a selective activation of the baroreceptor afferents in the carotid sinus, without spreading electrical current to adjacent tissues (*e.g.* carotid chemoreceptor afferents), explaining the lack of respiratory changes. This mechanism might also explain the small, or even absent, increase in the respiratory rate in response to the electrical stimulation of the carotid sinus in conscious dogs, as reported by Lohmeier et al. (2004), who described that the electrodes were also implanted around the carotid sinus wall.

With respect to the hypotensive response to the electrical stimulation of the CS, the results of the present study are in line with our previous report (Katayama et al., 2015). We have demonstrated that the

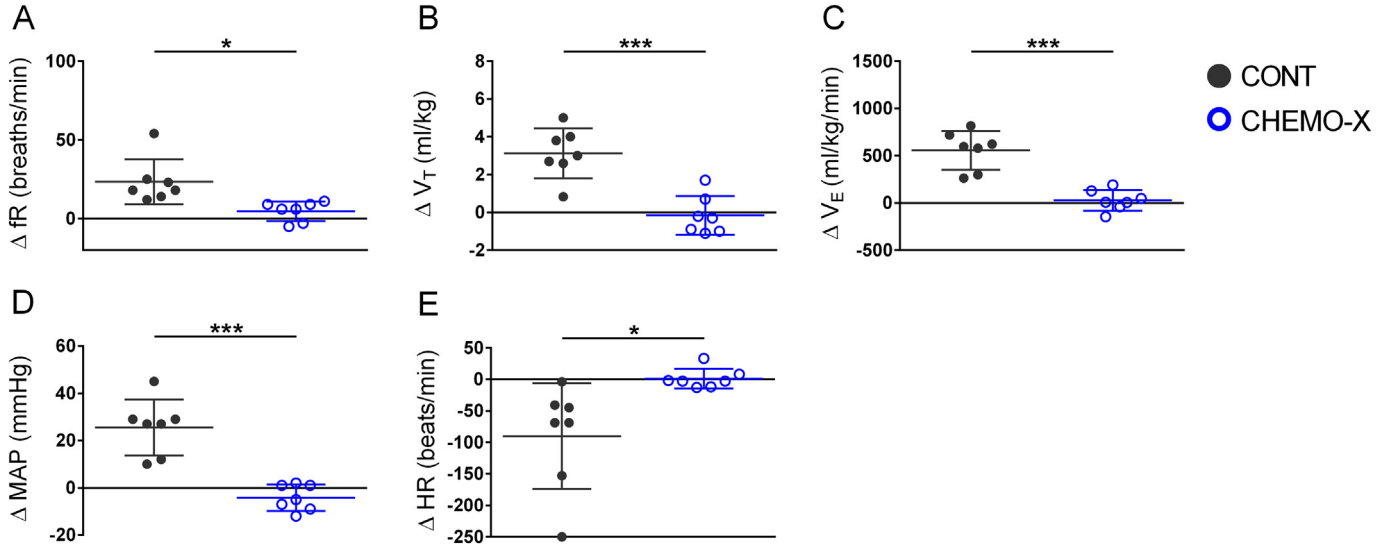

Fig. 6. Respiratory and cardiovascular responses to intravenous injection of KCN in control (CONT) and chemoreceptor deactivated (CHEMO-X) rats.
Changes in respiratory frequency (ΔfR; panel A), tidal volume (ΔV_T; panel B), minute ventilation (ΔV_E; panel C), mean arterial pressure (ΔMAP; panel D) and heart rate (ΔHR; panel E) in response to KCN (40 μg per rat, I.V.) in CONT and CHEMO-X rats. *P < 0.05; ***P < 0.001.

carotid chemoreceptor deactivation also potentiated the hypotensive response to electrical stimulation of the CS at the higher frequencies of stimulation (60 and 90 Hz) as compared to CONT rats. Also, the hypotensive response observed in the CONT rats of the present study are comparable to those reported in studies conducted in dogs (Lohmeier et al., 2004, 2007) and humans (Alnima et al., 2015; Illig et al., 2006; Tordoir et al., 2007). A new important observation of the present study was that the peak hypotensive response occurred earlier in CHEMO-X than in CONT rats. In addition, the hypotensive response appears to last the whole period of stimulation in CHEMO-X whereas it tends to return to baseline values in CONT during the last 5 s of stimulus. This data suggests that the carotid chemoreceptor activation in CONT affects not only the magnitude but also the kinetics of arterial pressure changes during the electrical stimulation of the CS.

However, the HR response to electrical stimulation of the carotid sinus is somewhat controversial. In the present study the changes in HR did not differ between CONT and CHEMO-X rats at any frequency of stimulation; however, in our previous report there was a difference between CONT and CHEMO-X rats at the frequency of 90 Hz (Katayama et al., 2015). Moreover, it is remarkable the absence of substantial bradycardia in CONT rats in both the present and previous study from our laboratory (Katayama et al., 2015); since both carotid baroreflex and chemoreflex activation should cause a reflex bradycardia (Franchini et al., 1996). These results are not in line with the studies performed in dogs (Lohmeier et al., 2004, 2007, 2010) and humans (De Leeuw et al., 2017; Illig et al., 2006; Tordoir et al., 2007; Wustmann et al., 2009), because they reported a significant bradycardic response to electrical stimulation of the carotid sinus. In contrast to the above-mentioned studies conducted in humans, Alnima et al. (2015) only observed a decrease in HR in 1 (60 Hz, 6 V) out of 6 different combinations of stimulation parameters applied in their study. As previously addressed in Katayama et al. (2015), the lack of HR responses to electrical stimulation of the CS in CONT rats could be supported by experiments in which simultaneous baroreflex/chemoreflex activation resulted in abolition of the expected bradycardic responses, potentially by centrally-mediated mechanisms (Kongo et al., 1999; Murata et al., 1999). Murata et al. (1999) showed that the baroreflex vagal bradycardia caused by electrical stimulation of the aortic depressor nerve, was markedly reduced by a simultaneous peripheral chemoreflex stimulation, accomplished by intracarotid injection of sodium cyanide in rats. This effect was abolished after carotid sinus nerve sectioning. In addition, the authors found that the stimulation of the peripheral chemoreflex had no effect on a peripherally originated vagal bradycardia elicited by electrical stimulation of a peripheral cut end of cervical vagus nerve. The results of Murata et al. (1999) support the notion that, in rats, peripheral chemoreflex stimulation inhibits the baroreflex vagal bradycardia and that this effect is dependent on the carotid sinus nerve and central mechanisms, what could help to explain the different HR responses observed in the present study and those reported by studies carried out in dogs (Lohmeier et al., 2004, 2007) and humans (De Leeuw et al., 2017; Illig et al., 2006; Tordoir et al., 2007; Wustmann et al., 2009).

It is important to highlight that the experimental model of electrical stimulation of the CS in rats, used in the present study, was developed by our group and first described in our previous work (Katayama et al., 2015). Therefore, we were not able to find standard parameters of CS stimulation for rats in the literature. However, based in the similar magnitude of the hypotensive responses, we believe that our rat model for electrical stimulation of the CS is comparable to the dog model described by Lohmeier et al. (2004) and, also, to human studies (Tordoir et al., 2007). Lohmeier et al. (2004) using an intensity of 6 V and a frequency of 100 Hz observed a 20 to 25 mm Hg decrease in mean arterial pressure in dogs. Regarding the human studies, Tordoir et al. (2007) reported maximal acute reductions of systolic blood pressure from 177 mm Hg to 141 mm Hg (−36 mm Hg) and diastolic blood pressure from 99 mm Hg to 78 mm Hg (−21 mm Hg) applying a mean voltage of 4.6 ± 1.7 V and a frequency of 100 Hz. In our first study (Katayama et al., 2015) we stimulated CS with pulses of 5 V at 15, 30, 60 and 90 Hz. At 90 Hz, the reductions in mean arterial pressure were of 19 mm Hg in CONT rats and 33 mm Hg in CHEMO-X rats. In the present study, based in previous tests, we applied pulses of 3 V with 15, 30, 60 and 90 Hz which produced similar results to those observed using 5 V: at 90 Hz, the decrease in mean arterial pressure was of 21 mm Hg in CONT and 40 mm Hg in CHEMO-X rats.

Finally, it is worth mentioning that the baseline respiratory and cardiovascular parameters were not affected by carotid body deactivation procedure, as suggested by previous studies (Barros et al., 2002; Ribeiro et al., 2013). Barros et al. (2002) reported that 1 day after bilateral deactivation of the carotid bodies, the MAP and HR were found similar as compared to the values measured before the procedure in Wistar rats. Ribeiro et al. (2013) showed that carotid body denervation did not affect neither the V_T nor the MAP of control rats but normalized these parameters in rats fed with high-fat or high sucrose diets. These data suggest that the carotid bodies may not contribute to the control of respiration and arterial pressure in physiological conditions but might play an important role in pathological states such as metabolic syndrome (Ribeiro et al., 2013), hypertension (Pijacka et al., 2018) and heart failure (Marcus et al., 2014).

5. Conclusion

In conclusion, electrical stimulation of the CS in conscious rats activated both carotid baro- and chemoreceptors, triggering a reflex increase in ventilation and decrease in AP. The observed respiratory responses to electrical stimulation of the carotid sinus were dependent on the integrity of carotid chemoreceptors and were abolished after chemoreceptor deactivation. Carotid chemoreceptor deactivation potentiated the hypotensive response observed in CONT rats, by allowing the exclusive carotid baroreflex activation. It is important to stress that, in the present study, both the left carotid chemoreceptor integrity in CONT and the left carotid chemoreceptor deactivation in CHEMO-X rats were confirmed at the end of the experimental protocols. Despite no evidence of carotid chemoreceptor activation in other models of electrical stimulation of the carotid sinus (dogs and humans), this possibility is not completely ruled out and is currently under investigation in drug-resistant hypertensive patients (Tank J. et al. clinicaltrials.gov, NCT02587533). As mentioned by Alnima et al. (2015), even though the electrical stimulation of the carotid sinus did not affect the respiratory parameters of humans, a potential activation of the carotid chemoreflex is still possible and should be further explored.

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Conflict of interest

None.

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