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Cardiovascular responses elicited by continuous versus intermittent electrical stimulation of the aortic depressor nerve in conscious rats

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ABSTRACT

Aims: Short-term (seconds or minutes) continuous electrical activation of the aortic depressor nerve (ADN) in conscious rats has been successfully used to investigate baroafferent function in experimental hypertension, heart failure, and peripheral inflammation. The aim of this study was to characterize the hemodynamic responses elicited by longer periods (60 min) of continuous or intermittent electrical baroreflex activation.
Main methods: Wistar rats were implanted with an electrode around the left ADN and a catheter into a femoral artery. The systolic, diastolic and mean arterial pressure and heart rate were recorded in subjects randomly assigned to continuous or intermittent electrical stimulation. The time-course of cardiovascular responses in conscious rats was examined during longer-term (60 min) continuous ($n = 6$) or intermittent (5 s ON/3 s OFF; $n = 10$) electrical stimulation (0.5 mA; 0.25 ms; 30 Hz) of the ADN.
Key findings: The prompt (20 s) hypotensive response was greater under continuous stimulation, but no difference was detected in the bradycardic response. The hypotensive response was sustained only by continuous stimulation while no sustained bradycardia was observed in either protocol.
Significance: These findings indicate that continuous stimulation of the ADN is more effective in reducing arterial pressure over a longer period (60 min) of stimulation. Nevertheless, both protocols - continuous or intermittent - were unable to elicit a sustained bradycardia.

1. Introduction

The arterial baroreflex is a vital short-term regulatory system responsible for the maintenance of blood pressure in a relatively narrow range of oscillation [1, 2]. The baroreflex promotes inhibition of the sympathetic drive combined with activation of parasympathetic activity; therefore, baroreflex activation shifts the sympathovagal balance toward parasympathetic predominance [1, 2].

Electrical activation of baroreceptor afferents within the aortic depressor nerve (ADN) in conscious rats is a reliable technique for studying baroreflex function under physiological and pathophysiological conditions [3]. Studying the effects of electrical stimulation of the ADN in conscious rats permits evaluation of reflex bradycardia and hypotension without the undesirable effect of anesthesia [4].

Baroreflex activation therapy (BAT) successfully reduces systolic arterial pressure (SAP) in patients with drug-resistant hypertension [5–10]. This maneuver is proving to be a novel, safe and effective therapeutic approach to the treatment of hypertension [11–15]. Electrical activation of the baroreflex in conscious rats - particularly via activation of baroafferents within the ADN - may bring new insights into the mechanisms responsible for BAT and provide effective stimulus paradigms.

Recently, our laboratory showed, for the first time, that the activation of the baroreflex by electrical stimulation of the ADN modulates the innate immune system, attenuating joint inflammation in experimental arthritis [16]. In this study, to ensure the efficacy of the activation of the baroreflex, we examined the hemodynamic responses promoted by short-term (2 min) continuous stimulation of the ADN. Although several studies from our laboratory [3, 16, 17] have shown that short-term periods of electrical stimulation of the ADN (seconds to minutes) can be successfully used in conscious rats, further investigation is required to validate this technique during a more prolonged (60 min) stimulation in these animals. Thus, the aim of this study was to characterize the changes in arterial pressure and heart rate (HR) in conscious rats during continuous or intermittent electrical stimulation of the ADN for 60 min.

2. Materials and methods

2.1. Experimental animals

The experiments were conducted on male Wistar rats (250–280 g) maintained under controlled temperature (22°C), constant 12:12 h

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light-dark cycle, and receiving food and water ad libitum. All procedures were reviewed and approved by the Committee of Ethics in Animal Research from the Medical School of Ribeirão Preto (University of São Paulo) - Protocol number 93/2012.

2.2. Surgical procedures

The rats were anesthetized with thiopental sodium (40 mg/kg ip), and then subjected to surgical procedures to isolate the left ADN for implantation of electrodes and catheterization of the femoral artery. In brief, the rats were subjected to ventral neck surgery, and the left ADN was isolated at the point where it entered the superior laryngeal nerve. The ADN was implanted with a bipolar stainless steel electrode with an inter-leads distance of 2 mm. The electrodes were constructed by attaching two 40 mm long stainless steel wires (0.008 in. bare, 0.011 in. Teflon coated; model 791400; A-M Systems, Sequim, WA, USA) to a small plug (GF-6; Microtech, Boothwyn, PA, USA). The bared tips of the electrodes consisted of a 2 mm length forming hooks that were implanted around the ADN. The electrodes were tunneled through the sternocleidomastoid muscle and a small plug was exteriorized at the nape of the neck. The short segment of the ADN that was implanted with the bipolar electrodes was carefully covered with silicone impression material (Kwik-Sil silicone elastomer; World Precision Instruments, Sarasota, FL, USA). A few minutes were allowed for the complete polymerization of the silicone impression material. The femoral artery was then catheterized with polyethylene tubing (PE-50 soldered to PE-10 polyethylene tube; Intramedic, Clay Adams, Parsippany, NJ, USA) for pulsatile arterial pressure (PAP) recording. The femoral arterial catheter was exteriorized at the back of the nape of the rats, and surgical incision sites were closed by sutures.

2.3. Assessment of the hemodynamic parameters

Twenty-four hours after the surgical procedures, the PAP and HR of the conscious rats were recorded. Briefly, the arterial catheter was connected to a pressure transducer (MLT844; ADInstruments, Bella Vista, Australia) and the signal amplified (ML224; ADInstruments, Bella Vista, Australia) and sampled by an IBM/PC computer (Core 2 Duo, 2.2 GHz, 4 GB RAM) equipped with an analog-to-digital interface (2 kHz; ML866; ADInstruments, Bella Vista, Australia). A quiet environment was maintained to avoid stress, and only rats that showed no signs of discomfort during electrical stimulation of the ADN were assigned to the study. The electrodes were connected to an external stimulator (1M1C; AVS Projetos, São Carlos, SP, Brazil) and ADN was subjected to electrical stimulation for 60 min. PAP recordings were processed with computer software (LabChart 7.0; ADInstruments, Bella Vista, Australia) capable of detecting inflection points and generate mean arterial pressure (MAP), SAP, diastolic arterial pressure (DAP) and HR beat-by-beat time series.

2.4. Experimental procedures

The rats were randomly assigned into two groups. One group was subjected to continuous electrical stimulation (intensity of 0.5 mA; pulse length of 0.25 ms; frequency of 30 Hz). The other group to intermittent stimulation (intensity of 0.5 mA; pulse length of 0.25 ms; frequency of 30 Hz; ON: 5 s; OFF: 3 s). Fig. 1 illustrates the two different approaches. The experimental protocol consisted of a basal recording of SAP, DAP, MAP and HR for 30 min, followed by electrical stimulation of the ADN for 60 min under continuous ($n = 6$) or intermittent ($n = 10$) protocol. After the electrical stimulation protocol, the cardiovascular parameters were recorded for a further 30 min. Data from repeated measurements (i.e. continuous or intermittent stimulation protocol was employed in the same animal, 24 h apart) were collected to preclude any problem with the interface of the electrode with the ADN (Figs. 1 and 2 in Supplementary Material).

2.5. Statistical analysis

A two-way analysis of variance (ANOVA) for repeated measures, followed by Student-Newman-Keuls post hoc test was used to compare continuous and intermittent protocols at baseline conditions and the prompt hemodynamic responses to electrical stimulation of the ADN. To evaluate the time course of the hemodynamic responses to electrical stimulation of the ADN in conscious rats, the recordings were split into twelve segments of 5 min each and data were analyzed with two-way ANOVA for repeated measures, followed by Student-Newman-Keuls post hoc test. Differences were considered significant when $P < 0.05$. The results are shown as mean ± standard error of the mean (SEM).

3. Results

3.1. Typical tracings of arterial pressure and heart rate during electrical stimulation of the ADN

Fig. 2 shows typical tracings of PAP (white line represents MAP) and HR of representative subjects from continuous (Fig. 2, panel A and B) and intermittent (Fig. 2, panel C and D) groups. Continuous stimulation was characterized by a robust fall in SAP and DAP that showed minor oscillations (Fig. 2, panel A and B). In contrast, the intermittent stimulation elicited falls in SAP and DAP that we associated with substantial oscillations (Fig. 2, panel C and D). Fig. 3 shows typical tracings of MAP and HR during the first 20 s of electrical stimulation of the ADN. It is noticeable that at the end of the 3 s period without (OFF) stimulation the MAP recovered expressively toward the basal level, being interrupted by initiation of the next 5 s period of stimulation (ON).

3.2. Prompt cardiovascular responses to electrical stimulation of the ADN

Baseline SAP, DAP, MAP, and HR were similar between the groups subjected to continuous or intermittent stimulation (Fig. 4, black and clear bars). The electrical stimulation of the ADN elicited a prompt (first 20 s) decrease in SAP, DAP, MAP and HR in both continuous or intermittent stimulation groups (Fig. 4). However, the hypotensive response was greater under continuous as compared to intermittent stimulation (Fig. 4 A, B, and C).

3.3. Time course - 60 min - of the hemodynamic responses to electrical stimulation of the ADN

The prompt hypotensive (SAP, DAP and MAP) response with intermittent (ON/OFF) stimulation was transitory, while under continuous (CONT) stimulation the hypotension was sustained throughout the 60 min period of stimulation (Table 1; Fig. 5 A, B, and C). Moreover, the time course of the hemodynamic responses revealed a transient bradycardia that did not differ between groups (Table 1; Fig. 5 D).

4. Discussion

Before the onset of continuous or intermittent ADN stimulation, the cardiovascular parameters - SAP, DAP, MAP, and HR - accessed directly from the femoral artery were similar between the two groups of rats. The baseline values were consistent with data from conscious Wistar rats from our [3, 17, 18] and other [19–21] laboratories. In the present study, the prompt hypotensive response elicited by continuous electrical stimulation of the ADN was greater than that elicited by intermittent stimulation. The hypotension elicited by continuous stimulation was sustained throughout the entire period (60 min). In contrast, both continuous and intermittent stimulation of the ADN elicited similar transient reductions in HR.

The ADN of rats comprises a relatively pure population of baroreceptor afferents encompassing A-fibers and C-fibers [22–25], with C-fibers representing 80% of the total fibers [26]. During graded electrical