Hemodynamic responses to electrical stimulation of the aortic depressor nerve in awake rats

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De Paula, Patrícia M., Jaci A. Castania, Leni G. H. Bonagambha, Helio C. Salgado, and Benedito H. Machado. Hemodynamic responses to electrical stimulation of the aortic depressor nerve (ADN) were evaluated in conscious freely moving rats. Platinum electrodes were implanted into the ADN of all rats studied, and some of these animals were also implanted with miniaturized Doppler probes around the superior mesenteric artery and inferior abdominal aorta (hindquarter). In both groups, the femoral artery and vein were catheterized one day before the experiments. In the first group of rats (n = 7), the control ES of the ADN in the range from 0.5 to 3.0 V (50 Hz, 10 ms) produced bradycardia and hypotension in an intensity-dependent manner, and treatment with methylatropine (intravenously) blocked the bradycardia but produced no significant changes in the hypotensive response. In a second group (n = 6), ES of the ADN was performed with the intensity fixed at 3 V and the frequency of the stimuli varying from 10 to 50 Hz. In this group, the hypotensive response was frequency dependent, whereas the bradycardic response was not. In a third group of rats (n = 6), ES of the ADN (2.5 V) produced hypotension (~35 ± 4 mmHg), minor changes in the mesenteric (+5 ± 14%), and vasodilation in hindquarter (~32 ± 6%) vascular beds. The data show that 1) ES of the ADN produces a fall in pressure, bradycardia, and vasodilation in the hindquarter, and no changes in the mesenteric vascular resistance; 2) methylatropine blocked the bradycardia and produced no effect on the hypotensive response to ES of the ADN, and 3) the baroreceptor afferent fibers involved in the hypotensive response to ES of ADN are sensitive to the variation of the frequency of the stimuli, whereas the fibers involved in the bradycardic response are not.

arterial baroreceptors; heart rate; blood pressure; blood flow; vascular resistance; baroreflex; frequency; intensity

THE BAROREFLEX is the most important regulatory system involved in the moment-to-moment regulation of the arterial pressure, and it has been extensively studied under different experimental conditions (13, 15, 26). The activation of the baroreflex produces sympathetic inhibition and parasympathoexcitation at the brain stem level. The sympathetic inhibition can be quantified by recording the sympathetic nerve discharge (11) or by the direct measurement of arterial pressure, whereas the parasympathoexcitation can be evaluated by the reduction of heart rate (HR). Most of the studies related to the activation of the baroreflex performed in conscious as well as in anesthetized animals have used phenylephrine, an α1-adrenergic receptor agonist, to produce vasoconstriction and an increase in arterial pressure with consequent activation of the baroreceptors. In this case, the reflex autonomic responses may be quantified by the measurement of the sympathetic nerve discharge, usually in anesthetized animals, and by the reflex bradycardia. Electrical stimulation of the arterial baroreceptors in anesthetized animals also has been widely used, permitting the evaluation of the fall in arterial pressure and HR as well as the measurement of the sympathetic nerve discharge (25) and of changes in the regional vascular resistance (17).

The use of phenylephrine or any other vasoconstrictor agent to produce activation of the baroreflex in conscious animals presents the inconvenience of its direct vasoconstrictor effects, which mask the reflex vasodilator responses and prevent the evaluation of the hypotensive response and the changes in the regional vascular resistance. For these reasons, in the present study we developed a reliable technique to produce electrical stimulation of the aortic depressor nerve (ADN) in conscious freely moving rats, which permits the evaluation of the bradycardic and hypotensive responses as well as the changes in the vascular resistance of the regional beds in awake rats. Chronic electrical stimulation of the carotid sinus nerve has been successfully performed in studies on conscious hypertensive dogs and humans (9, 10, 22). However, electrical stimulation of the ADN in awake rats has not been performed previously. In the present study we evaluated the effect of the variation of the intensity (from 0.5 to 3.0 V) or frequency (from 10 to 50 Hz) on the hypotensive and bradycardic responses to the electrical stimulation of the ADN to determine how these afferent fibers respond to the variation of the characteristics of the stimuli in unanesthetized rats.

With respect to the regulation of regional vascular resistance by the arterial baroreceptors, we previously suggested that aortic baroreceptors play a predominant role in the regulation of hindquarter vascular resistance, considering that bilateral carotid occlusion in conscious rats with aortic baroreceptor deafferentation presented a larger increase in mean arterial pressure (MAP) than in control rats because of a major increase in hindquarter vascular resistance (16). We also documented that electrical stimulation of the ADN in anesthetized rats produced greater vasodilation in the
hindquarter than in the renal and mesenteric vascular beds (17). Therefore, in the present study we used a new technique that allows us to produce electrical stimulation of the ADN in awake rats to evaluate whether our previous evidence obtained with bilateral carotid occlusion in aortic-denervated rats or with electrical stimulation of the ADN in unanesthetized rats is consistent with the hemodynamic responses to electrical stimulation of the ADN in conscious freely moving rats.

**METHODS**

Male Wistar rats weighing 250–270 g were used in the present study. Under pentobarbital sodium anesthesia (40 mg/kg ip) the rats were submitted to ventral neck surgery with the purpose of isolating the left ADN. After isolation, the ADN was placed on a bipolar platinum microelectrode and the electroneurographic recording of baroreceptor activity was obtained with an oscilloscope (Tektronics) after the signal had been properly amplified (20). An electronic device permits us to follow the sound of the potentials and the combination of the records of the train of potentials in the oscilloscope, and the sound of the potentials with the direct measurement of the pulsatile arterial pressure was used for identification of the ADN. Only rats that presented clear electroneurographic recording of the ADN were implanted with chronic electrodes. After identification of the ADN under the microscope, the bipolar platinum electrode supporting a short segment of the ADN was carefully covered with silicone gel (Wacker Sil Gel, 604, Wacker, Munich, Germany) or with dental covering material ( Coltene President/Coltene Whaledent). A critical procedure carried out before covering the electrode and the ADN was to determine if the vessel that irrigates the nerve was patent, because the success of the experiments performed 24 or 48 h later seems to depend on the integrity of nerve irrigation. After covering the electrode and the nerve, at least 30 min were allowed to elapse for complete polymerization of the gel and again the electroneurographic recording was performed to verify the integrity of the nerve. Once the integrity of the nerve was confirmed, the fine platinum wires of the electrodes were exteriorized on the back of the rat and soldered to a small plug that was later connected with the wires from the electrical stimulator.

Under the same anesthesia, the femoral artery and vein were catheterized for recording pulsatile arterial pressure and for drug administration (intravenous), respectively. Twenty-four hours after the end of this surgery the rats were connected to the recording system (polygraph and pressure transducer, Narco Bio-Systems, Austin, TX) and to the electrical stimulator (Department of Physiology, School of Medicine of Ribeirão Preto Bioengineering Facilities), and the experiment was performed in an acoustically isolated room in which the rats were maintained after they recovered from anesthesia.

In the first experimental group the protocol consisted of electrical stimulation of the ADN with the following parameters: frequency, 50 Hz; pulse duration, 10 ms; and voltage intensity, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 V in a random sequence. Each stimulus was maintained for 5 s at intervals of at least 3 min. Pulsatile arterial pressure (PAP), MAP, and HR were recorded on the polygraph. After a series of intensity (voltage) response experiments, the rats received methylxatropine (2 mg/kg iv) to evaluate the changes in MAP after blockade of the parasympathetic component of the baroreflex. To determine if the hypotensive and bradycardic responses to electrical stimulation of the ADN were intensity and/or frequency dependent, a second group of rats was submitted to stimulus intensities varying from 0.5 to 3.0 V. In the second part of this protocol the intensity was fixed at 3.0 V, and the frequency was varied from 10 to 50 Hz. A third group of rats also responsive to electrical stimulation of the ADN on the first day after the implant of the electrodes was submitted to a new surgery under pentobarbital sodium anesthesia for the implantation of miniaturized Doppler probes around the superior mesenteric artery and/or inferior abdominal aorta (hindquarter) to measure the changes in regional blood flow in response to electrical stimulation of the ADN. After this second surgery, the rats were allowed to recover from anesthesia for 24 h and then were connected to a Hewlett-Packard polygraph for recording of PAP, MAP, and mesenteric and hindquarter blood flow using a Doppler flowmeter (University of Iowa, Bioengineering Facilities). The experimental protocol for electrical stimulation of the ADN was similar to that used on the previous day for the same animals. With this approach it was possible to evaluate the effect of electrical stimulation of the ADN on the regulation of regional vascular resistance.

Doppler technology (12) has been useful in measuring blood flow in small animals, and despite its limitations it permitted evaluation of the changes in regional vascular resistance. In the present study. In addition, a study by Haywood et al. (12) documented that the Doppler method is adequate in measuring the velocity of blood flow and shows a linear relationship with the square wave of the electromagnetic flowmeter. Considering that in addition to the flow we were also measuring the MAP, we calculated the percent changes in resistance produced by each stimulation.

In all experimental protocols we tested the baroreflex responses as well as the changes in regional blood flow using an intravenous injection of phenylephrine (0.5 µg/kg iv) to verify the integrity of the baroreflex and to check the Doppler probe responses to vasoconstriction. The intensity-response curves in the control group as well as in the methylxatropine-treated group submitted to electrical stimulation of the ADN were analyzed by two-way multivariate analysis of variance (MANOVA) for repeated measures (P < 0.05), whereas the frequency-response curves were analyzed by one-way MANOVA for repeated measures (P < 0.05). With respect to the changes in regional vascular resistance in response to the increasing intensity of electrical stimulation of the ADN we used the nonparametric test of Friedman because the percent changes did not follow normal distribution. Changes in the hindquarter and mesenteric vascular resistances in relation to baseline were compared by one sample t-test (1 tailed) to verify whether the average change for one stimulus intensity differed from a specific constant.

**RESULTS**

Electrical stimulation of ADN and changes in MAP and HR. Figure 1 presents tracings of one rat representative of the group in which increasing intensity of voltage produced an intensity-dependent reduction in HR and MAP. In this case, as well as in most of the other rats, the threshold was at 0.5 V and the maximal reduction in MAP and HR was in the range of 2.5 and 3.0 V. It is important to note that the reduction of HR was very sharp in response to all intensities, whereas the reduction in MAP was slower. In the rat presented in Fig. 1, the injection of phenylephrine produced a pressor response and reflex bradycardia, indicating that the baroreflex was intact.

Figure 2 presents the tracings of the same rat as shown in Fig. 1 in the protocol in which electrical stimulation of the ADN was performed under the effect...
of methylatropine (2 mg/kg iv). The tracings show that methylatropine produced a significant increase in basal HR and no major changes in basal MAP. In addition, the bradycardic responses to electrical stimulation of the ADN were completely blocked by methylatropine, whereas the hypotensive responses, especially at the highest intensity used (3.0 V), were not affected. In this rat treated with methylatropine, the reflex bradycardia in response to the pressor response produced by phenylephrine (intravenous) was blocked, indicating the efficacy of the dose used.

Figure 3 summarizes the data obtained with this group of rats (n = 7) and shows that electrical stimulation of the ADN in the control condition produced an
intensity-dependent reduction in HR and MAP and that treatment with methylatropine blocked the intensity-dependent pattern of the bradycardic response but produced no significant changes in the intensity-dependent pattern of the hypotensive response, considering that the two curves for the hypotensive responses were not statistically different. It is important to note that there is no statistical significance for the interaction term for treatment versus time.

Figure 4 presents the tracings of one rat submitted to electrical stimulation of the ADN in which the intensity of the stimuli was fixed at 3 V and the frequency varied from 10 to 50 Hz. The tracings show that the hypotensive response was frequency dependent, whereas the bradycardic response was not. The data for this group (n = 6) are summarized in Fig. 5, which shows that the hypotensive but not the bradycardic response was frequency dependent.

Electrical stimulation of ADN and changes in blood flow in hindquarter and mesenteric vascular beds. Figure 6 presents a typical tracing for one rat representative of the group showing the changes in MAP, PAP, and in hindquarter and mesenteric blood flow in response to electrical stimulation of the ADN. On the first day of the experimental protocol this rat was responsive to electrical stimulation of the ADN (bradycardia and hypotensive responses). Under anesthesia, the Doppler probes were implanted into the abdominal aorta and superior mesenteric arteries and on the subsequent day the rat was submitted again to electrical stimulation of the ADN. The mean blood flow is presented in Fig. 6, and the changes in vascular resistance were calculated considering the peak changes.
in mean blood flow and in MAP. In the panels related to electrical stimulation at intensities of 1.5, 2.0, and 2.5 V, we can see that the reduction in MAP was linked to an increase in hindquarter blood flow, whereas a reduction or no change in mesenteric blood flow occurred.

Figure 7 summarizes the data for this group of animals (n = 6), showing the changes in MAP and hindquarter and mesenteric vascular resistance. Electrical stimulation of the ADN at the 0.5- to 2.5-V range produced a reduction in MAP, which seems to be related to the vasodilatation observed in the hindquarter vascular beds, particularly in the range from 1.5 to 2.5 V. No significant differences in mesenteric vascular resistance were observed at any intensity of stimulation, indicating that stimulation of the ADN produced no major changes in the resistance of the mesenteric vascular beds.

DISCUSSION

Intravenous injection of phenylephrine is used to evaluate the reflex bradycardia induced by the activation of the baroreflex, but the sympathoinhibitory component of this reflex (hypotension) is masked by the direct vasoconstrictor effect of this agonist. To avoid this problem we developed a technique to perform electrical stimulation of the ADN in conscious freely moving rats. The data obtained in the present study show that electrical stimulation of the ADN in conscious rats produces hypotension and bradycardia. After intravenous injection of methylatropine, the bradycardic response was abolished and the hypotensive
response was not affected. The data show that the fall in MAP in response to the electrical stimulation of the ADN seems to be purely due to the sympathoinhibitory component of the baroreflex, because blockade of the bradycardia did not affect the hypotensive response.

Electrical stimulation of the ADN in conscious freely moving rats produced a fall in pressure, bradycardia, vasodilation in the hindquarter, and no change in mesenteric vascular resistance. In addition, the hypotensive response to electrical stimulation of the ADN was not affected by blockade of the bradycardic response with methylatropine. These data indicate that the bradycardia is due to parasympathoexcitation, and the fall in arterial pressure may involve different sympathoinhibitory pathways. The tracings of the rat representative of the group presented in Fig. 1 show that the injection of phenylephrine produced a pressor response and reflex bradycardia, indicating that the baroreflex was intact. Electrical stimulation of the ADN did not induce a behavioral or stressful response in this rat or in any of the other animals responsive to stimulation. Several rats implanted with the electrodes were not responsive to electrical stimulation of the ADN, probably because of problems with the insulation of the electrode or preservation of the nerve, and presented slight contractions of the neck muscles in response to low-intensity stimuli. For this reason, they were not further evaluated and were excluded from the study.

The variation of the stimulus voltage was used in the present study as a first step in the development of this technical approach as well as to compare the findings in unanesthetized rats with the data previously obtained in urethan-anesthetized rats (17), to which we also applied electrical stimulation in the ADN with variation of the stimulus voltage. The data related to the variation of the intensity of the stimuli indicate that both hypotensive and bradycardic responses to the electrical stimulation of the ADN were intensity dependent. On the other hand, the data related to the variation of the frequency of the stimuli indicate that the hypotensive response (sympathoinhibitory component) was frequency dependent, whereas the bradycardic response (parasympathoexcitatory component) was not.

With respect to the effect of the frequency of stimulation on the activity of the baroreceptors, a previous study by Chapleau and Abboud (4) documented that the increase in activity of the carotid sinus nerve in dogs after pulsing is related to the duration of the pulsing period and amplitude and frequency of the pressure pulses. In addition, pulse pressure has been considered a more effective stimulus of the baroreceptors than static pressure (1), an effect that was attributed to the recruitment of additional afferent fibers during the systole (3). The variation of the frequency of the stimuli in particular showed that the gain of the ADN activity increased two to three times as the frequency of the changes in pressure increased (21). There is also experimental evidence showing that the reflex changes in peripheral vascular resistance occur at a lower level of arterial pressure than do the HR changes, which may be associated with the characteristics of the different baroreceptor fibers (14, 23). Therefore, the findings of the present study related to the different responses of hypotension and bradycardia to the variation of the frequency of the stimuli are in agreement with previous studies and suggest that the afferent fibers of the aortic baroreceptors involved in the mediation of the sympathoinhibitory component of the baroreflex are more sensitive to the variation of the frequency of the stimuli than the fibers involved in the mediation of the parasympathoexcitatory component of the baroreflex.

In the protocols of the present study related to the changes in vascular resistance, we used only the varia-
tion of the intensity of the stimuli because the fall in MAP in response to electrical stimulation of the ADN was similarly dependent on the frequency and intensity of the stimuli. In addition, a study by Smith and Barron (24) using the variation of the frequency of the stimulus documented changes in MAP and in the mesenteric and iliac vascular resistance similar to those observed in the present study and in a previous one by our group (17) using variation of stimulus voltage.

The data related to vascular resistance obtained in the present study in conscious freely moving rats are in accordance with our previous study (17) in which we showed that the ADN plays a larger role in the regulation of the hindquarter than in the mesenteric vascular bed. In previous studies (16) we also observed that bilateral carotid occlusion produced an increase in vascular resistance associated with the innervation of specific vascular beds. The vascular resistance obtained in conscious rats and that after selective denervation of the aortic baroreceptors the increase in MAP was greater and was mainly related to a large increase in hindquarter vascular resistance, indicating that aortic baroreceptors play a more major role in the regulation of blood flow to skeletal muscle than to visceral territories. These findings differ from those reported by Creager et al. (5), who demonstrated that aortic baroreceptors regulate splanchnic and renal but not limb vascular resistance in patients with congestive heart failure, suggesting selective regulation of regional blood flow that may contribute to the redistribution of blood flow in congestive heart failure. Our evidence obtained with rats indicates that activation of aortic baroreceptors plays a predominant role in the regulation of the vascular beds of skeletal muscle compared with other vascular territories. Previous studies also showed that the aortic baroreceptors of dogs are more effective in regulating HR than are carotid baroreceptors (27).

In a previous study from our laboratory, performed on urethan-anesthetized rats, we observed a significant increase in mesenteric vascular resistance in response to electrical stimulation of the ADN at the lower current intensity (17), whereas in the present study performed in unanesthetized rats we observed no changes in mesenteric vascular resistance. With respect to the increase in mesenteric vascular resistance observed in anesthetized rats, it is possible that mesenteric vasoconstriction is secondary to the fall in MAP produced by electrical stimulation of the ADN and we may suggest that hypotension could induce the activation of the peripheral chemoreceptors with consequent sympathoexcitation. In studies performed by Smith and Barron (24) on urethan-anesthetized rats with sinoaortic deafferentation (SAD), electrical stimulation of the ADN produced mesenteric vasodilation. In this case the different findings may be related to the fact that the surgery for SAD can damage the carotid chemoreceptors with consequent dysfunction of the arterial chemoreceptors. With respect to the regulation of renal vascular resistance, DiBona et al. (8) demonstrated that the majority of renal sympathetic nerve fibers is responsive to the stimulation of arterial baroreceptors. However, in our previous study (17) we verified that electrical stimulation of the ADN in intact as well as in selective arterial baroreceptor-denervated rats produced minor changes in renal vascular resistance probably due to the activation of autoregulatory mechanisms at this level. Therefore, the precise mechanisms for such a selective role of the ADN in the regulation of the vascular resistance of each territory require further and careful investigation.

The selective regulation of regional vascular resistance by the ADN may be related to a possible selective modulation of sympathovasomotor neurons located in the rostral ventrolateral medulla (RVLM), which, in accordance with several studies, are topographically distributed in this area (6, 7, 18, 19). More recently, Campos and McAllen (2) also showed a topographic distribution of sympathetic neurons in the RVLM involved in the regulation of vascular resistance and support the concept that ADN plays a predominant role in the regulation of hindquarter vascular resistance in relation to the mesenteric vascular bed. The hypotensive response to electrical stimulation of the ADN was not affected by blockade of the bradycardic response by methylatropine, indicating that the hypotension was due to the sympathoinhibitory response produced by ADN stimulation. The data also indicate that the hypotensive response to electrical stimulation of the ADN in conscious rats is dependent on the frequency and intensity of the stimuli, whereas the bradycardic response is not dependent on the frequency of the stimuli.

Perspectives

The findings of the present study supporting the concept that aortic baroreceptors play a predominant role in the regulation of the hindquarter vascular resistance when compared with the mesenteric vascular bed open new and interesting possibilities for understanding the function of specific baroreceptor afferents in the neurmodulation of the sympathetic nerve activity generated by RVLM neurons as well as the autonomic processing of the baroreceptor afferents at the level of the NTS. Additional studies using microinjections of excitatory amino acids (L-glutamate) into different subregions of the RVLM in unanesthetized rats are required to evaluate a possible topographic distribution of presympathetic neurons associated with the innervation of specific vascular beds. The data, showing that the sympathoinhibitory response
(hypotension) to electrical stimulation of the ADN was frequency dependent, whereas the parasympathoexcitatory response (bradycardia) was not, open new possibilities for further studies on the neurotransmission of these different afferent fibers in the NTS, the site of the first synapse of these afferents in the central nervous system. The technique of electrical stimulation of the ADN in unanesthetized rats used in the present study also will be useful in an experimental approach combining electrical stimulation of the ADN with microinjections into the NTS and/or RVLM of different antagonists, particularly of excitatory amino acid receptors, to evaluate the neurotransmitters and receptors involved in the autonomic processing of the baroreflex afferents in the brain stem.

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