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Effect of carotid and aortic baroreceptors on cardiopulmonary reflex: the role of autonomic function

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Abstract

We determined the sympathetic and parasympathetic control of heart rate (HR) and the sensitivity of the cardiopulmonary receptors after selective carotid and aortic denervation. We also investigated the participation of the autonomic nervous system in the Bezold-Jarish reflex after selective removal of aortic and carotid baroreceptors. Male Wistar rats (220-270 g) were divided into three groups: control (CG, N = 8), aortic denervation (AG, N = 5) and carotid denervation (CAG, N = 9). AG animals presented increased arterial pressure (12%) and HR (11%) compared with CG, while CAG animals presented a reduction in arterial pressure (16%) and unchanged HR compared with CG. The sequential blockade of autonomic effects by atropine and propranolol indicated a reduction in vagal function in CAG (a 50 and 62% reduction in vagal effect and tonus, respectively) while AG showed an increase of more than 100% in sympathetic control of HR. The Bezold-Jarish reflex was evaluated using serotonin, which induced increased bradycardia and hypotension in AG and CAG, suggesting that the sensitivity of the cardiopulmonary reflex is augmented after selective denervation. Atropine administration abolished the bradycardic responses induced by serotonin in all groups; however, the hypotensive response was still increased in AG. Although the responses after atropine were lower than the responses before the drug, indicating a reduction in vagal outflow after selective denervation, our data suggest that both denervation procedures are associated with an increase in sympathetic modulation of the vessels, indicating that the sensitivity of the cardiopulmonary receptors was modulated by baroreceptor fibers.

Key words: Carotid denervation; Aortic denervation; Bezold-Jarish reflex

Introduction

The physiological role of arterial baroreceptors in the maintenance of circulatory homeostasis has been well established. The surgical deafferentation of these baroreceptors as described by Krieger (1) leads to a marked increase in arterial pressure variability (APV), providing an experimental model of the complete absence of baroreceptor function, since the arterial baroreceptor reflex plays an important role in preventing short-term wide fluctuations (2-4). However, there is a different degree of baroreflex impairment in the presence of cardiovascular diseases such as hypertension, myocardial infarction and heart failure, causing a reduction of baroreflex sensitivity associated with increased APV (5,6). Indeed, the reduction of baroreflex sensitivity has

been demonstrated to be a significant predictor of cardiac mortality (7,8).

The reflex control of the cardiovascular system also depends on the activation of cardiopulmonary receptors (9). The Bezold-Jarish reflex is one of the cardiac reflexes that contribute to cardiovascular control. This reflex is characterized by an increase in parasympathetic efferent activity associated with inhibition of sympathetic efferent activity resulting in hypotension and bradycardia. The chemical stimulation of cardiopulmonary receptors with serotonin is an effective method for inducing the Bezold-Jarish reflex in animals (10,11). The sensitivity of the Bezold-Jarish reflex evaluated with serotonin was increased after sinoaortic

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denervation (SAD), suggesting that there is an important functional role in the maintenance of cardiovascular homeostasis by the cardiopulmonary receptors in the absence of the baroreceptors (12).

The cardiovascular effects observed in complete (nonselective) SAD depend on the net effect of eliminating excitatory and inhibitory influences (chemoreceptor and baroreceptor elimination, respectively). However, there is no information about the separate effects of each of the two sets of baroreceptors.

Thus, in the present study, we tested the hypothesis that only carotid or aortic denervation can selectively modulate the autonomic control of the circulation and the sensitivity of the cardiopulmonary receptors. Moreover, since there is some evidence that alterations in the characteristics of baroreflex sensitivity reflect alterations in autonomic control of the cardiovascular system (6), we also determined the participation of the autonomic nervous system in the Bezold-Jarish reflex after selective removal of aortic and carotid baroreceptors.

Material and Methods

Animals

The experiments were performed on male Wistar rats weighing 220-270 g, housed in cages with free access to water and food, and maintained in a room with a constant temperature (23°C) on a 12-h light/dark cycle. All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals and were approved by the Institutional Animals Care and Use Committee. The rats were randomly divided into three groups: carotid denervation (CAG, N = 9), aortic denervation (AG, N = 5), and control (CG, N = 8).

For the surgical procedures the animals were anesthetized intraperitoneally (*ip*) with a mixture of 80 mg/kg ketamine (Parke-Davis, Brazil) and 12 mg/kg xylazine (Bayer, Brazil). The control group was submitted to sham operation.

Baroreceptor denervation

The SAD method described by Krieger (1) was used, ex-

cept that the aortic fibers of the baroreceptors were preserved in the carotid denervation. A midline neck incision was made, and sternocleidomastoid muscles were reflected laterally, exposing the neurovascular sheath. The carotid bifurcation was exposed, the carotid fibers were interrupted bilaterally and the carotid body was resected.

For aortic denervation, a midline neck incision was made and the aortic fibers of the baroreceptors that travel along the sympathetic trunk or as isolated nerves were sectioned, keeping the carotid fibers and carotid body intact. Another contingent of baroreceptor aortic fibers located along the inferior laryngeal nerve was interrupted when sectioning the superior laryngeal nerve.

Catheterization and arterial pressure recording

One day after carotid and aortic denervation, arterial and venous catheters were placed in the right femoral artery and vein for direct measurements of arterial pressure (AP) and for drug administration. The catheters were exteriorized through the back of the neck.

The AP signals were recorded continuously in conscious, freely moving rats. The arterial catheter was connected to a transducer (Blood Pressure XDCR, Kent® Scientific, USA), and AP signals were recorded over a 20-min period by a microcomputer equipped with an analogy-to-digital converter board (CODAS, 2000 Hz sampling frequency, Dataq Instruments, Inc., USA). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean arterial pressure (MAP) and heart rate (HR).

AP and HR variability was evaluated on the basis of the standard deviation.

Gasometry

One day after carotid and aortic denervation, a small amount of blood (0.2 mL) was collected from the femoral artery and analyzed with an ABL-5 instrument (Radiometer, Copenhagen, Denmark) to determine pCO₂ and pO₂.

Bezold-Jarish reflex test

The responses to stimulation of chemosensitive cardiopulmonary receptors (Bezold-Jarish reflex) were determined in conscious rats. After recording 20 min of resting AP

Table 1. Mean arterial pressure (MAP), arterial pressure variability (APV), heart rate (HR), heart rate variability (HRV), and pCO₂ and pO₂ concentrations in the control group (CG), carotid group (CAG) and aortic group (AG).

Groups	MAP	APV	HR	HRV	pCO ₂	pO ₂
CG (N = 8)	110 ± 3.4	5.2 ± 0.4	364 ± 5.1	20 ± 2.8	36 ± 0.7	80 ± 2.1
CAG (N = 9)	92 ± 7 [#]	10.5 ± 1.3 [#]	351 ± 6.2	20 ± 3.6	48 ± 1.4 [#]	58 ± 2.2 [#]
AG (N = 5)	122 ± 3.3 [*]	10.4 ± 0.9 [*]	407 ± 8.9 [*]	26 ± 3.7	35 ± 0.8	78 ± 1.7

[#]P ≤ 0.001 for the CAG group versus the CG group; ^{*}P ≤ 0.001 for the AG group versus the CG group (one-way ANOVA).

and HR, the animals were successively injected with 2, 4, and 8 $\mu\text{g}/\text{kg}$ serotonin (5-HT; Sigma, USA) while MAP and HR were recorded. For data analysis, control and peak changes of MAP and HR after each dose were analyzed with a microcomputer (IBM-AT/CODAS, Brazil). Injections were not repeated until the recorded parameters had returned to pre-injection levels. The tests were repeated before and after pharmacological blockade of the parasympathetic nervous system.

Pharmacological blockade

Pharmacological blockade was performed in the same animals on 2 consecutive days.

On the 1st day of the study, immediately after the resting HR was recorded, 4 mg/kg atropine (Sigma), a cholinergic receptor antagonist, was injected intravenously (*iv*). After atropine injection, AP and HR were recorded for 15 min to permit the antagonists to exert their full effects and the Bezold-Jarish test was then repeated. Propranolol (4 mg/kg; Sigma), a beta-adrenergic receptor antagonist, was administered *iv* after the Bezold-Jarish test, and the AP and HR responses were recorded for 5 min. The intrinsic heart rate (IHR) was measured after simultaneous blockade with propranolol and atropine. On the 2nd day of the study, propranolol was administered first to obtain the inverse sequence of blockade.

The vagal effect was the difference between the maximum HR after atropine injection and the control HR, on the 1st day. The sympathetic effect was the difference between the control HR and minimum HR after propranolol injection on the 2nd day. The vagal tone was calculated as the difference between the IHR and the HR after propranolol injection on the 2nd day. The sympathetic tone was the difference between the HR after atropine injection and the IHR, on the 1st day.

Statistical analysis for the comparison of MAP, APV, HR, HR variability, pCO_2 , pO_2 , IHR, vagal, and sympathetic

effects was performed by one-way ANOVA followed by the Newman-Keuls test. The responses of the reflex after successive injections of serotonin were also compared by two-way ANOVA followed by the Newman-Keuls test. The results are reported as means \pm SEM.

Results

Aortic denervation increased MAP while carotid denervation decreased it. The APV of CAG and AG was increased compared with CG ($P = 0.001$ and $P = 0.0005$, respectively). The HR of the AG was increased when compared with CG ($P = 0.001$). CAG rats presented a reduction of pO_2 ($P = 0.0001$) accompanied by an increase of pCO_2 ($P = 0.0001$) compared with CG animals (Table 1).

The IHR obtained after pharmacological blockade was lower in CAG than in CG (344 ± 8.0 vs 401 ± 6.9 bpm; $P = 0.0003$), and the vagal effect was decreased in AG ($P = 0.009$) and CAG ($P = 0.03$) compared with CG (AG = 50 ± 11.2 , CAG = 58 ± 8.3 vs CG = 114 ± 13 bpm). The sympathetic effect was higher in AG compared with CG (83 ± 2.1 vs 41 ± 5 bpm; $P = 0.0002$; Figure 1A). Vagal and sympathetic tonus was lower in CAG when compared with CG ($P = 0.04$), as shown in Figure 1B.

As expected, serotonin induced significant bradycardia and hypotension. The bradycardic response was higher in AG than CG (275 ± 15 vs 163 ± 17 bpm, respectively; $P = 0.001$; Figure 2B).

The hypotensive response to serotonin (decreased MAP) was increased in AG ($P = 0.0001$) and CAG ($P = 0.0008$) compared with CG (AG = 46 ± 5 and CAG = 36 ± 4 vs CG = 19 ± 2 mmHg, respectively; Figure 2A).

The hypotensive response after atropine administration was higher in AG ($P = 0.0001$) than in CG (AG = 11 ± 2 vs CG = 4.5 ± 0.7 mmHg) and the bradycardic response did not differ between groups (Figure 2A,B).

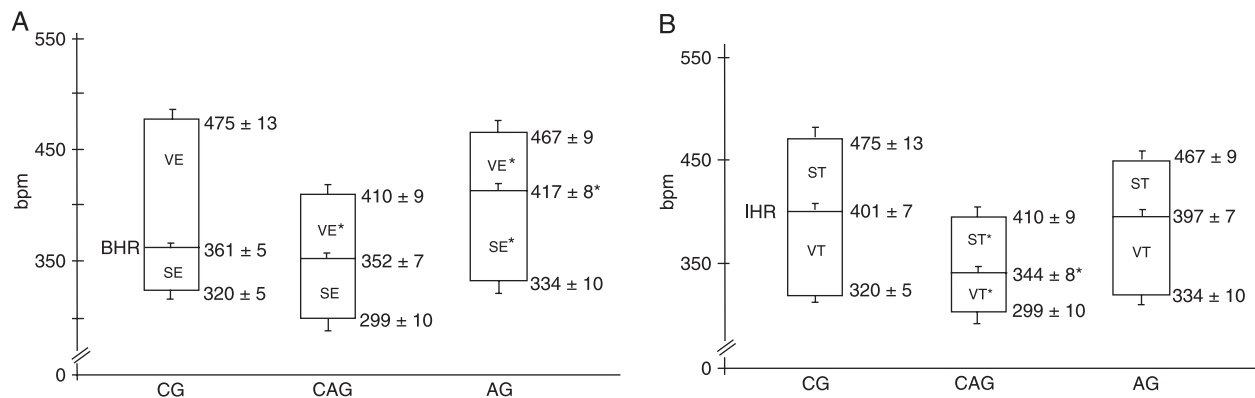


Figure 1. A, Vagal (VE) and sympathetic (SE) effect and B, vagal (VT) and sympathetic (ST) tonus of the control group (CG), carotid group (CAG) and aortic group (AG). IHR = intrinsic heart rate; BHR = basal heart rate. * $P < 0.05$ versus CG (one-way ANOVA).

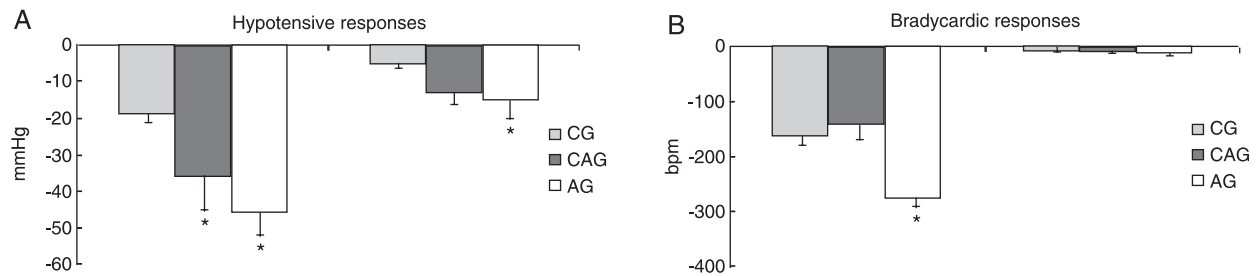


Figure 2. A, Mean hypotensive response obtained with all doses of serotonin before and after atropine administration; B, mean bradycardic response obtained with all doses of serotonin before and after atropine administration for the control group (CG), carotid group (CAG) and aortic group (AG). *P < 0.05 versus CG (two-way ANOVA).

Discussion

Clinical and experimental studies have demonstrated the role of cardiopulmonary receptors in normal and pathophysiological conditions, showing the importance of these receptors in cardiovascular control (9,11,13-16). In fact, the Bezold-Jarish reflex obtained by serotonin administration has been evaluated in several experimental studies and was found to be impaired in spontaneously hypertensive rats (17) and in renal hypertensive rats (18). In contrast, this reflex is enhanced in hypertensive rats by inhibition of nitric oxide synthase (19) and after acute myocardial infarction (16) and is still preserved in diabetic rats (11). These studies have confirmed the idea that the neural reflex control of circulation depends not only on baroreflex input but also on cardiopulmonary reflexes (9,13,14).

The interplay between arterial and cardiopulmonary baroreflexes has been demonstrated in several experimental studies (10,12,17,20). Tonic inhibition of both carotid chemoreflex and arterial baroreflex is produced by cardiopulmonary vagal afferent activity (21). However, the role of the cardiopulmonary reflex in the control of the circulation in the absence of baroreflex control after SAD is not completely understood. In dogs, it was suggested that cardiopulmonary afferents could be counteracting the increase in sympathetic activity after removal of arterial baroreceptors (22,23). In addition, Chianca Júnior and Machado (12) showed a significant increase in the sensitivity of the Bezold-Jarish reflex in both acute and chronic sinoaortic denervated rats. However, there is no information about the separate participation of the aortic baroreceptors or of the carotid baroreceptors in Bezold-Jarish sensitivity. Thus, in the present study, we evaluated the sensitivity of the Bezold-Jarish reflex after acute (24 h) selective aortic or carotid denervation.

In the present study, we have confirmed that the animals submitted to aortic denervation had increased MAP and HR while animals submitted to carotid denervation had a reduction in MAP with no changes in HR, as previously demonstrated (24-30). Moreover, APV was higher in both groups of animals submitted to denervation when compared

with intact controls. Studies have shown that sinoaortic denervated rats had hypertension, tachycardia, and increased APV in the acute phase, whereas in the chronic phase HR and AP returned to normal and APV remained increased (4). Regarding the arterial blood gases, CAG animals showed significantly reduced arterial pO₂ and significantly increased pCO₂. Huckstorf et al. (31) and Franchini and Krieger (30) obtained similar data when comparing carotid body-denervated and sham-operated control rats.

The stimulation of the cardiopulmonary receptors by the injection of serotonin usually induces bradycardic and hypotensive responses (Bezold-Jarish effect) (10-12). In the present experiment, these responses were increased in AG compared with CG, suggesting that the sensitivity of the cardiopulmonary reflex is augmented in AG. Parasympathetic blockade with atropine caused a marked reduction in the bradycardic and hypotensive response induced by serotonin in all groups, confirming data from Chianca Júnior and Machado (32), which showed that the hypotensive response induced by the Bezold-Jarish reflex was due to both sympathetic withdrawal and decreased cardiac output produced by vagal-induced bradycardia. However, the hypotensive response was still augmented in AG when compared with CG. The higher hypotensive response in AG after atropine blockade suggests an intense withdrawal of peripheral sympathetic activity in this group after serotonin stimulation. Indeed, this finding is consistent with studies that demonstrated an increase in sympathetic activity in animals with aortic denervation (33), as well as a reduced baroreceptor function of carotid baroreceptors, as previously described in rats (34).

Our data indicate that atropine administration abolished serotonin-induced bradycardia responses while hypotensive responses were maintained. Although the responses after atropine were lower than the responses before administration of the drug, the blockade indicated that both denervation procedures are associated with an increase in sympathetic modulation of the vessels. Indeed, after atropine blockade all groups showed reduced hypotensive responses, although the AG response was more marked than that of the other groups. This finding suggests an overactivity of

the sympathetic system in this group. On the other hand, hypotension in CG and CAG animals was predominantly due to parasympathetic influence on the heart.

The chemoreflex induced by activation from peripheral chemoreceptors located in the aortic and carotid bodies has been extensively studied as one of the neural controls of blood pressure regulation (35-37). These chemosensitive cells are excited by decreases in pO₂ or increases in pCO₂ or pH that will stimulate the terminal innervations of the carotid sinus nerve (38). Our data showed a marked reduction in IHR associated with a decrease in vagal function after selective carotid denervation. These results could be explained at least in part by the hypoxia observed after carotid denervation, since these animals showed a decrease in pO₂ and pH simultaneously with an increase in pCO₂.

The relationship between hypoxia and the autonomic nervous system has been demonstrated in several animal studies, which have evaluated the cardiovascular autonomic

responses to hypoxia. In fact, recently, Sugimura et al. (39), using spectral analysis methods, have demonstrated that acute progressive hypoxia inhibits cardiac parasympathetic activity in hypertensive rats. Therefore, a possible mechanism for the alterations observed in cardiac parasympathetic control in CAG could be related to hypoxia itself (40).

In conclusion, our data showed that both carotid and aortic baroreceptors selectively modulated the sensitivity of the cardiopulmonary receptors. This modulation was associated with the effects of chemo- and baroreceptor fiber activities coming from the carotid region or with tonic inhibition of sympathetic activity depending on aortic nerve input to the central nervous system.

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