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AUTOCRINE/PARACRINE MODULATION OF BARORECEPTOR ACTIVITY AFTER ANTIDROMIC STIMULATION OF AORTIC DEPRESSOR NERVE *IN VIVO*

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Abstract

Activation of the sensory nerve endings of nonmyelinated C-fiber afferents evokes release of autocrine/paracrine factors that cause localized vasodilation, neurogenic inflammation, and modulation of sensory nerve activity. The aims of this study were to determine the effect of antidromic electrical stimulation on afferent baroreceptor activity *in vivo*, and investigate the role of endogenous prostanooids and hydrogen peroxide (H₂O₂) in mediating changes in nerve activity. Baroreceptor activity was recorded from the left aortic depressor nerve (ADN) in anesthetized rats before and after stimulating the ADN for brief (5–20 s) periods. The rostral end of the ADN was crushed or sectioned beforehand to prevent reflex changes in blood pressure. Antidromic stimulation of ADN using parameters that activate both myelinated A-fibers and nonmyelinated C-fibers caused pronounced and long-lasting (>1 min) inhibition of baroreceptor activity (n=9, P<0.05), with the magnitude and duration of inhibition dependent on the duration of the stimulation period (n=5). Baroreceptor activity was only transiently inhibited after selective stimulation of A-fibers. The inhibition of activity after antidromic stimulation of A and C fibers was prolonged after administration of the cyclooxygenase inhibitor indomethacin (5 mg/kg, IV, n=7) and abolished after administration of PEG-catalase (10⁴ units/kg, IV, n=7), an enzyme that catalyzes the decomposition of H₂O₂ to water and oxygen. The results demonstrate a long-lasting inhibition of baroreceptor activity after antidromic stimulation of ADN and suggest that

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endogenous prostanoids and H₂O₂ oppose and mediate the inhibition, respectively. These mechanisms may contribute to rapid baroreceptor resetting during acute hypertension and be engaged during chronic baroreceptor activation therapy in patients with hypertension.

Keywords

Baroreceptor resetting; hypertension; prostacyclin; reactive oxygen species; post-excitatory depression

1. Introduction

In addition to transmitting sensory signals to the central nervous system, the sensory terminals of nonmyelinated C-fiber afferents serve local effector functions. For example, activation of sensory nerve endings in skin releases the neuropeptides calcitonin gene-related peptide (CGRP), substance P, and neurokinin A that cause vasodilation and neurogenic inflammation (Holzer, 1998). This mechanism mediates the well-described 'axon reflex' where action potentials initiated in one branch of a sensory nerve are propagated to adjoining branches leading to release of neuropeptides from their terminals (Holzer, 1998).

The same or similar mechanisms are operant in other tissues including the heart, kidneys, pulmonary airways, gastrointestinal tract, and urinary bladder (Franco-Cereceda, 1988; Maggi, 1995). Furthermore, additional substances are produced in the vicinity of the activated sensory nerve endings including prostanoids, reactive oxygen species (ROS), nitric oxide, bradykinin, histamine and cytokines (Maggi, 1995; Holzer, 1998). These factors may originate from the sensory nerve endings themselves and/or from nearby cells such as leukocytes, mast cells, epithelial cells, and vascular endothelium (Maggi, 1995; Kopp et al., 1996; Holzer, 1998; Chien et al., 2003; Kopp et al., 2004). Moreover, some of these factors modulate the activity of the sensory nerves in an autocrine/paracrine manner (Holzer, 1998; Kopp et al., 1996; Kopp et al., 2004).

Baroreceptors are specialized sensory nerve endings in carotid sinuses and aortic arch that are sensitive to changes in arterial blood pressure (BP) (Kirchheim, 1976; Chapleau, 2012). Approximately 80–90% of the afferent fibers in the carotid sinus and aortic depressor nerves (ADN) are nonmyelinated (Andresen et al., 1978; Fazan et al., 1997). Neuronal cultures derived from nodose ganglia, where the soma of aortic arch baroreceptor neurons reside, produce prostacyclin and reactive oxygen species (ROS) in response to chemical and neuronal activation (Weinreich et al., 1995; Snitsarev et al., 2004; Snitsarev et al., 2005). Previous studies by members of our group have shown that repetitive injection of depolarizing current pulses causes a subsequent, long-lasting inhibition of neuronal excitability in a subpopulation of isolated baroreceptor neurons, and that this inhibition is prolonged by the cyclooxygenase inhibitor indomethacin and prevented by the ROS hydrogen peroxide (H₂O₂) scavenger PEG-catalase (Snitsarev et al., 2004; Snitsarev et al., 2005). Furthermore, exogenous prostacyclin increases the excitability of isolated baroreceptor neurons *in vitro* (Snitsarev et al., 2007). Our group and others have demonstrated that prostacyclin (and other prostanoids) and ROS also exert excitatory and

inhibitory influences on afferent baroreceptor activity, respectively (McDowell et al., 1989; Chen et al., 1990; Xie et al., 1990; Wang et al., 1993; Li et al., 1995; Li et al., 1996).

Based on the results described above, we hypothesized that transient activation of baroreceptor sensory terminals leads to H₂O₂-dependent inhibition of afferent baroreceptor activity that is opposed by an excitatory influence of endogenous prostanoids. To our knowledge, the effect of direct activation of baroreceptor C-fiber afferents on subsequent afferent baroreceptor activity has not been investigated previously. The specific aims of the study were to determine the effect of antidromic electrical stimulation of the ADN *in vivo* on afferent baroreceptor activity recorded after the period of stimulation, and to investigate the role of endogenous prostanoids and H₂O₂ in mediating the changes in nerve activity that are observed.

2. Materials and Methods

Experiments investigating responses to electrical stimulation of A-fibers vs. C-fibers in the ADN were performed at the University of Iowa in Iowa City, Iowa; and experiments testing responses to ADN stimulation before and after indomethacin, polyethylene glycolated (PEG)-catalase, and isotonic saline were performed at the University of Sao Paulo in Ribeirao Preto, Brazil. The methods used were similar in both laboratories. Differences between laboratories are described where applicable. All procedures were reviewed and approved by either the University of Iowa Animal Care and Use Committee or the Committee of Ethics in Animal Research of the School of Medicine of Ribeirão Preto (University of São Paulo, Brazil).

Male Wistar rats (270–340g) were studied at the University of Sao Paulo. The rats were anesthetized with thiopental sodium (40 mg/kg, IP; Sigma, St. Louis, MO, USA). Supplemental doses of thiopental (10 mg/kg, IV) were administered as needed. A cervical midline incision was performed and the trachea was cannulated with polyethylene tubing (PE-240 Intramedic, Clay Adams, Parsippany, NJ, USA) to facilitate ventilation during spontaneously breathing. The carotid artery and femoral vein were catheterized with polyethylene tubing (PE-50, Intramedic, Clay Adams, Parsippany, NJ, USA) for recording of arterial BP and intravenous drug administration, respectively. BP was measured with a pressure transducer (P23Gb; Statham Instruments, Hato Rey, Puerto Rico, USA).

Male Sprague-Dawley rats (250–400g) were studied at the University of Iowa. The rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and mechanically ventilated through a trachea cannula. PE catheters were placed in a femoral artery and vein for measurement of BP (Gould Statham P23 pressure transducer and Beckman type 9853 strain gauge) and administration of supplemental anesthetic, respectively. BP data were sampled at 200 Hz and recorded digitally on either a MacLab data acquisition system or an IBM/PC equipped with an analog-to-digital interface.

In both sets of experiments, the level of anesthesia and need for supplemental injections of either thiopental or pentobarbital were assessed by monitoring paw withdrawal responses to pinch and fluctuations in blood pressure and heart rate. The rats breathed or were ventilated with room air; supplemental oxygen was not administered in either set of experiments.

2.1 Recording of Baroreceptor Activity from the Aortic Depressor Nerve (ADN)

The left ADN of each rat was exposed by a ventral midline incision in the cervical region and was isolated from surrounding connective tissue below its juncture with the superior laryngeal nerve using a dissecting microscope. The ADN was placed on a bipolar stainless steel or bipolar platinum electrode in a pool of mineral oil. The procedures for recording nerve activity from the ADN have been described elsewhere (Ma et al., 2002; Salgado et al., 2006). The correct identification of the nerve was confirmed by its typical pattern of discharge synchronous with the arterial pressure pulse. The nerve activity signal was digitally sampled (10 kHz) and input along with the BP signal to the MacLab data acquisition system or the IBM/PC. ADN activity (voltage) was rectified and integrated with electrical noise (measured post-experiment) subtracted electronically in the experiments performed at the University of Sao Paulo, or counted as spikes/s using a window discriminator to eliminate electrical noise in the experiments performed at the University of Iowa (Ma et al., 2002; Salgado et al., 2006).

2.2 Electrical Stimulation of the ADN

The electrode used to record ADN activity was also used to stimulate the nerve. The electrode was connected to a stimulator (Grass, model s44/SIU5; or AVS-100, AVS Projetos, São Paulo, SP, Brazil) by means of a switch that enabled rapid conversion from recording mode to stimulus mode, and vice versa (Fig. 1). Measurement of reflex decreases in BP and heart rate (HR) during stimulation of the intact ADN confirmed correct identification of the ADN and efficacy of the chosen stimulus parameters. Before beginning the protocols, the ADN was crushed or sectioned rostral to the electrode to prevent a reflex decrease in BP during ADN stimulation (Fig. 1).

2.3 Drugs

Thiopental sodium, sodium pentobarbital, indomethacin, and PEG-catalase were purchased from Sigma, Co (St. Louis, MO, USA).

2.4 Protocols

Responses to A-fiber vs. A+C fiber stimulation—Arterial BP and ADN activity were recorded for at least 1 minute before and for 5 minutes after stimulating the ADN for 5 seconds using parameters that selectively activate myelinated A-fibers (0.2ms, 5V pulses at 80Hz) and parameters that activate both A and C fibers (2ms, 5V pulses at 20Hz) (Douglas et al., 1956; Numao et al., 1985; Fan & Andresen, 1998; Fan et al., 1999) (n=9). The order of testing was randomized. The effect of changes in the duration of A+C fiber stimulation (5, 10 and 20 s) on the changes in afferent nerve activity measured after the period of stimulation was determined in an additional group of rats (n=5).

Contributions of prostanoids and H₂O₂ to changes in baroreceptor activity after ADN stimulation—To determine the role of endogenous prostanoids in mediating the changes in afferent baroreceptor activity following transient stimulation of A and C fibers in ADN (2ms, 5V pulses at 20Hz for 10 s), BP and ADN activity were recorded for 2 minutes before and for 5 minutes after the 10 second period of ADN stimulation, both under

control conditions and again ~15–20 minutes after IV injection of the cyclooxygenase inhibitor indomethacin (5mg/kg, IV, n=7). In a second group of rats, the role of H₂O₂ in mediating the changes in baroreceptor activity was determined by measuring responses to ADN stimulation before and after administration of PEG-catalase (10⁴ U/kg, IV, n=7), an enzyme that catalyzes the decomposition of H₂O₂ to water and oxygen. To demonstrate that responses to ADN stimulation are reproducible with repeated testing, responses to ADN stimulation were measured before and after administration of the saline vehicle solution (0.1 ml of 0.9% NaCl/100g body wt, IV) in a third group of rats (n=6).

2.5 Data Analysis

ADN activity was normalized as a percentage of the baseline activity measured before the period of ADN stimulation. Group data for mean BP and normalized ADN activity are presented as means $\pm$ SE. The effects of ADN stimulation on BP and ADN activity measured over time post-stimulation (vs. baseline) and differences between treatment groups were analyzed by two-factor ANOVA with repeated measures design. When ANOVA was significant, Tukey's post hoc test was used to assess the differences between means. Differences were considered significant when $P < 0.05$.

3. Results

3.1 Responses to A-fiber vs. A+C Fiber Stimulation

As expected, section or crush of the ADN rostral to the electrode abolished the reflex changes in BP and HR during ADN stimulation (Figs. 2 and 3). The recordings of ADN activity revealed transient inhibition of baroreceptor activity after selective antidromic stimulation of A-fibers that dissipated within 30 seconds (Fig. 3). In contrast, the inhibition of baroreceptor activity was pronounced (>50%) and sustained (>1min) after combined antidromic stimulation of A and C fibers (Figs. 2 and 3). The magnitude and duration of the inhibition of activity after ADN stimulation were increased with increases in the duration of the stimulation period from 5 to 10 to 20 seconds (Fig. 4).

3.2 Contributions of Prostanoids and H₂O₂ to Changes in Baroreceptor Activity

Mean arterial BP was unaffected by treatment of rats with saline, indomethacin, or PEG-catalase, or by stimulation of the ADN caudal to the site of nerve crush or section (Figs. 5 and 6). The results from 3 representative experiments depicting effects of saline, indomethacin, and PEG-catalase on the nerve activity responses to ADN stimulation are shown in Figure 5. The summarized data from each experimental group are shown in Figure 6. The magnitude of inhibition of nerve activity was not altered by saline injection, was prolonged after injection of indomethacin, and was abolished after injection of PEG-catalase (Figs. 5 and 6). Indomethacin did not significantly affect the initial inhibition of baroreceptor activity after the period of ADN stimulation, but instead slowed the recovery of nerve activity over time. Baroreceptor activity did not fully return to basal levels within the 4 minute period of observation in indomethacin-treated rats.

4. Discussion

The major novel findings of this study are: (1) Antidromic electrical stimulation of C and A-fiber afferents in the ADN leads to prolonged inhibition of baroreceptor activity *in vivo*, an effect that persists much longer than that observed after selective antidromic activation of A-fiber afferents only; (2) The cyclooxygenase inhibitor indomethacin does not augment the maximal inhibition but prolongs its duration indicating that endogenous prostanoids contribute to the recovery of nerve activity; and (3) The inhibition of baroreceptor activity is abolished by PEG-catalase thereby identifying H₂O₂ as the mediator of the inhibition of activity.

In the remaining discussion we address the relationship of our results to the previously described phenomenon of 'post-excitatory depression' after a period of increased intravascular pressure, the evidence supporting the roles of prostanoids and H₂O₂ in sensory signaling, and the physiological significance of our findings.

4.1 Post-excitatory Depression of Baroreceptors

Activation of baroreceptors by an increase in non-pulsatile pressure within the isolated aortic arch or carotid sinus causes 'post-excitatory depression' of single-fiber activity when pressure is rapidly returned to the baseline pressure (Bronk & Stella, 1935; Landgren, 1952; Saum et al., 1976). The duration of post-excitatory depression (neural silence) ranges from ~100 ms to ~5–8 seconds after rapidly decreasing the pressure, with the duration dependent on the level and duration of the preceding pressure step and the number of action potentials evoked during the period of increased pressure (Landgren et al., 1952; Saum et al., 1976). This transient post-excitatory depression has been attributed to activation of an electrogenic Na⁺/K⁺ pump and consequent hyperpolarization of the nerve endings (Saum et al., 1976). Baroreceptor activity recovers quickly from inhibition, approaching a steady-state level of activity within ~5 seconds after the first action potential is generated (Saum et al., 1976). Thus, classical post-excitatory depression is much shorter in duration than the prolonged inhibition of baroreceptor activity (>60s) we observed after low-frequency antidromic electrical stimulation of A- and C-fibers in ADN *in vivo* (Figs. 2–6).

We propose that activation of nonmyelinated sensory C-fibers is necessary to evoke long-lasting inhibition of baroreceptor activity. While myelinated A-fibers can be selectively activated by electrical stimulation with short duration (.02–.2ms) and/or low voltage (<3V) pulses, activation of C-fibers requires longer pulse durations and/or higher voltages (Douglas et al., 1956; Numao et al., 1985; Fan & Andresen, 1998; Fan et al., 1999). Furthermore, low-frequency (5–20Hz) stimulation of C-fibers in the ADN evokes robust reflex decreases in sympathetic nerve activity and BP, whereas higher frequencies of stimulation are required to evoke A-fiber mediated reflexes (Douglas et al., 1956; Numao et al., 1985; Fan & Andresen, 1998; Fan et al., 1999). We confirmed that low-frequency (20Hz) stimulation of the intact ADN with 5 V, 2 ms pulses elicited substantial reflex decreases in BP and heart rate, while high-frequency stimulation of A-fibers (5V, .2ms, 80Hz) was required to evoke the reflex (data not shown). These results support our choice of stimulus parameters and our proposal that activation of C-fibers was responsible for the prolonged inhibition of baroreceptor activity in our study.

The only previous study to use antidromic electrical stimulation to evoke baroreceptor post-excitatory depression did not report the stimulus parameters used (Saum et al., 1976). Thus, C-fibers may not have been activated in those experiments. Furthermore, the effectiveness of step increases in non-pulsatile pressure in isolated carotid sinus and aortic arch preparations to activate baroreceptor C-fibers is limited, as many of these fibers have high pressure thresholds and show pronounced adaptation of spike firing frequency during sustained step increases in non-pulsatile pressure (Thoren et al., 1977; Thoren et al., 1999). In addition, viscoelastic changes in the arterial wall reduce the tension transmitted to the nerve endings during sustained changes in BP making it difficult to separate mechanical vs. neuronal contributions to changes in nerve activity (Kirchheim, 1976; Krieger et al., 1982; Coleridge et al., 1984). For these reasons, we chose to activate baroreceptor afferent fibers directly by electrical stimulation.

Advantages of the *in vivo* antidromic ADN stimulation/recording preparation include the ability to selectively activate afferent fiber types in a controlled manner in the absence of a change in BP and to record baroreceptor activity under the physiological conditions of an intact blood-perfused circulation and pulsatile arterial BP. Limitations of the preparation include the unphysiological, synchronous activation of all or nearly all A or A + C fibers during electrical stimulation and the inability to determine the relative contribution of A-fibers and C-fibers to the inhibition of ADN activity observed after antidromic nerve stimulation. Single-fiber recordings will be required to answer the latter question. The prolonged inhibition of nerve activity after antidromic stimulation of A + C fibers is consistent with release of a chemical mediator.

It is important to address the possibility that electrical stimulation of the ADN may activate chemoreceptor as well as baroreceptor afferents. To our knowledge, there is no evidence of efferent nerves in the rat ADN. While the functional significance of aortic chemoreceptor reflexes in rats has been challenged and debated (Sapru & Krieger, 1977; Sapru et al., 1981; Kobayashi et al., 1999), the presence of glomus tissue innervated by chemosensitive afferent fibers in the ADN has been convincingly demonstrated in rats (Cheng et al., 1997; Brophy et al., 1999).

We cannot rule out a possible contribution, but believe it is unlikely that activation of chemoreceptor afferents was responsible for the subsequent inhibition of baroreceptor activity in our study. In previous studies we demonstrated that activation of sensory neurons isolated from nodose ganglia (including baroreceptor neurons) generates reactive oxygen species and decreases neuronal excitability after the period of stimulation, and that PEG-catalase abolishes these responses (Snitsarev et al., 2004; Snitsarev et al., 2005). The similarity of our *in vivo* and *in vitro* results along with the relatively low number of chemoreceptor afferents in the rat ADN (Cheng et al., 1997; Kobayashi et al., 1999) suggest that antidromic activation of baroreceptor terminals initiated the subsequent inhibition of baroreceptor activity we report here. Future studies are needed to further define the mechanisms involved.

4.2 Contributions of Prostanoids and H₂O₂ to Sensory Signaling

Activation of sensory C-fibers leads to release of numerous factors in diverse tissues, including the neuropeptides CGRP and substance P, prostanoids, ROS, nitric oxide, bradykinin, histamine and cytokines (Franco-Cereceda, 1988; Maggi, 1995; Holzer, 1998). The neuropeptides are produced in the sensory nerves, but multiple cell types including the nerve endings may contribute to release of the other factors *in vivo* (Kopp et al., 1996; Holzer, 1998; Chien et al., 2003; Kopp et al., 2004). Substance P has been shown to generate ROS in sensory neurons isolated from dorsal root ganglia (Linley et al., 2012). While direct evidence of release of prostanoids and ROS from baroreceptor nerve terminals is lacking, production of these mediators has been demonstrated in response to chemical and electrical activation of neurons isolated from nodose ganglia, where soma of aortic baroreceptors reside *in vivo* (Weinreich et al., 1995; Snitsarev et al., 2004; Snitsarev et al., 2005).

Previous studies from our laboratory and others have demonstrated that prostanoids and ROS exert excitatory and inhibitory influences on the activity measured in baroreceptor afferent fibers, respectively (McDowell et al., 1989; Chen et al., 1990; Xie et al., 1990; Wang et al., 1993; Li et al., 1995; Li et al., 1996). The finding that indomethacin inhibited baroreceptor activity recorded from the isolated carotid sinus after removal of the endothelium (Wang et al., 1993) is consistent with a neuronal source of endogenous prostanoids.

In the present study, administration of PEG-catalase abolished the inhibition of ADN activity after the period of ADN stimulation, suggesting that H₂O₂ is the mediator (Figs. 5 and 6). H₂O₂ is produced in multiple cell types during periods of increased metabolic activity and participates in cell signaling in the nervous system (Saitoh et al., 2006; Gamper et al., 2006; Riquelme et al., 2011; Rice, 2011; Linley et al., 2012). It has been shown to hyperpolarize neurons and decrease neuronal excitability, primarily by increasing K⁺ channel activity (Vega-Saenz de Miera & Rudy, 1992; Gamper et al., 2006; Dyavanapalli et al., 2010; Rice, 2011). Activity-induced oxidation of cysteine and/or methionine residues in K⁺ channels has been proposed to provide negative feedback regulation of neuronal excitability (Hoshi & Heinemann, 2001; Gamper et al., 2006; Linley et al., 2012). Therefore, H₂O₂-sensitive K⁺ channels are prime candidates for mediating the inhibition of baroreceptor activity after antidromic stimulation of the ADN (Figs. 2–6). Indeed, large conductance Ca²⁺-activated BK, A-type, and M-type K⁺ channels are important regulators of excitability in baroreceptor neurons (Chapleau et al., 1993; Snitsarev et al., 2007; Wladyka et al., 2008).

Activation of the Na⁺/K⁺ pump may contribute to the inhibition of baroreceptor activity after ADN stimulation. The Na⁺ pump mediates transient post-excitatory depression of activity in a variety of sensory nerves (Nakajima & Takahashi, 1966; Sokolove & Cooke, 1971) including baroreceptor terminals (Saum et al., 1976), and its activation is enhanced by H₂O₂ (Matsuoka et al., 1990; Rounds et al., 1998). The stability of H₂O₂ may account for the relatively prolonged period of inhibition of baroreceptor activity observed after antidromic stimulation of ADN.

The duration of inhibition of baroreceptor activity after ADN stimulation was increased (>4 min post-stimulation) after administration of the cyclooxygenase inhibitor indomethacin (Figs. 5 and 6), suggesting that endogenous prostanoids facilitate the restoration of activity. The results are consistent with previous studies demonstrating generation of prostacyclin in neuronal cultures derived from nodose ganglia (Weinreich et al., 1995; Snitsarev et al., 2005), and excitatory effects of prostanoids on baroreceptor activity recorded from the carotid sinus nerve (McDowell et al., 1989; Chen et al., 1990; Xie et al., 1990; Wang et al., 1993; Li et al., 1995) and membrane excitability of isolated nodose sensory neurons (Weinreich et al., 1995; Li et al., 1997; Snitsarev et al., 2005; Snitsarev et al., 2007).

The *in vivo* results reported here are remarkably similar to those obtained in previous *in vitro* studies (Snitsarev et al., 2004; Snitsarev et al., 2005) in which: (1) electrical stimulation of nodose neurons in culture decreased neuronal excitability after the period of stimulation associated with increased production of ROS and prostacyclin; (2) the inhibition of excitability after the period of neuronal activation was abrogated by PEG-catalase and prolonged by indomethacin; and (3) the recovery of excitability after the period of neuronal activation was accelerated by the stable prostacyclin analog carbacyclin in the continued presence of indomethacin.

4.3 Perspectives and Significance

The results of the present study demonstrate that antidromic activation of nonmyelinated C-fiber afferents in the ADN causes a prolonged inhibition of afferent baroreceptor activity after the period of stimulation that appears to be mediated by H_2O_2 and opposed by prostanoids. These autocrine/paracrine mechanisms may contribute to rapid baroreceptor resetting during acute hypertension (Krieger et al., 1982; Coleridge et al., 1984) and be engaged during chronic baroreceptor stimulation therapy in patients with drug-resistant hypertension (Taylor & Bisognano, 2010).

To our knowledge, a possible contribution of H_2O_2 or other ROS to resetting of the baroreceptor pressure-activity relationship to higher pressures during acute hypertension has not been investigated. The role of prostanoids has been studied with variable results. Systemic administration of indomethacin to anesthetized rats did not significantly affect mean BP or the magnitude of baroreceptor resetting after 30 minutes of hypertension induced by aortic coarctation (Salgado et al., 2006). In contrast, resetting of the pressure threshold of myelinated baroreceptor fibers induced by 15-minute exposures of the isolated carotid sinus of dogs to different 'conditioning' pressures was decreased in the presence of indomethacin (Wang et al., 1993), not increased as one would have expected from our results.

Reasons for the different results are not clear, but may relate to: (1) failure of pressure changes to sufficiently activate baroreceptor C-fibers; (2) differences in the intensity or duration of baroreceptor activation; (3) differential effects of increased BP and neuronal activation on generation of prostanoids; and/or (4) the complex interactions between neuronal and vascular prostanoid, ROS and nitric oxide systems (Salgado et al., 2006; Félétou et al., 2010). Further studies are needed to determine the contributions of C-fiber

activation and autocrine/paracrine actions of H₂O₂ and prostanoids to baroreceptor resetting during acute hypertension.

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Figure 1.

Experimental preparation. Shown is a diagram of the preparation that enables rapid conversion from stimulating the ADN to recording ADN activity and vice versa. Before performing the protocols, the ADN was crushed or sectioned rostrally to the electrode to prevent reflex changes in arterial BP during and after the period of nerve stimulation.

Figure 2.

Recordings of arterial pressure and the ADN neurogram before and after antidromic stimulation of the ADN using parameters designed to activate both A and C fibers at low frequency (2ms 5V pulses at 20Hz for 10s). Marked inhibition of the pulse synchronous ADN activity is evident 5 and 30 seconds post-stimulation. Arterial pressure was not affected by the nerve stimulation. The suggestion of a lower pulse pressure 180 seconds post-stimulation was the result of dampening of the pressure recording. Pulse pressure was restored after flushing the catheter with saline.

Figure 3.

Comparison of the magnitude and duration of the inhibition of baroreceptor activity after antidromic stimulation of A-fibers vs. A + C fibers. Representative traces from one experiment (Panel A) and group data (Panel B, n=9) are shown. Stimulation parameters for A-fibers: 0.02ms 5V pulses at 80Hz for 5s; for A+C fibers: 2ms 5V pulses at 20Hz for 5s. * Significant change from baseline (pre-stimulation) nerve activity, $P<0.05$. † Significant difference between activity measured after stimulation of A + C fibers vs. after stimulation of A fibers, $P<0.05$.

Figure 4.

The magnitude of inhibition of baroreceptor activity after antidromic stimulation of A + C fibers is dependent on the duration of the stimulation period. *Panel A* shows the magnitude and time course of the responses after stimulating the ADN (2ms 5V pulses at 20Hz) for 5, 10 and 20s periods (n=5). *Panel B* shows the level of activity measured 30s after stimulating for 5, 10 and 20s (n=5). * Significant change from baseline nerve activity, $P < 0.05$. † Significant difference between activity measured after 10 or 20s of ADN stimulation vs. after 5s of stimulation, $P < 0.05$.

Figure 5.

Shown are recordings of arterial pressure and mean ADN activity from 3 representative experiments that depict effects of saline (A), indomethacin (B), and PEG-catalase (C) on ADN stimulation-induced inhibition of baroreceptor activity. ES = electrical stimulation of ADN.

Figure 6.

Shown are average values of mean blood pressure (BP) and ADN activity (% of baseline, mean $\pm$ SE) under baseline conditions (B) and after antidromic ADN stimulation in rats treated with saline (n=6), indomethacin (n=7), and PEG-catalase (n=7). Results obtained before and after the 3 treatments are indicated by solid and open symbols, respectively. The ADN was stimulated with 2ms, 5V pulses at 20Hz for 10min. * Significant change from baseline nerve activity, $P < 0.05$. † Significant difference between activity measured before vs. after drug treatment, $P < 0.05$.