Baroreceptor and chemoreceptor contributions to the hypertensive response to bilateral carotid occlusion in conscious mice


Baroreceptor and chemoreceptor contributions to the hypertensive response to bilateral carotid occlusion in conscious mice. Am J Physiol Heart Circ Physiol 299: H1990–H1995, 2010. First published September 17, 2010; doi:10.1152/ajpheart.00315.2010.—This study aimed to characterize the role played by baroreceptors and chemoreceptors in the hypertensive response to bilateral carotid occlusion (BCO) in conscious C57BL mice. On the day before the experiments the animals were implanted with pneumatic cuffs around their common carotid arteries and a femoral catheter for measurement of arterial pressure. Under the same surgical approach, groups of mice were submitted to aortic or carotid sinus denervation or sham surgery. BCO was performed for 30 or 60 s, promoting prompt and sustained increase in mean arterial pressure and fall in heart rate. Compared with intact mice, the hypertensive response to 30 s of BCO was enhanced in aortic-denervated mice (52 ± 4 vs. 41 ± 4 mmHg; P < 0.05) but attenuated in carotid sinus-denervated mice (15 ± 3 vs. 41 ± 4 mmHg; P < 0.05). Suppression of peripheral chemoreceptor activity by hyperoxia [arterial partial pressure of oxygen (PaO₂) > 500 mmHg] attenuated the hypertensive response to BCO in intact mice (30 ± 6 vs. 51 ± 5 mmHg in normoxia; P < 0.05) and abolished the bradycardia. It did not affect the hypertensive response in carotid sinus-denervated mice (20 ± 4 vs. 18 ± 3 mmHg in normoxia; P < 0.05). The attenuation of the hypertensive response to BCO by carotid sinus denervation or hyperoxia indicates that the hypertensive response in conscious mice is mediated by both baro- and chemoreceptors. In addition, aortic denervation potentiates the hypertensive response elicited by BCO in conscious mice.

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Baroreflex; chemoreflex; carotid sinus denervation

Improvements in gene characterization and the development of techniques enabling genetic manipulation have led to advances in our understanding of physiological mechanisms (4, 7, 9). The cardiovascular system in mice has become increasingly interesting to researchers because of the availability of genetic tools that can be applied in this species. For this reason, several methods of cardiovascular investigation have been adapted from larger animals to mice.

Previous studies have used drug-induced changes in arterial pressure (AP) to investigate baroreflex function in mice. Nevertheless, repeated injections of vasoactive drugs such as phenylephrine or sodium nitroprusside may increase inappropriately the small blood volume of mice, limiting the number of times the baroreflex can be evaluated (18, 22). Bilateral carotid occlusion (BCO) has been used as a reliable approach for examining baroreflex function in anesthetized mice (11, 17, 25, 26, 27). During BCO the intrasinus pressure falls and elicits temporary cessation of carotid barosensory discharges that in turn triggers a reflex increase in AP (1, 5). In conscious rats, BCO lasting 60 s induces a pressor response that can be divided into two components: an initial peak of higher magnitude that develops within the first 20 s and a sustained response of lower magnitude that develops within the next 40 s (2, 3, 20). In contrast, the hypertensive response to BCO in conscious dogs (10, 29), cats (30), or rabbits (15, 31) reaches an upper plateau without decaying over time throughout the occlusion.

It is well documented that the cardiovascular effects of BCO involve not only the baroreceptors but also the chemoreceptors (5). The pressor response produced by BCO is reduced during artificial ventilation with 100% oxygen in anesthetized cats (1, 6) and mice (26), indicating that carotid chemoreceptors play a role in this hypertensive response. In anesthetized rats, there is evidence that the second component of the hypertensive response to BCO requires intact carotid chemoreceptors at carotid bodies on the external carotid arteries near the common carotid bifurcation (16).

To our knowledge, the cardiovascular responses triggered by BCO in mice have only been examined under anesthesia (11, 26, 27). Therefore, the present study aimed to examine AP and heart rate (HR) responses to BCO in conscious mice and to describe the roles played by baroreceptors and chemoreceptors in these responses.

METHODS

The experimental protocols used in this study were reviewed and approved by the Committee of Ethics in Animal Research of the School of Medicine of Ribeirão Preto, University of São Paulo (Protocol No. 007/2006). The experiments were performed on male C57BL/6J mice weighing 25–30 g. Mice were housed individually with free access to food and water and were maintained on a 12:12-h light-dark cycle. At the end of the experiments, the mice were killed with an intravenous overdose of tribromoethanol.

Bilateral Carotid Occlusion

BCO in conscious, freely moving mice was performed with pneumatic cuffs that were implanted 1 day before the experiment under tribromoethanol anesthesia (250 μg/g ip). Pneumatic cuffs were constructed as described elsewhere for rats (3) and adapted to mice. Briefly, the tip of a 4-cm length of polyethylene tubing (PE-10) was slightly dilated by heating. Both tips of a second piece of tubing (PE-190), 0.5 cm in length, were also diluted by heating. The PE-190 tubing was then cut along its longitudinal axis, providing two edges. A hole was placed in the center of the opposite wall of the edges of the PE-190 tubing. Next, a rubber membrane was fixed with suture thread over the dilated tip of the PE-10 tubing, which becomes a balloon when inflated with water. The rubber membrane was then

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pushed through the hole of the PE-190 tubing, completing the construction (Fig. 1). The pneumatic cuff was fitted around each carotid artery through an incision in the ventral cervical region, and the PE-10 tubing from the cuff was tunneled and fixed on the back of the neck. For simultaneous occlusion of both carotid arteries, both cuffs were connected to a 3-ml syringe filled with water by Y-shaped polyethylene tubing.

Selective Denervation

During the same surgical procedure in which the pneumatic cuffs were implanted, selective carotid sinus (baro- and chemoreceptors) or aortic baroreceptor denervation was performed as described by Krieger (14) for rats and adapted to mice. Briefly, each animal was placed in a supine position, and a ventral midline incision was made in the neck. For selective carotid sinus denervation the carotid bifurcation was exposed. The adventitia and associated connective tissue were stripped from the carotid sinus region including the adjacent internal, external, occipital, and common carotid arteries. After this procedure was completed bilaterally, the cuffs were implanted and the incision was sutured closed. For selective aortic denervation the sympathetic trunk, superior cervical ganglion, and superior laryngeal nerves were visualized and removed. Sham-operated mice—referred to below as “intact”—underwent a similar surgical procedure involving bilateral exposure of the carotid bifurcation, sympathetic trunk, superior cervical ganglion, and superior laryngeal nerves without damaging the innervation, followed by bilateral pneumatic cuff implantation and suture of the incision.

Femoral Artery Cannulation and Blood Pressure Recording

A polyethylene catheter (PE-0.08 connected with PE-50) was inserted into the left femoral artery and exteriorized between the scapulae of the mouse immediately after carotid sinus or aortic denervation followed by pneumatic cuff implantation around the carotid arteries. AP was recorded by means of a pressure transducer (P23XL, Statham Instruments, Valley View, OH) connected to the polyethylene catheter inserted into the left femoral artery. During the experiment, the pulsatile AP was continuously sampled (2 kHz) with an IBM computer equipped with an analog-to-digital interface (DI-220, Dataq Instruments). HR was calculated from pressure recordings on a beat-by-beat basis by measuring the intervals between successive diastolic pressure values.

Experimental Protocols

One day after surgery, the mice were taken to the recording room at least 1 h before the protocol began to adapt them to the experimental environment. Four protocols were carried out.

Protocol 1. BCO for 60 s in intact mice. After a basal AP recording for 5 min, conscious intact mice (n = 6) were submitted to two periods of BCO (60 s) with an interval of 30 min between each period. The results of this protocol demonstrated that BCO elicited a prompt (within 10 s) increase in AP that leveled off until the end of the 60-s period. This finding led us to carry out BCO for 30 s in the next two protocols, while the hypertensive response was measured 15–20 s after the beginning of BCO.

Protocol 2. BCO for 30 s in intact or aortic- or carotid sinus-denervated mice. After a basal AP recording for 5 min, three groups of mice consisting of intact (n = 7), aortic-denervated (n = 7), and carotid sinus-denervated (n = 5) mice were submitted to BCO for 30 s.

Protocol 3. BCO under normoxia and hyperoxia in intact or carotid sinus-denervated mice. In two distinct groups of mice, i.e., intact and carotid sinus denervated, the suppression of chemoreceptor activity by hyperoxia was used to evaluate the role of the chemoreflex in the hypertensive response to BCO. In this approach the chemoreflex component was calculated by subtracting the hypertensive response to BCO during hyperoxia from the hypertensive response during normoxia. The animals were placed inside an acrylic chamber (10-cm diameter × 15-cm length), and hyperoxia was produced by flushing the chamber with 100% oxygen (1 l/min) for 5 min. The time frame of 5 min was chosen on the basis of preliminary studies in which oxygen concentrations were measured by an oximeter (OA 272, Taylor Servomex) placed inside the chamber.

Before the experiment, intact (n = 7) or carotid sinus-denervated (n = 6) mice were allowed to adapt to the acrylic chamber’s environment for 1 h while atmospheric air flowed through the chamber. BCO was applied twice in each mouse, i.e., during normoxia and hyperoxia. The order of normoxia or hyperoxia for applying the BCO was randomly chosen.

Protocol 4. Arterial blood gases and pH. In a separate group (n = 6), mice were anesthetized with tribromoethanol (250 μg/g ip) and a polyethylene catheter (PE-10 soldered with PE-50) was introduced into the carotid artery, exteriorized, and fixed on the nape of the mouse. The next day, the mouse was placed inside the acrylic chamber and was ventilated with atmospheric air. After a 1-h period of adaptation, arterial blood samples were taken from the arterial catheter for measurement of the partial pressure of oxygen (Pao2), partial pressure of carbon dioxide (Paco2), and pH (Cobas b 121, Roche Diagnostics, São Paulo, Brazil). Next, after a 30-min period of recovery from the first arterial blood collection, the chamber was flushed with 100% oxygen (1 l/min) for 5 min and arterial blood samples were collected again for analysis of Pao2, Paco2, and pH.

Statistical Analysis

Data are expressed as means ± SE. Basal mean arterial pressure (MAP) and HR and their responses to BCO were analyzed by two-way analysis of variance (ANOVA) for repeated measurements followed by the Tukey test. Statistical significance was considered for P < 0.05.

Fig. 1. Photography showing the pneumatic cuff used to perform bilateral carotid occlusion (BCO) in mice. A lateral view is shown at top, while details of the cuff empty and filled with water are shown at middle and bottom, respectively.
RESULTS

BCO for 60 s in Intact Mice

Figure 2 shows a representative tracing of pulsatile AP and HR of a conscious intact mouse submitted to BCO for 60 s. BCO caused a sustained rise in AP and fall in HR followed by a prompt return to baseline levels after cessation of BCO. Figure 3 shows group data of the hypertensive and bradycardic responses to BCO for the two sets of occlusion. The average baseline values for MAP and HR before BCO were 115 ± 3 mmHg and 621 ± 17 beats per minute (bpm) for the first set and 115 ± 2 mmHg and 594 ± 41 bpm for the second set of BCO. The two sets of BCO produced similar AP and HR responses characterized by a marked and sustained increase in MAP (40 ± 5 and 42 ± 11 mmHg for 1st and 2nd sets of BCO, respectively) and a fall in HR (−134 ± 40 and −102 ± 31 bpm for 1st and 2nd sets of BCO, respectively).

BCO for 30 s in Intact, Aortic-Denervated, and Carotid Sinus-Denervated Mice

Figure 4 shows group data for the hypertensive and bradycardic responses to 30 s of BCO in conscious intact, aortic-denervated, and carotid sinus-denervated mice. The average baseline values of MAP and HR were not different in the three groups (intact mice: 112 ± 2 mmHg and 587 ± 43 bpm; aortic-denervated mice: 118 ± 2 mmHg and 567 ± 30 bpm; carotid sinus-denervated mice: 118 ± 2 mmHg and 427 ± 37 bpm). The hypertensive response to BCO was enhanced in aortic-denervated mice (52 ± 4 vs. 41 ± 4 mmHg; P < 0.05) but was attenuated in carotid sinus-denervated mice (15 ± 3 vs. 41 ± 4 mmHg; P < 0.05). Aortic-denervated mice presented a transient tachycardia during the onset of BCO (25 ± 11 bpm), followed by a bradycardic response that was smaller than that observed in intact mice (−62 ± 29 vs. −110 ± 38 bpm; P < 0.05). BCO did not change HR in carotid sinus-denervated mice.

BCO for 30 s Under Normoxia and Hyperoxia in Intact and Carotid Sinus-Denervated Mice

Table 1 shows that during hyperoxia there was a remarkable increase in PaO₂, while PaCO₂ and pH were the same as during normoxia.
normoxia. Figure 5 shows the group data for MAP and HR responses to 30 s of BCO in intact mice during normoxia (21% oxygen) and hyperoxia. Suppression of chemoreceptor activity by hyperoxia did not affect basal MAP and HR of intact mice (117 ± 2 vs. 120 ± 2 mmHg and 543 ± 40 vs. 519 ± 40 bpm during hyperoxia) or carotid sinus-denervated mice (115 ± 2 vs. 115 ± 4 mmHg and 518 ± 15 vs. 520 ± 26 bpm during hyperoxia). Compared with normoxia, hyperoxia attenuated the hypertensive response to BCO and prevented the fall in HR in intact mice. MAP and HR during BCO were not affected by hyperoxia in carotid sinus-denervated mice.

### Discussion

The results of the present study demonstrated the following: 1) BCO elicited a remarkable rise in AP and fall in HR that attained a plateau within 10–20 s; 2) compared with intact mice, aortic-denervated mice, and carotid sinus-denervated mice, the hypertensive response to BCO was markedly reduced compared with intact mice and no change in HR was observed; 3) suppression of chemoreceptor activity by hyperoxia attenuated the hypertensive response and abolished the bradycardic response to BCO in intact mice but did not affect either the MAP or HR responses in carotid sinus-denervated mice.

The two sets of BCO demonstrated that the hypertensive response was reproducible. Both sets of BCO elicited a prompt increase in AP that leveled off throughout the period of occlusion. A similar pattern of response was observed in conscious dogs (10, 29), cats (30), and rabbits (15, 31). In anesthetized intact mice, BCO elicits a modest increase in systemic AP of 13–20 mmHg (11, 26), which is smaller than the response observed in the present study in conscious mice. It is well documented that anesthesia affects the reflex hypertensive response to BCO (3). In conscious intact mice, the hypertensive response due to BCO was ~40 mmHg, providing support to the notion that anesthesia blunts this hypertensive response. Thus the undesirable effects of anesthesia might be a reasonable explanation for the differences among the hypertensive responses obtained by Sun et al. (26), Jung et al. (11), and the present study.

### Table 1

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<th>PaO₂, mmHg</th>
<th>PaCO₂, mmHg</th>
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<tr>
<td>Normoxia</td>
<td>104 ± 2</td>
<td>29 ± 3</td>
<td>7.4 ± 0.04</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>567 ± 6*</td>
<td>33 ± 3</td>
<td>7.4 ± 0.03</td>
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Values are means ± SE for n = 6 mice. PaO₂, arterial partial pressure of oxygen; PaCO₂, arterial partial pressure of carbon dioxide. *P < 0.01 compared with normoxia.

### Figure 5

MAP and HR responses to 30 s of BCO in intact (n = 7) and carotid sinus-denervated (n = 6) mice during normoxia and hyperoxia. *P < 0.05 group effect (normoxia vs. hyperoxia; 2-way ANOVA for repeated measurements); significant (P < 0.05) time effect was shown (2-way ANOVA for repeated measurements) for both groups (intact and carotid sinus denervated) in both situations (normoxia and hyperoxia).
of the two components of the pressor response to BCO in rats being responsible for the AP decrease toward a lower plateau. In the present study, the hypertensive response to BCO in aortic-denervated mice was ~21% greater than that in intact mice, while in conscious rats aortic denervation enhanced the pressor response to BCO by ~70% (20). On the basis of this finding, we can speculate that, in contrast to rats, the aortic baroreceptors have a smaller contribution in blunting the pressor response to BCO in mice.

A transient rise in HR accompanied the onset of the hypertensive response to BCO in aortic-denervated mice (Fig. 4). As the BCO was maintained, the tachycardia was reversed to a modest bradycardia (Fig. 4). The absence of aortic baroreflex-mediated bradycardia in aortic-denervated mice likely unmasked the initial tachycardia and attenuated the subsequent bradycardia, presumably mediated by chemoreceptor activation given its absence after carotid sinus denervation (Fig. 4).

The pressor response (15 ± 3 mmHg) to BCO in conscious carotid sinus-denervated mice was substantially attenuated compared with the response in intact mice but was not totally suppressed. It is well known that afferents from the carotid sinus contain baro- and chemoreceptor fibers, and the usual procedure for carotid sinus denervation eliminates both types of fibers (8, 28). Thus the residual increase in AP after carotid sinus denervation in mice might be associated with a nonspecific ischemic mechanism involving the central nervous system. It is well documented that BCO reduces cerebral blood flow in mice (13, 21, 32). Therefore, when carotid occlusion is performed to examine baroreceptor function, this central ischemic component should also be taken into account. Accordingly, the residual pressor response to BCO observed in carotid sinus-denervated mice probably does not involve the baro- or chemoreflex but, presumably, cerebral ischemia. Denervation of the carotid sinus region completely prevented any change in HR during BCO.

In intact mice, hyperoxia attenuated the hypertensive response to BCO and completely abolished the bradycardia. It is well known that peripheral chemoreceptors are stimulated by decreases in PaO2 (19). The activation of chemoreceptors during BCO can be effectively suppressed by high PaO2, as achieved during 100% oxygen ventilation (1, 18, 26). In the present study, PaO2 achieved values around 560 mmHg after the experimental chamber was flushed with 100% oxygen for 5 min. When BCO was performed during hyperoxia, the hypertensive response was less intense and the fall in HR did not occur, suggesting that chemoreceptor activation mediated the bradycardia and contributed to the pressor response in intact mice. Moreover, hyperoxia did not affect the hypertensive or bradycardic response in carotid sinus-denervated mice. The similarity of results obtained under normoxia and hyperoxia in carotid sinus-denervated mice precludes the hypothesis that hyperoxia, by itself, affects the pressor response to BCO by influencing central neurotransmission, vascular responsiveness to sympathetic nerve activity, or cardiac output. Therefore, the difference between the AP and HR responses to BCO performed while intact mice were breathing atmospheric air or 100% oxygen represents the contribution of chemoreceptor activation.

Overall, the approaches used in the present study, i.e., carotid sinus denervation and/or hyperoxia, showed that both baro- and chemoreceptors are important contributors to the hypertensive response to BCO in conscious mice. In addition, the significant residual hypertensive response to BCO observed in carotid sinus-denervated mice could be attributed to progressive cerebral ischemia.

**Perspectives**

With advances in genomic sciences, the mouse is receiving special attention because of its unique susceptibility to genetic manipulation. Nevertheless, much of the normal physiology, as well as pathophysiological aspects well established for other species, remains unknown for the mouse. Previous studies have used drug-induced changes in AP to investigate baroreflex function in mice. However, repeated injections of vasoactive drugs may increase inappropriately the small blood volume of mice, limiting the evaluation of baroreflex function (18, 22). An additional assay of baroreflex function, the BCO reflex, avoids these limitations. The results from the present study demonstrate that BCO is a reliable tool for examining baroreflex and chemoreflex function in conscious mice. Furthermore, the hypertensive response due to BCO from intact and/or baroreceptor-denervated mice can be compared with other species such as rat, dog, and rabbit, providing valuable information concerning the relative roles played by the aortic and carotid baroreceptors in the control of AP among species.

**ACKNOWLEDGMENTS**

We thank Prof. Dr. Mogens L. Glass, Humberto Giusti, Mirian Bassi, and Glauber S. F. da Silva, from the Department of Physiology of the School of Medicine of Ribeirão Preto, University of São Paulo, for the arterial blood gas analysis.

**GRANTS**

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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