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RESEARCH ARTICLE | *Integrative Cardiovascular Physiology and Pathophysiology*

Plasticity of cardiovascular chemoreflexes after prolonged unilateral carotid body denervation: implications for its therapeutic use

Jaime Eugenín,[1,3]* Carolina Larraín,[2,3] and Patricio Zapata[2,3]*

[1]*Laboratorio de Sistemas Neurales, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile;* [2]*Centro de Fisiología Celular e Integrativa, Facultad de Medicina, Clínica Alemana, Universidad del Desarrollo, Santiago, Chile; and* [3]*Laboratorio de Neurobiología, Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile*

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Eugenín J, Larraín C, Zapata P. Plasticity of cardiovascular chemoreflexes after prolonged unilateral carotid body denervation: implications for its therapeutic use. *Am J Physiol Heart Circ Physiol* 318: H1325–H1336, 2020. First published April 24, 2020; doi: 10.1152/ajpheart.00451.2019.—Unilateral carotid body denervation has been proposed as treatment for sympathetic-related human diseases such as systolic heart failure, hypertension, obstructive sleep apnea, and cardiometabolic diseases. The long-term therapeutic effects of carotid body removal will be maintained if the remnant "buffer nerves," that is, the contralateral carotid nerve and the aortic nerves that innervate second-order neurons at the solitary tract nuclei (NTS), do not modify their contributions to the cardiovascular chemoreflexes. Here, we studied the cardiovascular chemoreflexes 1 mo after unilateral carotid body denervation either by excision of the petrosal ganglion (petrosal ganglionectomy, which eliminates central carotid afferents) or exeresis of a segment of one carotid nerve (carotid neurectomy, which preserves central afferents). Cardiovascular chemoreflexes were induced by intravenous (iv) injections of sodium cyanide in pentobarbitone-anesthetized adult cats. After 1 mo of unilateral petrosal ganglionectomy, without significant changes in basal arterial pressure, the contribution of the contralateral carotid nerve to the chemoreflex increases in arterial pressure was enhanced without changes in the contribution provided by the aortic nerves. By contrast, after 1 mo of unilateral carotid neurectomy, the contribution of remnant buffer nerves to cardiovascular chemoreflexes remained unmodified. These results indicate that a carotid nerve interruption involving denervation of second-order chemosensory neurons at the NTS will trigger cardiovascular chemoreflex plasticity on the contralateral carotid pathway. Then, unilateral carotid body denervation as therapeutic tool should consider the maintenance of the integrity of carotid central chemoafferents to prevent plasticity on remnant buffer nerves.

NEW & NOTEWORTHY Unilateral carotid body denervation has been proposed as treatment for sympathetic hyperactivity-related human disorders. Its therapeutic effectiveness for maintaining a persistent decrease in the sympathetic outflow activity will depend on the absence of compensatory chemoreflex plasticity in the remnant carotid and aortic afferents. Here, we suggest that the integrity of central afferents after carotid body denervation is essential to prevent the emergence of plastic functional changes on the contralateral "intact" carotid nerve.

arterial pressure control; cardiovascular chemoreflexes; carotid body denervation; heart rate control; petrosal ganglionectomy

INTRODUCTION

The "buffer nerves," the carotid (Hering's sinus) nerves (branches of glossopharyngeal nerves) and the aortic (Cyon's depressor) nerves (branches of vagus nerves), carry both baro- and chemosensory afferents, both of which are essential for cardiorespiratory homeostasis (21).

Arterial blood pressure (AP) is continuously sensed by baroreceptors located in carotid sinuses and aortic arch. Increased AP at the carotid sinus results in immediate reflex sympathetic inhibition, leading to reductions in both AP and heart rate (HR) (60). On the other hand, arterial blood gases are monitored by aortic and carotid body chemoreceptors, the latter being considered as the main oxygen and carbon dioxide sensors of arterial blood and contributing to the adaptation of breathing to physiological demands (21). Stimulation of aortic and carotid body chemoreceptors leads to cardiovascular reflexes through sympathetic activation, resulting in AP and HR increases (39).

Because buffer nerves carry both baro- and chemosensory nerve impulses, their interruption involves opposite effects on the sympathetic outflow. Whereas acute carotid sinus barodenervation leads to impairment of sympathetic inhibition induced by increased AP (68), acute carotid nerve chemodenervation leads to impairment of sympathetic activation induced by carotid body stimulation (21). Thus, continuous recordings of AP through implanted aortic catheters in conscious cats revealed increases in mean AP (MAP) by 30–40 mmHg, tachycardia (to 250 beats/min), and suppression of baroreceptor reflex evoked by phenylephrine immediately after bilateral section of carotid and aortic nerves, as expected in baro-denervated animals (70). Otherwise, the hypoventilation and reduction of hypoxia-induced ventilatory chemoreflexes, observed after a crush or section of carotid nerves is performed, are attributed to the interruption of chemosensory afferents (7, 19, 48, 57, 65).

Carotid baro- and chemosensory activities are recovered within a few days of carotid nerve crush or section, as regenerating carotid nerve fibers re-establish contact with carotid sinus adventitia and glomus cells, respectively (2, 66, 70, 82). In cats, activity in carotid chemosensory afferents reinitiates

* J. Eugenín and P. Zapata contributed equally to this work.
Correspondence: J. Eugenín (jaime.eugenin@usach.cl).

after 6 days of carotid nerve crush performed 1–2 mm from carotid body (82). In rats subjected to bilateral carotid denervation, eupneic Pa_{CO_2} returns to basal values within 15–23 days (48). Thus, reinnervation of peripheral receptor sites cannot be discarded as an explanation for the partial recovery of cardiovascular and ventilatory basal values and chemoreflexes that follow their initial reduction after carotid nerve crush or section (48, 79, 80, 82). In fact, reinnervation of carotid body and sinus occurs even when the carotid sinus nerve has been sectioned and its central stump has been displaced and sutured as far away as possible from its original destination (80).

Carotid body chemoreceptors have been suppressed either by carotid body excision (glomectomy), cryocoagulation (73, 74), or ischemia (47, 55), but such treatments fail when a few glomus cells are preserved. Therefore, the only secure way to achieve irreversible carotid body and carotid sinus denervation, as well as degeneration of central carotid sinus nerve fibers, is by excision of the petrosal ganglion, where the somata of carotid nerve afferents are located (22).

Carotid body ablation in humans has been used as therapy for carotid body tumors (35), bronchial asthma (27), and chronic obstructive pulmonary disease (77). In both pulmonary diseases, carotid body surgery was discontinued because the surgical approach did not have a clear advantage over the medical (not surgical) approach. However, recently, this procedure has brought a renewed interest. A growing body of evidence indicates that sympathetic hyperactivity, resulting from an increased carotid body chemosensory discharge, contributes to the development and progression of diverse pathologies (58) such as systolic heart failure (HF) (54), neurogenic and essential hypertension (44, 45), obstructive sleep apnea (OSA) (31, 51), and metabolic diseases (12). For instance, in humans with heart failure, transient carotid body inhibition reduces indexes of sympathetic outflow and clinical signs of poor prognosis (53, 54). Similarly, in rats with heart failure with reduced ejection fraction (HFrEF), carotid body ablation normalizes indexes of sympathetic outflow, such as baroreflex sensitivity and breathing alterations, and also improves the survival rate (15). In a recent prospective study, 6 mo after unilateral carotid body resection, the ambulatory systolic AP dropped by 26 mmHg in eight of 15 humans with drug-resistant hypertension (50). This beneficial effect was associated with a reduction in both muscle sympathetic outflow and ventilatory response to hypoxia (50). Initial results in another prospective study indicate that 1 mo after carotid body resection in 10 patients with advanced HFrEF and overactive chemoreceptors, the peripheral ventilatory response to hypoxia and the muscle sympathetic nerve activity were reduced, whereas the exercise time and quality of life of these patients improved (53, 54).

What happens to the cardiovascular chemoreflexes after chronic chemo-deafferentation will define whether a carotid afferent interruption will have transient or permanent therapeutic effect. This is relevant since unilateral instead of a bilateral carotid body ablation has been thought to be a better treatment for hypertension or heart failure in humans (50, 53, 54). Although bilateral carotid body denervation will result in a more profound blunting of cardiovascular chemoreflexes than unilateral carotid body denervation, it has a higher risk of nighttime desaturations and hypoxemia (50, 53, 54).

Here, we report the evolution of the cardiovascular chemoreflexes after 1 mo of carotid deafferentation by unilateral petrosal ganglionectomy or unilateral carotid neurectomy in anesthetized cats. The consequences of such partial prolonged deafferentation on basal ventilatory parameters and on the ventilatory chemoreflex responses to cytotoxic hypoxia (NaCN) and transient chemosensory silencing by hyperoxic exposure have already been reported (20). After unilateral petrosal ganglionectomy, the delayed chemoreflex ventilatory recovery has been explained by increased chemoafferent contribution of remnant intact buffer nerves or by plastic changes in the central chemosensory pathway (5, 20, 79). But changes in basal cardiovascular parameters and cardiovascular chemoreflexes went unreported and are analyzed here.

METHODS

Studies were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* endorsed by the National Institutes of Health. Experimental protocols were approved by the Bioethics and Biosafety Committee, Pontificia Universidad Católica de Chile.

General Procedures for the First and Second Surgeries

All studies were performed in adult cats of either sex, initially anesthetized with sodium pentobarbitone 40 mg/kg ip. Cats were placed in supine position over a thermoregulated pad, with body temperature being maintained at 38.0 ± 0.1°C. Cats breathed spontaneously through a flexible pediatric endotracheal cuffed tube (3.5 mm id, 7.0 mm od) introduced per os. Additional doses of 6 mg were given intravenously (iv) through a heparinized cannula (50 IU/mL in saline solution) inserted into the right saphenous vein when required to maintain a light level of surgical anesthesia (stage III, plane 2), ascertained by persistence of patellar reflexes, in absence of digital, corneal, and ear-whisker reflexes. The left femoral artery was catheterized, inserting a cannula (PE-100) filled with heparin 50 IU/mL in saline solution.

Cats were separated into two different experimental groups: those with exeresis of one petrosal ganglion (unilateral ganglionectomy) and those with excision of the distal portion of one carotid nerve (unilateral carotid neurectomy).

Anesthetic agents can alter the autonomic control of cardiovascular variables (6, 25, 72, 76). However, we used general anesthesia with pentobarbitone to obtain steady-state levels of cardiovascular and respiratory basal conditions and to have surgical access to buffer nerves, subjected to reversible blockade through local application of lidocaine during the first surgery session and for their sectioning during the second surgery session.

Each supplemental dose of pentobarbitone given iv induced a mild decrease in arterial pressure, subsiding within 3 min, with full recovery of ventilatory and cardiovascular reflex effects induced by stimulation of carotid bodies chemoreceptors. Similar levels of basal AP, basal HR, and baroreflex gain have been observed in cats anesthetized with either chloralose or pentobarbitone (24, 63), but basal recordings are more stable under pentobarbitone. Vagolytic actions of pentobarbital sodium are inconsistent in dogs (25), and they are not supported in cats, at the anesthetic level used here, because respiratory sinus arrhythmia (RSA) and the fast frequency component of heart rate variability (HRV) were preserved, indicating vagal modulation of sinoatrial node, and the bradycardic effects of vagal stimulation at different frequencies were inalterable. It should also be noted that basal arterial pressure values in cats are higher than those reported in other mammals. These high values have also been reported in another series of pentobarbitone-anesthetized cats (61), in intact α-chloralose anesthetized cats (38, 40), in paralyzed decerebrate cats (43), and in resting conscious cats (41).

Cardiovascular Recordings

Systemic AP was recorded from one femoral artery through a cannula filled with 50 IU/mL heparin in saline solution and connected to a Statham pressure transducer. MAP was obtained by electronic filtering of the upper frequencies of pulsatile AP. Instantaneous HR was obtained through tachography either from differential pressure recordings or from ECG signals at Einthoven's lead II. Recordings were continuously displayed on a polygraph and stored on either magnetic or video tapes for later analyses.

Ventilatory Recordings

Tracheal air flow was recorded by connecting the endotracheal tube to a heated Fleisch pneumotachograph head 00 and this to a differential volumetric pressure transducer. The air flow signal was integrated electronically to obtain tidal volume (V_T) and introduced into a tachograph for obtaining the instantaneous respiratory frequency (f_R). End-tidal CO_2 pressure (PET_{CO_2}) was monitored cycle to cycle through a fine sampling line inserted deep into the endotracheal tube and connected to an infrared gas analyzer.

Physiological variables, i.e., V_T, f_R, PET_{CO_2}, and AP, were simultaneously displayed on a polygraph and a multiple-beam oscilloscope. Raw signals (tracheal air flow, PET_{CO_2}, and AP) were also stored on magnetic tape through an FM recorder (frequency response, DC to 312 Hz) for later analyses.

Surgical Sessions

First surgical session. After the general procedures mentioned above were performed, a ventral longitudinal incision of the neck, strict aseptic conditions, and blunt dissection under stereomicroscope visualization allowed the exposure of both carotid artery bifurcations and of both carotid sinus and glossopharyngeal nerves. A petrosal ganglionectomy or a carotid neurectomy was performed unilaterally through a ventral incision of the neck. For the ganglionectomy, the left glossopharyngeal nerve was followed up to the jugular foramen, and after a trephine perforation of the bulla tympanica, the bone was removed with bone rongeurs and fine breaking forceps, exposing the petrosal ganglion, which was excised along with adjacent distal and central segments of the glossopharyngeal nerve. Damage to the adjacent vagal nerve central trunk was avoided, and fluctuations in AP and HR occurred during manipulation of carotid nerve afferents. Carotid neurectomy consisted in the exeresis of a piece of the carotid nerve from ~4 mm from the carotid body up to its entrance into the glossopharyngeal nerve. Reversible interruption of impulse traffic along the remnant intact buffer nerves was achieved by applying minute sheets of filter paper embedded in lidocaine hydrochloride 20 mg/mL below and above each nerve. Before blockade, a parafilm paper sheet was located under the nerve segment to avoid diffusion of lidocaine into the surrounding tissues. This procedure produced a quick (within 5 s) blockade of nerve impulses and was maintained until anesthetic sheets were removed and the site washed with saline drops.

At the end of the first surgical session, dipyrone (25 mg/kg) was administered iv for pain relief via saphenous vein cannula and then per os during surgery recovery (SOS). The cannulas, inserted into the right saphenous vein and the left femoral artery, were removed and the vessels ligated with linen thread to avoid bleeding. Linen thread sutures closed the muscular layer, and Michel clips coapted the wound edges at the skin incision after application of iodine polyvinylpyrrolidone. Antibiotic prophylaxis was given by im injection of penicillin (200,000 IU benzathine, 100,000 IU procaine, 100,000 IU potassium). Cats recovered from anesthesia in a thermostable box and were placed later in isolated cages with water and food ad libitum. They were observed daily for detecting signs of pain and discomfort such as general attitude, mood, placement in the cage, pain vocalization (groaning, growling, and crying), bended or flatten ear position, and muzzle alteration. Special attention was given to daily examination looking for painful areas or wound infestation and unresponsiveness or aggressiveness to stroking.

Second surgical session. Cats survived in good conditions without signs of trophic lesions and minimal (<1°C) temperature difference between right and left hind paws due to the previous occlusion of the catheterized left femoral artery. They maintained pre-surgery weight, the surgical wounds were healed, and their general attitude was that of a normal cat.

After 4 wk, cats with unilateral carotid neurectomy or petrosal ganglionectomy were re-operated, again under pentobarbitone anesthesia, exposing the buffer nerves and monitoring the AP, MAP, and HR, as described before. Now, remnant buffer nerve deafferentation was performed by nerve sections. Because sectioning a buffer nerve induces an initial barrage of afferent impulses (injury potentials) discharged from the nerve's central end, which is responsible for a brief period of reflex hypotension and hyperventilation, such sections were performed after applying local lidocaine hydrochloride as described above. At the end of the second surgical session, cats were euthanized by an overdose of pentobarbitone.

Testing the Cardiovascular and Ventilatory Chemoreflexes

Cardiovascular and ventilatory chemoreflexes were elicited by increasing doses of sodium cyanide (NaCN), from 0.5 to 100 μg/kg, injected iv, in boluses of 0.2 mL followed by 0.2 mL of saline. Dose-response curves were performed during the first surgical session, initially under control (CO, buffer nerves intact) conditions; then, after acute interruption of unilateral carotid nerve afferences by unilateral petrosal ganglionectomy (AUPG), or unilateral carotid neurectomy (AUCN); and finally, after completing reversible bilateral carotid deafferentation by lidocaine block of the contralateral carotid nerve (+UCB). After 4 wk of the first surgery, basal values and contribution of the remaining buffer nerves to cardiovascular chemoreflexes were initially evaluated under unilateral chronic deafferentation, either chronic unilateral petrosal ganglionectomy (CUPG) or chronic unilateral carotid neurectomy (CUCN), then after additional unilateral carotid neurotomy (+UCN) preceded by unilateral carotid nerve block (+UCB), and, finally, after bilateral aortic nerve section (+BAN).

The above procedures allowed us to explore the contribution of the aortic nerves in presence or absence of the functional contralateral carotid nerve input. The contribution of the contralateral remnant carotid nerve was assessed by subtracting the values of variables or NaCN-evoked effects obtained during reversible blockade or neurotomy of the carotid nerve from the values obtained when the contralateral carotid nerve (and both aortic nerves) was intact.

The additional bilateral aortic nerve section performed at the end of the second surgery session allowed us to assess any remnant cardiovascular and ventilatory chemoreflexes evoked by NaCN iv injections. In all the cases, the bilateral carotid and aortic nerve deafferentation abolished the chemoreflex induced by 100 μg/kg iv NaCN, which confirms previous work (63, 64). In addition, the appearance of prolonged vasodepressor responses was assessed at the dose of 400 μg/kg (not shown). This response is provoked by direct effects of cyanide on vascular smooth muscle, and it is observed in absence of chemoreflex counterbalancing.

Data Analysis

Baseline cardiovascular (MAP and HR) and ventilatory (V_T, f_R, and PET_{CO_2}) values were determined and averaged during 1 min of steady-state conditions after allowing enough time for their stabilization by ≥5–10 min. In control conditions (4 buffer nerves intact), this time varied between 20 and 30 min; after petrosal ganglionectomy or carotid neurectomy, this time varied from 1 to 2 h (includes petrosal and carotid surgery); after contralateral carotid blockade or neurotomy, this time was ~30 min. The

responses to cytotoxic hypoxia were represented by the maximal value of the variable evoked by NaCN iv.

Variables were expressed as raw values or as percentages of their respective basal values; average and dispersion were expressed as arithmetic means ± SE; ED_{50}s were averaged as geometric means.

Each dose-response curve obtained for each cat at each experimental condition was fitted to a symmetrical sigmoidal function (16) using the simplex algorithm (33). The general mathematical expression for the equation describing the dose-response curves is

$$R = R_{max} + \frac{(Bas - R_{max})}{\left[1 + \left(\frac{D}{ED_{50}}\right)^{S}\right]}$$

where R is response, Bas is basal value, D is the arithmetic dose of NaCN that is able to induce that response, R_{max} is the maximal response or "reactivity," ED_{50} is the median effective dose or "sensitivity," and S is the slope factor of each curve.

Basal values of MAP for each cat at each condition were introduced in the logistic equation as "Bas" parameter, whereas the raw peak values of MAP induced by iv NaCN injections were used by the software as "R" variable determined by each D dose to obtain through fitting the best values for R_{max}, ED50, and slope. Alternatively, normalization of dose-response curves was attained by expressing each NaCN-evoked cardiovascular response "R" as percentage of the basal value and fixing the Bas parameter in 100%. This constraining for the curve fitting tries to represent the dose-response under similar initial conditions to reveal differences in the other curve parameters (R_{max}, ED50, and slope).

Because the physiological responses to hypoxia in cats present high interindividual but low intraindividual variations (75), each cat served as its own control. Thus, multiple comparisons among these dependent samples were assessed with Friedman's test, followed by paired comparisons through Conover's post hoc test.

RESULTS

Basal MAP and HR After Petrosal Ganglionectomy

Figure 1 summarizes the basal levels of MAP (Fig. 1*A*) and HR (Fig. 1*B*) recorded immediately after acute unilateral petrosal ganglionectomy (AUPG) and after 4 wk of chronic unilateral petrosal ganglionectomy (CUPG). A tendency to a transient fall in MAP during the laborious procedure of petrosal ganglionectomy, likely due to repeated injuries to nerve fibers, may hide an expected increase in AP shortly after unilateral carotid nerve deafferentation. Thus, unilateral petrosal ganglionectomy did not significantly change the basal MAP or the basal HR in the short term. This is not surprising since the contralateral carotid sinus may rapidly reset its baroreceptor activity to acute changes in prevailing AP in cats (37). Nevertheless, local anesthesia of the contralateral carotid nerve to achieve bilateral carotid nerve block (+UCB) provoked statistically significant increases in both basal MAP and basal HR.

Four weeks after unilateral petrosal ganglionectomy, basal MAP was not significantly different from that observed under control conditions or immediately after unilateral petrosal ganglionectomy. Otherwise, basal HR under CUPG was higher than that observed in CO. Completing bilateral carotid deafferentation (+UCN) resulted again in a level of MAP significantly above that recorded in CO and AUPG and in a level of basal HR above CO, AUPG, and CUPG. Additional bilateral aortic nerve section (+BAN) did not provoke further increases in MAP and HR. Thus, acute or chronic unilateral carotid deafferentation did not significantly increase MAP, whereas bilateral carotid deafferentation did.

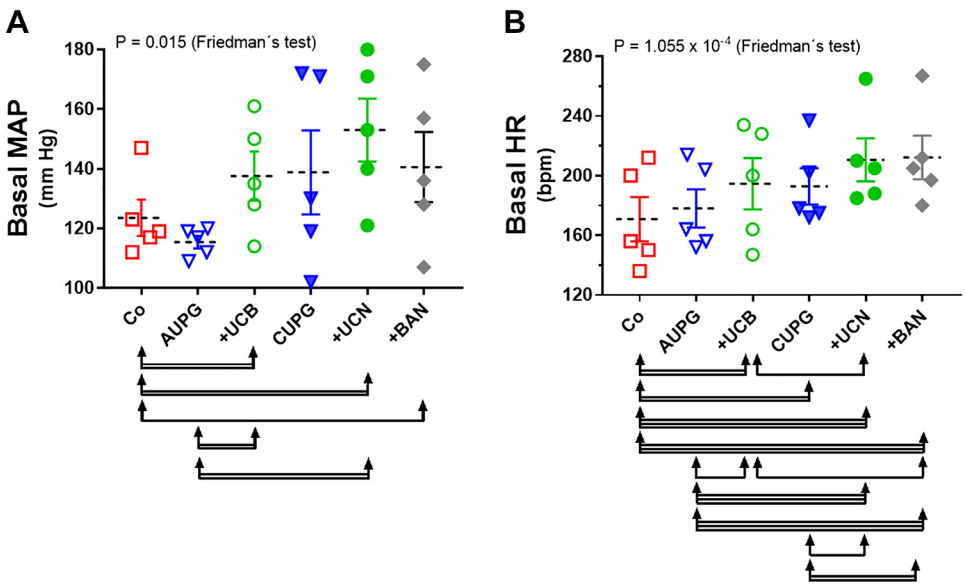

Fig. 1. Basal levels of mean arterial pressure (MAP; *A*) and heart rate (HR; *B*) recorded from 5 cats under acute and chronic unilateral carotid deafferentation by petrosal ganglionectomy. Control conditions (Co; open red squares), after acute unilateral petrosal ganglionectomy (AUPG; open blue inverted triangles), and additional unilateral carotid nerve block (+UCB, open green circles). After 4 wk, recordings were performed after chronic unilateral petrosal ganglionectomy (CUPG; filled blue inverted triangles) and additional unilateral carotid nerve section (+UCN; filled green circles) and bilateral section of aortic nerves (+BAN; filled gray diamonds). Symbols, basal values for each cat; horizontal dotted and vertical solid lines: means and SE, respectively. Statistical differences for multiple dependent samples assessed by Friedman's test (*P* indicated for each variable), followed by paired comparisons through Conover's tests (single, double, and triple lines between arrows connecting 2 experimental conditions indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$). Absence of horizontal lines connecting groups indicates no significant statistical difference between them.

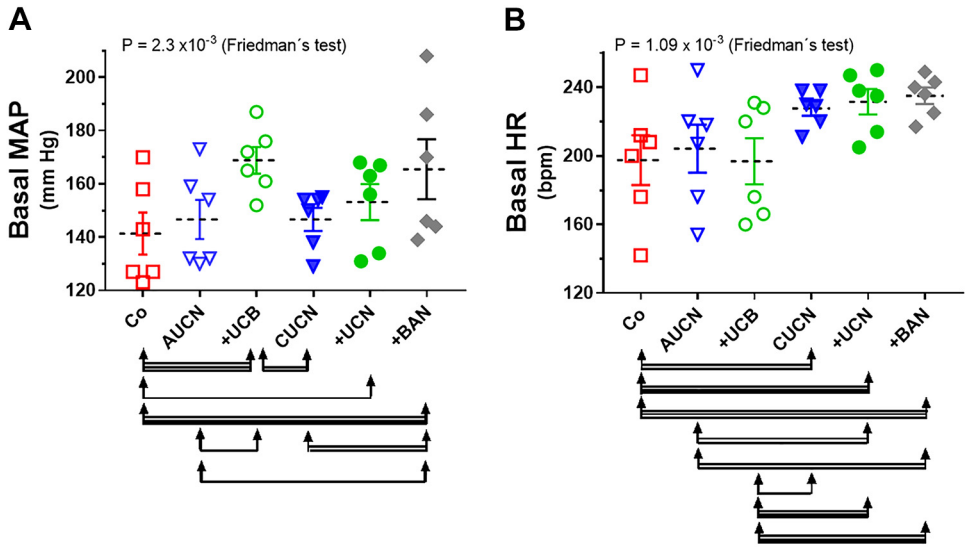

Fig. 2. Basal levels of mean arterial pressure (MAP; *A*) and heart rate (HR; *B*) recorded from 6 cats under acute and chronic unilateral carotid deafferentation by carotid nerve neurectomy. Control conditions (Co; open red squares), after acute unilateral carotid nerve neurectomy (AUCN; open blue inverted triangles), and additional unilateral carotid nerve block (+UCB; open green circles). After 4 wk, recordings were performed after chronic unilateral carotid nerve neurectomy (CUCN; filled blue inverted triangles), additional unilateral carotid nerve section (+UCN; filled green circles), and bilateral section of aortic nerves (+BAN; filled gray diamonds). Symbols, basal values for each cat; horizontal dotted and vertical solid lines: means and SE, respectively. Statistical differences for multiple dependent samples assessed by Friedman's test (*P* indicated for MAP and HR), followed by paired comparisons through Conover's tests (single, double, and triple lines between arrows connecting 2 experimental conditions indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$). Absence of horizontal lines connecting groups indicates no significant statistical difference between them.

Basal MAP and HR After Carotid Neurectomy

Figure 2 shows that basal levels of MAP (Fig. 2*A*) and of HR (Fig. 2*B*) observed shortly after AUCN were not significantly different from those observed in control conditions. However, acutely completing carotid deafferentation by anesthetic block of the contralateral carotid nerve (+UCB) resulted in a significant increase in basal MAP but not in basal HR. Four weeks after unilateral carotid nerve neurectomy, the basal MAP was not different from those observed under CO and AUCN conditions, whereas basal HR was higher than that observed in CO condition. A further increase in MAP, but not in HR, was provoked by section of the remnant carotid nerve (+UCN), a neural pathway that had only been transiently blocked in the initial session. Additional bilateral aortic nerves section (+BAN) did not further enhance the increased levels in MAP and HR observed after bilateral carotid denervation.

Contribution of Remaining Buffer Nerves to the Cardiovascular Chemoreflex After Petrosal Ganglionectomy

Apart from the changes in steady-state levels of cardiovascular parameters reported above, we evaluated whether chronic partial chemo- and baro-deafferentation can affect the dynamic vascular reflex responses. Figure 3 shows cardiovascular recordings along a series of iv injections of increasing doses of NaCN in a pentobarbitone-anesthetized cat with intact buffer nerves. The increases in AP, MAP, and HR were dose dependent. Because HR increases induced by sodium cyanide were small, especially after unilateral and bilateral carotid nerve deafferentation, a good determination of curve parameters was impaired. Therefore, analysis of cardiovascular reflexes was restricted to changes in MAP.

Figure 4*A* illustrates normalized dose-response curves for increases in MAP induced by NaCN iv in five cats. As compared with controls in acute conditions, a reduction in

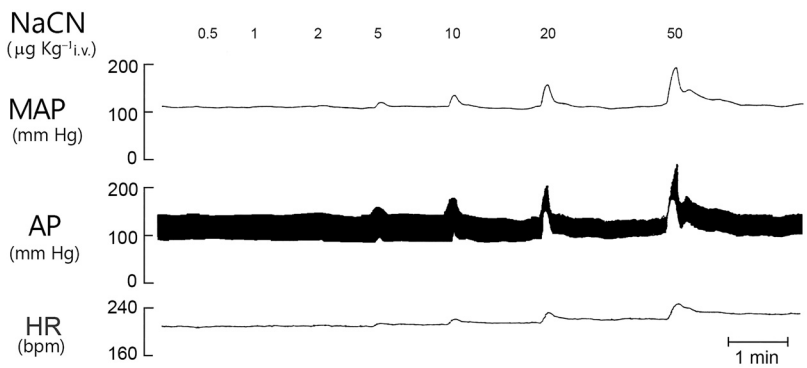

Fig. 3. Cardiovascular chemoreflexes in an adult anesthetized cat with intact buffer nerves (control). The chemoreflex was induced by right saphenous vein injections of 0.2-mL boluses of saline containing sodium cyanide (NaCN, 0.5 to 50 μg/kg). AP, arterial pressure; HR, heart rate; MAP, mean arterial pressure.

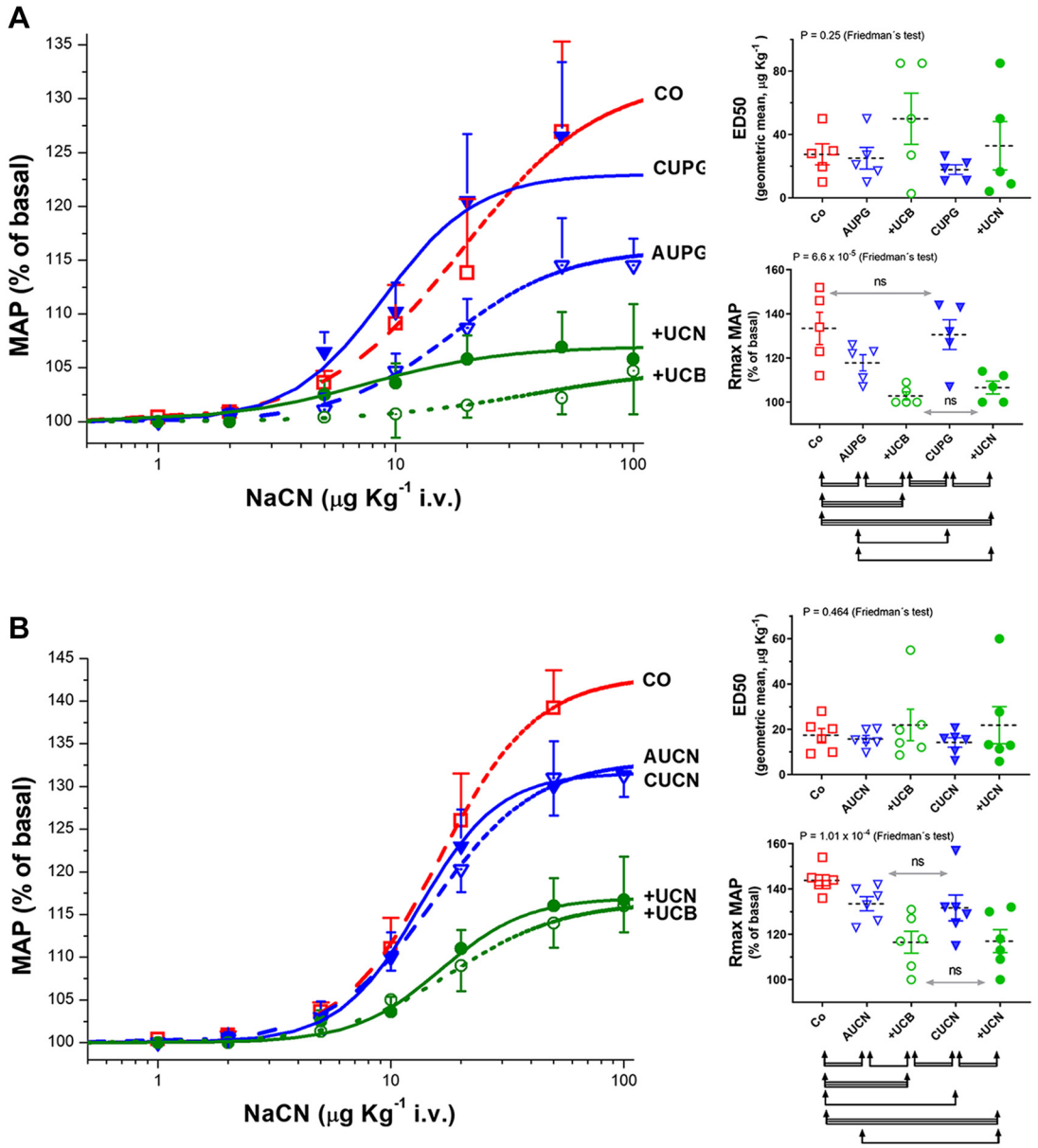

Fig. 4. Evaluation of reactivity and sensitivity of cardiovascular chemoreflexes in ganglionectomized ($n = 5$; *A*) and neurectomized ($n = 6$; *B*) adult pentobarbitone-anesthetized cats. *Left*: normalized dose-response curves for increases in mean arterial pressure (MAP) induced by intravenous (iv) doses of sodium cyanide (NaCN). *Right*: scatter plots for reactivity (R_{max}) and sensitivity (ED_{50}) of the curved lines, with each symbol representing a given animal. Acute conditions: control (CO; red open squares and dashed curved line), acute unilateral petrosal ganglionectomy (AUPG; blue open inverted triangles and dashed curved line; *A*), or acute unilateral carotid neurectomy (AUCN; blue open inverted triangles and dashed curved line; *B*) and contralateral carotid nerve blockade (+UCB, green open circles and dashed curved line; *A*). Chronic conditions: after 1 mo of petrosal ganglionectomy (CUPG, blue filled inverted triangles and solid curved lines; *A*) or neurectomy (CUCN; blue filled inverted triangles and solid curved lines; *B*), followed by contralateral carotid nerve neurotomy (+UCN, green filled circles and solid curved lines; *B*). *A*: reactivity but not sensitivity of vascular chemoreflexes supported by remnant contralateral carotid nerve was enhanced after chronic unilateral carotid denervation by petrosal ganglionectomy. *B*: by contrast, after chronic carotid neurectomy, neither reactivity nor sensitivity of vascular chemoreflexes supported by the contralateral carotid nerve showed significant changes. Normalized data (expressing MAP values as %basal MAP) was fitted to sigmoid function, as described in the text. Overall *P* values for statistical differences between multiple dependent samples assessed by Friedman's tests are indicated. Paired comparisons through Conover's tests, with significant differences between experimental conditions indicated by single ($P < 0.05$), double ($P < 0.01$), and triple ($P < 0.001$) horizontal lines joining arrows. Note that the R_{max} of CO and CUPG groups was not different as well as between +UCB and +UCN groups (indicated by gray joining arrows).

reactivity and no change in sensitivity were observed after unilateral petrosal ganglionectomy (AUPG). Additional silencing of contralateral carotid afferents by local nerve block (+UCB) reduced the reactivity even more. Thus, acutely, the contralateral carotid nerve does not compensate for the excised carotid afferents. However, 4 wk after unilateral petrosal ganglionectomy (CUPG), no statistically significant difference in the reactivity for MAP between control and chronic ganglionectomized cats was observed, suggesting that remnant carotid chemoafferents compensate for the deficit. Evaluation of chemoreflexes based on both aortic nerves results in similar changes after acute or chronic ganglionectomy, suggesting that

contribution of remnant aortic nerves to chemoreflex changes in MAP remains unaltered after petrosal ganglionectomy. Additional bilateral aortic nerve sectioning abolished the increase in MAP induced by 100 μg/kg iv NaCN.

For evaluating the chemoreflex drive of MAP, we obtained R_{max} by fitting each dose-response curve to the sigmoid function using the peak raw values of MAP evoked by each dose of cyanide (Fig. 5). The difference between R_{max} and the respective basal MAP was considered an index of the chemoreflex drive of MAP since it represents the maximal capacity for increasing MAP over the respective basal value. As illustrated in Fig. 5*A*, the chemoreflex drive was reduced by petrosal ganglionectomy with respect to control and by contralateral carotid nerve blockade with respect to AUPG. Under CUPG, the chemoreflex drive increased and became similar to that under control conditions. By contrast, chemoreflex drive exerted by aortic nerves was unmodified 4 wk after ganglionectomy.

Contribution of Remaining Buffer Nerves to the Cardiovascular Chemoreflex After Carotid Neurectomy

Figure 4*B* illustrates the observations in six cats subjected to unilateral carotid neurectomy. Analyses of the dose-response curves for the increases in MAP induced by cyanide injections reveal an acutely reduced reactivity after such procedure, which became more pronounced upon additional contralateral carotid nerve blockade (+UCB). However, after 4 wk of carotid neurectomy (CUCN), no recovery in the reactivity was observed. Furthermore, a comparison of the effects of suppressing the activity of the contralateral carotid nerve reveals that the contribution of the remnant aortic nerves afferences persisted unmodified.

Likewise, the chemoreflex drive of MAP, which was reduced by the unilateral carotid neurectomy, persisted low and unmodified after 4 wk (Fig. 5*B*). Neither the chemoreflex drive of MAP exerted by aortic nerves evidenced any change under chronic conditions (Fig. 5*B*).

After chronic neurectomy, like after chronic ganglionectomy, additional bilateral aortic nerve sectioning performed at the end of the second surgical session, abolished the increase in MAP induced by 100 μg/kg iv NaCN, suggesting that no effective carotid body nerve regeneration or carotid body reinnervation had occurred.

DISCUSSION

Plasticity of Cardiovascular Chemoreflexes

Our results demonstrate that after 1 mo of petrosal ganglionectomy, the contralateral carotid nerve, but not the aortic nerves, increases its contribution to the pressor chemoreflex evoked by cytotoxic hypoxia. By contrast, after 1 mo of carotid neurectomy, a procedure preserving central projections and prone to peripheral regeneration and carotid body reinnervation does not change pressor chemoreflexes.

The carotid body and carotid sinus afferent fibers project exclusively to NTS in the cat (11, 69), with the projections being predominantly ipsilateral to the rostral nuclei, but also contralateral to the caudal nuclei, where bilateral projections may overlap. The central projections of the entire glossopharyngeal nerve (degenerating after petrosal ganglionectomy) are

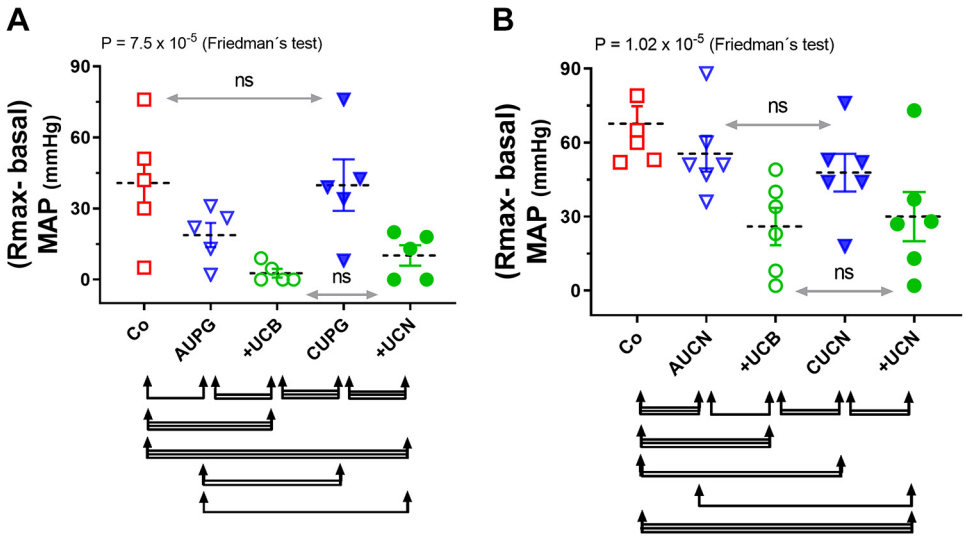

Fig. 5. Contribution of remnant carotid nerve to the chemoreflex drive of mean arterial pressure (MAP) is enhanced 4 wk after petrosal ganglionectomy (*A*) and persists unmodified 4 wk after carotid neurectomy (*B*). Sigmoid-curve parameters were obtained from dose-response curves for the raw peak values of MAP induced by intravenous (iv) sodium cyanide (NaCN) injections for each cat in each experimental condition. Parameters were calculated by fitting the dose-response curve for increases in MAP (expressed in mmHg) to the sigmoid function: $R = [Bas - R_{max}]/[1 + (D/ED_{50})^S] + R_{max}$, where R = MAP response (mmHg), Bas = basal MAP (mmHg), D = arithmetic dose to induce that response, R_{max} = maximal response of each curve, ED_{50} = median effective dose, and S = slope of the curve. Overall *P* values for statistical differences between multiple dependent samples assessed by Friedman's tests; multiple paired comparisons were performed through post hoc Conover's tests, and significant differences between experimental conditions are indicated by single ($P < 0.05$), double ($P < 0.01$), and triple ($P < 0.001$) horizontal lines joining arrows. Note that the chemoreflex drive (difference between R_{max} and Bas) in control (CO; all 4 buffer nerves intact) and chronic unilateral petrosal ganglionectomy (CUPG) groups are not different (indicated by gray arrow). Each symbol represents the (R_{max} − Bas) value obtained for each animal at different experimental conditions. Color and symbol code as described in legends of Figs. 1 and 2. AUCN, acute unilateral carotid neurotomy; AUPG, acute unilateral petrosal ganglionectomy; CUCN, chronic unilateral carotid neurotomy; NS, not significant; +UCB, adding unilateral carotid blockade; +UCN, adding unilateral carotid neurotomy.

considerably more extended (28). Therefore, second-order denervated neurons in the NTS (possibly by denervation supersensitivity) may be driven more powerfully by remnant carotid afferences and provide partial restoration of vascular chemoreflexes responsiveness. That the chemoreflex contribution of the contralateral carotid nerve was not enhanced after chronic carotid neurectomy suggests that degeneration of central processes of carotid nerve afferences ending upon NTS neurons is required for gating a reorganization of the vascular chemoreflex pathway.

Otherwise, aortic nerve (branch of vagus nerve) projections to the NTS (34), although bilateral, are not coincident with those of carotid nerve, making less probable a reorganization of partially denervated central neurons involved in vascular reflexes. In fact, only 19 out of 292 neurons recorded from the NTS in cats showed convergent inputs from carotid and aortic nerves (18).

Anesthetic block of impulse conduction along carotid nerves produces simultaneous transient baro- and chemo-denervation. But isolated interruption of carotid chemosensory impulses (by brief hyperoxic exposure) elicits bradycardia in normal or barodenervated cats (81) as well as in conscious normal men (67). Therefore, the immediate increases in MAP and HR provoked by acutely completing carotid deafferentation either by anesthetic block or neurotomy of the contralateral carotid nerve should be explained by a predominant withdrawal of carotid tonic baroreflexes. In addition, our observations suggest that the negative feedback on AP exerted via one carotid baroreflex is compensated by enhanced control exerted by the remnant carotid baroreflex, but suppression of both baroreflex paths is not compensated via baroreflexes from the aortic arch.

The finding that, after minutes of a ganglionectomy, the basal MAP and the basal HR show levels similar to those observed after 4 wk of ganglionectomy indicates that degeneration of the entire length of one glossopharyngeal nerve (carotid nerve included) and partial central denervation of NTS at one side are not compensated by an enhanced baroreflex influence from the contralateral carotid sinus nerve in basal steady-state conditions. This contrasts with the normal levels of resting alveolar ventilation (PET_{CO_2}) already reported in these same cats (20), suggesting that the remnant carotid nerve chemosensory afferents have increased their tonic reflex influence on resting ventilation after unilateral carotid denervation of NTS. The absence of significant changes in basal MAP or HR after aortic nerve section indicates that these nerves, with endings also impinging on NTS, have not assumed a controlling role upon basal MAP or HR when one NTS has been subjected to chronic carotid denervation. Therefore, the steady-state cardiovascular baroreflex does not exhibit the type of plastic adjustments reported for the steady-state respiratory chemoreflex regulatory system.

Plastic adjustments depend on the basal values at rest. In fact, ~60% of hypertensive patients undergoing unilateral carotid denervation, either due to carotid body tumor removal or as treatment for resistant hypertension, showed prolonged (12 mo) blood pressure reduction (23, 50). In patients with bronchial asthma or chronic obstructive pulmonary disease, bilateral removal of carotid bodies was followed by arterial pressure reduction observed 5 days after surgery and maintained for 6 mo only in hypertensive patients, whereas normotensive patients did not show this fall in blood pressure, and arterial pressure increased in hypotensive patients (49, 78).

Summary of Ventilatory Chemoreflex Plasticity After Unilateral Petrosal Ganglionectomy

Because ventilation can depress both cardiac vagal activity (1, 14) and sympathetic nerve outflow (71), which in turn can alter the cardiovascular chemoreflexes, a description of changes in ventilation after unilateral petrosal ganglionectomy is necessary to gain a more complete picture of the chemoreflex plasticity.

The plastic changes in ventilatory chemoreflexes after unilateral petrosal ganglionectomy, derived from observations obtained in the same animals reported here, have been described previously (20). In brief, during the first surgical session, the mean values of V_T, f_R, and PET_{CO_2} were not modified after unilateral petrosal ganglionectomy or after the additional reversible block of the contralateral carotid afferents. In the second surgical session performed after 1 mo of petrosal ganglionectomy, basal V_T was increased with respect to the Co and AUPG acute conditions, whereas basal values of f_R and PET_{CO_2} were not significantly modified (20). The basal V_T was slightly reduced by the additional contralateral carotid neurotomy but persisted above that observed under Co and AUPG acute conditions. This additional carotid neurotomy also failed to modify the basal values of f_R and PET_{CO_2}. Interestingly, after sectioning of all buffer nerves, basal V_T persisted elevated and not significantly different from that observed under +UCN. This suggests that the aortic buffer nerves are not contributing to the increase in basal V_T after chronic carotid denervation, and this likely corresponds to an adaptative change in the central nervous system (CNS) not dependent on remnant peripheral chemoreceptor input.

The analysis of the dose-response curves for the increases in V_T and f_R induced by NaCN iv obtained during the first surgical session, 1–2 h after the unilateral petrosal ganglionectomy, revealed that ED_{50}s for both variables were significantly displaced to the right. By contrast, the ED_{50}s obtained after 1 mo of chronic ganglionectomy were significantly displaced to smaller values of ED_{50}, which were not different from those in control (intact buffer nerves) conditions for V_T and f_R. These results indicate that the sensitivities of V_T and f_R dose-response curves are clearly enhanced in the chronic condition. Additionally, the block of the contralateral carotid nerve, during the first surgical session, did not accentuate the increases in ED_{50}s with respect to those observed in acutely ganglionectomized cats, suggesting that the contralateral carotid nerve was acutely unable to compensate, leading to restore ED_{50}s for V_T and f_R curves. By contrast, during the second surgical session, the neurotomy of the contralateral carotid nerve increased ED_{50}s to values similar to those observed in control and acutely ganglionectomized cats. Therefore, the enhanced sensitivities for V_T and f_R responses observed after CUPG depend on the afferences provided by the contralateral carotid nerve.

On the other hand, the analysis of the dose-response curves for increases in f_R induced by NaCN iv also revealed changes and adaptations in maximal reactivity. The maximal reactivity in f_R after 4 wk from ganglionectomy was significantly higher than those recorded in the three acute conditions (control, ganglionectomized, additional blockade of contralateral carotid

nerve). The sectioning of the contralateral carotid nerve in the chronic condition did not modify the maximal reactivity, which remained high, suggesting that increased maximal reactivity in f_R responses after chronic petrosal ganglionectomy depends on the afferences provided by aortic nerves.

In summary, the recovery of sensitivities for reflex changes in V_T and f_R required the presence of contralateral carotid afferents, whereas the increased reactivity in f_R depended on the integrity of aortic afferents. The results also suggest an enhanced contribution of central structures other than chemosensory inputs in respiratory control after partial deafferentation of the NTS.

Interactions Between Ventilation and Cardiovascular Baro- and Chemoreflexes

It should be noted that after 1 mo of unilateral carotid body and carotid sinus denervation by petrosal ganglionectomy, both ventilatory and cardiovascular chemoreflexes were simultaneously enhanced. It is conceivable that the increased ventilation elicited by an enhanced ventilatory chemoreflex may determine a larger inhibition of cardiac vagal activity (1, 14), leading to tachycardia that could support the increased MAP. However, increased ventilation can also inhibit the sympathetic nerve outflow (71), with opposite effects on MAP. On the other hand, increased AP or baroreflex stimulation inhibits ventilation (46). This is relevant since our ganglionectomized cats showed increased MAP after 1 mo of surgery. How much these ventilation/blood pressure or chemoreflex/baroreflex interactions are modulating the expression of our results is an open question.

Limitations of the Results

Extrapolation of our results to other species and experimental conditions should be done carefully. The cardiovascular response to carotid body chemoreceptor activation by hypoxia or cyanide is variable among different species. In conscious awake rats, the response consists of increased MAP associated with intense bradycardia (4), very similar to the responses described for fetal lambs (29), and conscious rabbits challenged by severe hypoxia (10). By contrast, in awake mice, there is a biphasic AP response to peripheral chemoreceptor activation, with a hypertensive phase followed by a hypotensive phase (8); nevertheless, the changes in HR among different studies using conscious mice are variable, from bradycardia (8), with no effect on blood pressure (17) to long-lasting tachycardia (59). In awake dogs and humans, peripheral chemoreceptor stimulation increases MAP and HR (26, 36), effects that are similar to the cardiovascular responses elicited by cyanide in anesthetized and spontaneously breathing cats reported here and in previous work (63). On the other hand, in the same species, the cardiovascular baro- and chemoreflexes depend on behavioral or functional state of the nervous system (14, 72), which would likely explain the small increase in HR we observed in anesthetized cats after NaCN injection. Chemosensory stimulation in anesthetized and spontaneously breathing dogs can increase or reduce HR, depending on the type of anesthesia and the intensity of stretch reflex from the lungs (14). Even the type of anesthesia may affect also the vagal tone on HR (25) and the pattern of response of the cardiovascular chemoreflex (14, 42). Furthermore, reflex control of cardiovascular variables is not uniformly affected by equipotent (abolished toe pinch and corneal reflexes) concentrations of the anesthetic substances (25).

Additionally, one should consider that the relative contribution of carotid and aortic buffer nerves to cardiovascular chemoreflexes varies among different species (3, 13, 32, 52, 63, 64, 68). In anesthetized Wistar rats, carotid bodies were proposed as the only functional peripheral chemoreceptors (62). However, after bilateral carotid neurotomy in Sprague-Dawley rats, chemoreflexes to cytotoxic and hypoxemic hypoxia still were present and showed reduced sensitivity and reactivity when compared with intact rats (9). In cats, dogs, and humans there is a clear predominance of carotid over aortic afferences in the chemoreflex control of ventilation and cardiovascular variables (13, 32, 52, 63, 64, 68). Besides, petrosal ganglionectomy or carotid neurectomy also affect carotid baroreflex pathway. Then, it is possible that baroreflexes also could present plastic changes, which were not evaluated in our experiments.

Translational Aspect

There is an increasing interest for considering carotid body denervation as a potential tool for the treatment of high-prevalence diseases having sympathetic hyperactivity in common (30, 44, 45, 50, 53, 54, 56, 68). Thus, carotid body denervation or chemosensory inhibition should reduce sympathetic overactivity, decrease hypertension in animal models of hypertension and OSA, eliminate cardiorespiratory instability, and improve animal survival in HF as well as restore insulin tolerance in cardiometabolic models. Human studies show some benefits of unilateral carotid body ablation, which could become a useful therapy for sympathetic hyperactivity. However, the beneficial effect of carotid body denervation could be transient if the interrupted chemoreceptor afferents are able to regenerate and reinnervate glomic tissue or if remnant buffer nerves increase their contribution to chemoreflexes. Reinnervation cannot occur after either total carotid body ablation or petrosal ganglionectomy. Ablation eliminates the glomus tissue, the natural target for chemoreceptor afferents, and petrosal ganglionectomy produces degeneration of peripheral and central processes of carotid nerve afferents, resulting in irreversible carotid body and sinus denervation as well as partial denervation of nuclei of the solitary tract (NTS) complex. Our present results call us to caution the possibility that plastic changes in cardiovascular chemoreflexes that relied on remnant buffer nerves could impose a restriction to long-term clinical benefits of carotid body denervation.

Conclusions

The cardiovascular effects of combined unilateral carotid baro- and chemo-deafferentation by either petrosal ganglionectomy or carotid neurectomy were studied at the time of surgeries and again 1 mo afterward. Dose-response curves for reflex changes in AP revealed increased reactivity after petrosal ganglionectomy but not after carotid neurectomy, differences that are attributed to the still intact contralateral carotid nerve. Changes in cardiovascular chemoreflexes are similar to those previously reported for chemoreflex changes in respiratory frequency (20). The results suggest that an effective chronic unilateral carotid nerve input reduction should be

based on maintaining the integrity of its central projections into the NTS to prevent plastic functional changes involving the contralateral carotid nerve.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.E. and P.Z. conceived and designed research; J.L.E. and C.L. performed experiments; J.L.E. and P.Z. analyzed data; J.L.E. and P.Z. interpreted results of experiments; J.L.E., C.L., and P.Z. prepared figures; J.L.E., C.L., and P.Z. drafted manuscript; J.L.E., C.L., and P.Z. edited and revised manuscript; J.L.E., C.L., and P.Z. approved final version of manuscript.

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