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Cardiovascular responses to peripheral chemoreflex activation and comparison of different methods to evaluate baroreflex gain in conscious mice using telemetry

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Braga VA, Burmeister MA, Sharma RV, Davisson RL. Cardiovascular responses to peripheral chemoreflex activation and comparison of different methods to evaluate baroreflex gain in conscious mice using telemetry. *Am J Physiol Regul Integr Comp Physiol* 295: R1168–R1174, 2008. First published July 30, 2008; doi:10.1152/ajpregu.90375.2008.—Peripheral chemoreceptors located in the carotid bodies are the primary sensors of systemic hypoxia. Although the pattern of responses elicited by peripheral chemoreceptor activation is well established in rats, lambs, and rabbits, the cardiovascular responses to peripheral chemoreflex activation in conscious mice have not been delineated. Here we report that stimulation of peripheral chemoreceptors by potassium cyanide (KCN) in conscious mice elicits a unique biphasic response in blood pressure that is characterized by an initial and robust rise followed by a decrease in blood pressure, which is accompanied by a marked reduction in heart rate. The depressor and bradycardic responses to KCN were abolished by muscarinic receptor blockade with atropine, and the pressor response was abolished by α-adrenergic receptor blockade with prazosin, suggesting that vagal and sympathetic drive to the heart and sympathetic drive to the vasculature mediate these cardiovascular responses. These studies characterized the chemoreflex in conscious mice and established the reliability of using them for studying hypoxia-related diseases such as obstructive sleep apnea. In another series of experiments, two methods for analyzing baroreflex sensitivity were compared: the classical pharmacological approach using phenylephrine and sodium nitroprusside (i.e., the Oxford technique) or the sequence method for analyzing spontaneous baroreflex activity. Our findings indicate that both methods are reliable, and the sequence method certainly has its benefits as a predictive tool in the context of long-term noninvasive studies using telemetry. However, for absolute determination of baroreflex function, analysis of spontaneous baroreflex activity should be complemented by the classical pharmacological method.

autonomic balance; hypoxia; sequence method; blood pressure

DURING SYSTEMIC HYPOXIA, activation of peripheral chemoreceptors located in the carotid bodies leads to autonomic and respiratory adjustments that function to restore oxygen levels to the normal physiological range. In conscious rats, the autonomic responses to peripheral chemoreflex activation consist of an elevation in blood pressure mediated by an increase in sympathetic drive to the vasculature, as well as a bradycardia mediated by a predominance of parasympathetic over sympathetic drive to the heart (18). Although low doses of potassium cyanide (KCN) have been reliably used as a pharmacological

tool to activate peripheral chemoreceptors to mimic hypoxia in rats (5, 6, 18), lambs (19), and rabbits (16), the cardiovascular responses to peripheral chemoreflex activation in conscious mice are not yet delineated.

In addition to the peripheral chemoreflex, the baroreceptor reflex is another key regulator of blood pressure. Baroreceptors located in the carotid sinuses and aortic arch detect changes in blood pressure and trigger reflex autonomic adjustments that buffer alterations in blood pressure. In pathological conditions such as hypertension and heart failure, there is impairment in the autonomic control of blood pressure, resulting in changes in the baroreflex sensitivity (9, 17, 36). For example, individuals suffering from chronic activation of the peripheral chemoreceptors during sleep apnea also often present with impaired autonomic balance and baroreflex function, which may collectively promote hypertension in these patients (11, 28, 38).

There are several methods to experimentally evaluate the baroreflex in commonly used laboratory animals, and there has been much debate about which one works best. The most frequent methods used are the classical vasoactive drug-induced activation of the baroreflex (3, 14, 39), which evaluates heart rate (HR) changes in response to pharmacological manipulations that increase or decrease blood pressure (i.e., the Oxford technique), and the spontaneous baroreflex analysis, which takes into consideration changes in HR produced by spontaneous fluctuations in blood pressure. In particular, the sequence method for evaluation of spontaneous baroreflex is based on the identification of sequences of four or more heart beats in which the systolic pressure and the pulse interval both progressively change in the same direction (1, 2, 23, 29).

The mouse has become the most commonly used animal for studying the genetic basis of cardiovascular and autonomic diseases, including hypertension and obstructive sleep apnea (8, 37). Furthermore, radiotelemetry for long-term recording of blood pressure in conscious, freely moving mice has proven to be a critical tool for understanding the physiology and pathophysiology of the cardiovascular system (21). Considering this, here we characterized the cardiovascular responses elicited by peripheral chemoreflex activation in conscious mice using telemetry. We hypothesized that stimulation of peripheral chemoreceptors would evoke similar cardiovascular responses in conscious mice as those seen in rats. Considering the potential clinical relevance of the interaction between both the chemoreflex and baroreflex, we also performed side-by-side compari-

sons of the classical pharmacological method and the sequence method for analyzing baroreflex function in the same mouse model.

EXPERIMENTAL PROCEDURES

Animals. Experiments were performed on adult (10–12 wk old) C57BL/6 male mice. Mice were individually housed in a temperature-controlled room set to a 12:12-h light-dark cycle and had access to standard mouse chow (Harlan Tek-Lad Diet 7912) and water ad libitum. All procedures were approved by the Cornell University Institutional Animal Care and Use Committee.

Radiotelemetry surgery. Mice were anesthetized (ketamine, 117 mg/kg ip in combination with xylazine, 17 mg/kg ip) and instrumented with TA11PA-C10 radio telemetry probes (Data Sciences International, Arden Hills, MN) as previously described (3, 7, 10, 23, 41). Briefly, the catheter of the telemeter was implanted in the thoracic aorta via the left common carotid artery, and the body of the probe was placed in a subcutaneous pocket created in the right flank. The wound was closed and sutured, and body temperature was maintained at 37°C using a heating pad until sternal recumbency had been recovered. Mice remained undisturbed in their home cages for 7 days before undergoing any subsequent experimentation so as to allow for full recovery of normal circadian rhythm and cardiovascular parameters (7).

Jugular vein catheterization. Following telemetry surgery (1 wk), mice were anesthetized (ketamine, 117 mg/kg ip in combination with xylazine, 17 mg/kg ip) and instrumented with jugular catheters for intravenous vehicle/drug administration. With the aid of a dissecting stereomicroscope (Olympus, SZ61), sterile heparinized saline-filled (50 U/ml) catheters (Microrenathane 0.1 cm OD $\times$ 0.06 cm ID; Braintree Scientific) were inserted in the jugular vein and fixed to the surrounding tissue with Vetbond veterinary adhesive. The free ends of the catheters were plugged, tunneled subcutaneously, and exteriorized and sutured at the dorsal surface of the neck. The catheters were flushed daily with heparinized saline to maintain patency.

Blood pressure recordings and drug administration. After a 5-day recovery period following implantation of the jugular catheters, telemetry probes were activated for continuous measurement of baseline arterial pressure (sampling frequency = 2,000 Hz). Mean arterial pressure (MAP) and HR were derived offline from the blood pressure tracing. Following baseline sampling, an intravenous injector was securely attached to each animal's indwelling jugular catheter; the intravenous injector consisted of microrenathane tubing connected to a syringe for injection of vehicle/drug. Animals were then returned to their home cages. The microrenathane tubing was extended outside of the cages so as to keep disturbance to the animals to a minimum. After stabilization and additional baseline sampling, drug or isotonic saline vehicle (30 μl) was delivered intravenously via the syringe/injector assembly. MAP and HR were analyzed before and after drug/vehicle administration.

Drugs and protocols. The peripheral chemoreflex in conscious mice was activated by administering KCN (Sigma, St Louis, MO) intravenously at a dose of 26 μg/g, as previously established for anesthetized mice (32). The peripheral chemoreflex was first activated before (control response) and after muscarinic receptor blockade with atropine (1 μg/g ip; Sigma). Following 40 min, the chemoreflex was activated again in the same animal after α-adrenergic receptor blockade with prazosin (1 μg/g ip atropine + prazosin; Sigma). One final KCN injection was performed 2 h after treatment with atropine and prazosin to verify the reversibility of these compounds' antagonistic effects. In additional studies, the chemoreflex was activated following α-adrenergic receptor blockade with prazosin alone (1 μg/g ip). Each drug was administered as an intravenous bolus in a volume of 30 μl, with isotonic saline as vehicle.

The baroreflex was evaluated in another series of experiments. Changes in blood pressure were evoked by intravenous bolus injec-

tions of phenylephrine (PE, 4 μg/kg; Sigma) and sodium nitroprusside (SNP, 10 μg/kg; Sigma); these doses were previously established in studies by Bissonnette et al. (3). After 40 min of baseline blood pressure recording, SNP was injected. After blood pressure returned to baseline levels, PE was injected before (control response) and after the muscarinic receptor antagonist atropine (1 μg/g, ip; Sigma). After 2 h, PE was injected again before and after β-adrenergic receptor blockade with propranolol (1 μg/kg, ip; Sigma). Similar to the chemoreflex studies, each drug was administered an intravenous bolus in a volume of 30 μl, with isotonic saline as vehicle.

The ambient air temperature of the room in which all studies were conducted was ~72°F (22°C). All studies were performed between the daytime hours of 1000 and 1400.

Chemoreflex and baroreflex analyses. Peripheral chemoreflex responses were analyzed as the peak of the increase and/or decrease in blood pressure and the peak of the decrease in HR following KCN injection during control and after pretreatment with either atropine or prazosin. Baroreflex responses were analyzed as the gain of the baroreflex (beats·min^{-1}·mmHg^{-1}) in response to SNP or PE injection. In addition, high-frequency data (2,000 Hz) were acquired during the blood pressure recordings to evaluate spontaneous baroreflex during normal fluctuations of systolic blood pressure 40 min before and 20 min after pretreatment with either atropine or propranolol using the sequence method analysis (time when animals were resting unhandled in their home cages). For comparative purposes in the same animal, the time range used to evaluate the spontaneous baroreflex (sequence analysis) was the baseline before SNP or PE administration. Baroreflex gain was calculated from spontaneous, reciprocal fluctuations in HR and MAP by the sequence method and analyzed using HemoLab software (kindly provided by Dr. Harald Stauss, University of Iowa, version 5.3). Each telemetry tracing was loaded in the analysis software and, if present, any artifact was removed. The region of interest along the tracing was selected based on minor or no fluctuations in blood pressure, and the baroreflex test was run obeying the following parameters: *1*) correlation coefficient of 0.85, *2*) pressure threshold of 15 mmHg, and *3*) lag of four beats as described by Chen et al. (10). A baroreflex sequence was defined by a series of at least four consecutive heart beats in which both systolic blood pressure and the following R-R interval either both increased or decreased. Conversely, a nonbaroreflex relationship was defined by sequences of at least four heart beats accompanied by opposite directional changes of R-R interval and systolic pressure of that beat. Only those baroreflex sequences for which changes in the two parameters had a correlation coefficient ≥0.85 were considered for analysis ($n = 20 \pm 4$ sequences). For the drug-induced baroreflex studies, the slope of the linear regression for changes in HR and blood pressure yielded the baroreflex gain (beats·min^{-1}·mmHg^{-1}), which was calculated from the following: ($\text{HR}_{maximum} - \text{HR}_{minimum}$) multiplied by (slope coefficient/4) as performed by Bissonnette et al. (3).

Statistical analysis. Results are expressed as means $\pm$ SE. The magnitude of the changes in cardiovascular parameters in response to reflex activation was compared before and after treatments by "one-way" repeated-measures ANOVA with subsequent Tukey posttest. Statistical significance was defined as $P < 0.05$.

RESULTS

Cardiovascular responses to peripheral chemoreceptor activation. The typical tracings shown in Fig. 1*A* illustrate changes in blood pressure and HR following peripheral chemoreflex activation in a conscious mouse before and after autonomic blockade with atropine and/or prazosin. Summary data from six mice presented in Fig. 1*B* show that activation of peripheral chemoreceptors with KCN produced a rapid yet transient increase in blood pressure ($+25 \pm 3$ mmHg, $P < 0.05$) that lasted for 5–8 s followed by a comparatively
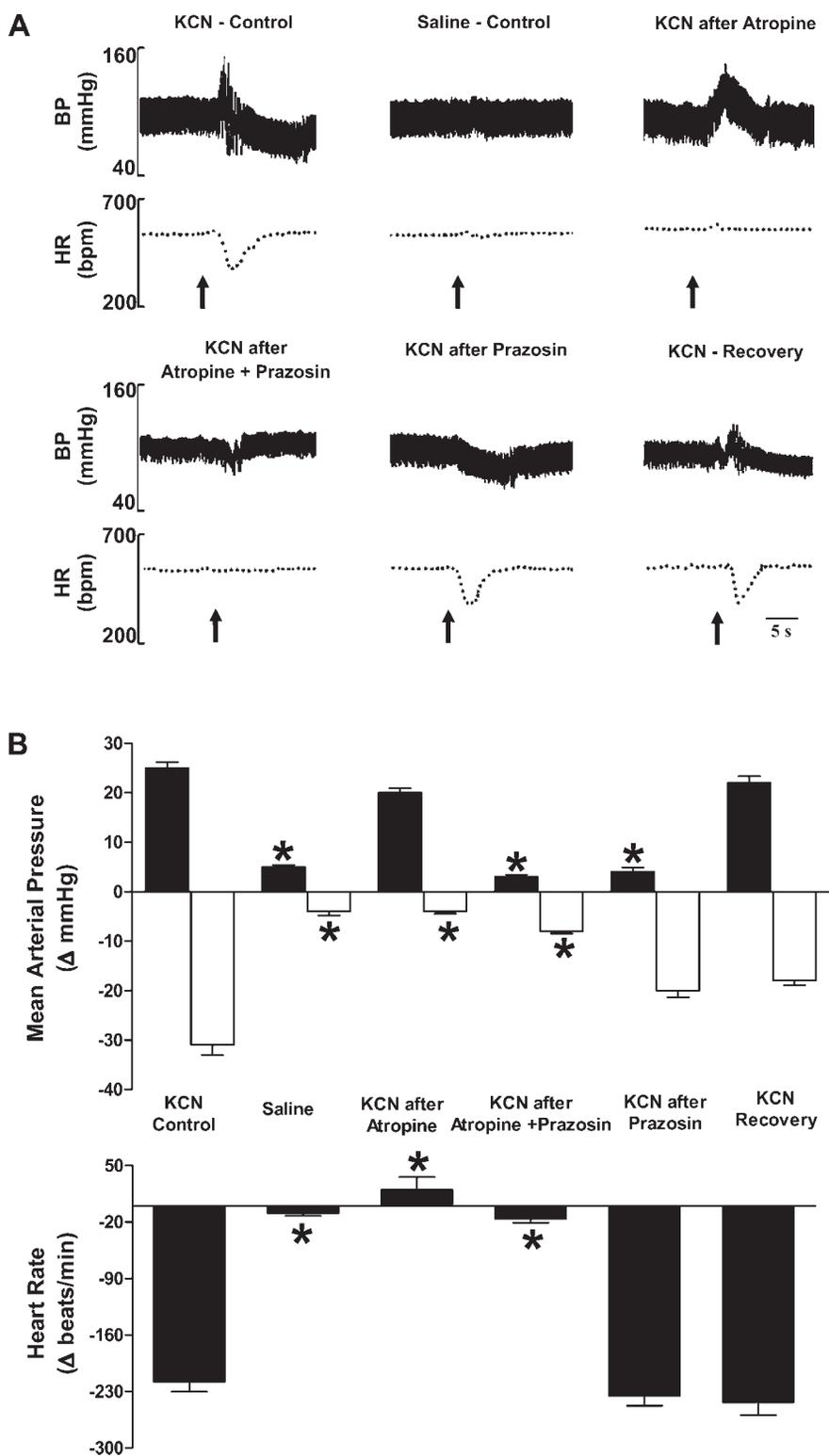
Fig. 1. Representative tracings and summary data of changes in blood pressure and heart rate (HR) produced by the iv bolus injection of potassium cyanide (KCN) in a conscious, telemetered C57BL/6 mouse before and after autonomic blockade with atropine and prazosin. *A*: for all tracings, mean arterial pressure (MAP) and HR were measured continuously via telemetry (2,000 Hz) during iv bolus KCN administration. Arrows indicate when KCN was administered. *B*: values are means ± SE and represent changes in MAP (solid bars: peak pressor response; open bars: peak depressor response) and HR produced by the iv bolus injection of either 26 μg/kg KCN before (control administration) and after pretreatment with atropine and prazosin or isotonic saline vehicle (30 μl). Data presented represent mean values for peak change immediately after iv KCN injection. *$P < 0.05$ compared with KCN control ($n = 6$).

prolonged decrease in blood pressure (-31 ± 5 mmHg, $P < 0.05$) that lasted for ~25 s. In addition to the changes in blood pressure, chemoreflex activation elicited a pronounced bradycardia (-218 ± 30 beats/min, $P < 0.05$). Overall, these responses were transient, since both blood pressure and HR had returned to baseline within 20–40 s following KCN injection. Moreover, 20-min pretreatment with the muscarinic receptor antagonist atropine (1 μg/g, ip) prevented the bradycardia produced by KCN ($+20 \pm 35$ vs. -218 ± 30 beats/min, $P < 0.05$), demonstrating that this response is mediated by an increase in the parasympathetic drive to the heart. Interestingly, the decrease in blood pressure in response to chemoreflex activation after atropine was also virtually abolished (-4 ± 1 vs. -31 ± 5 mmHg, $P < 0.05$), suggesting that it was, at least

Table 1. *Changes in of MAP and HR evoked by drug-induced activation of the baroreflex*

	Baseline	SNP	Baseline	PE	Baseline	PE + Atropine	Baseline	PE + Propranolol
MAP, mmHg ($n = 5$)	124 ± 4	67 ± 2*	112 ± 5	147 ± 5*	119 ± 4	165 ± 5*	111 ± 4	159 ± 3*
HR, beats/min ($n = 5$)	622 ± 18	765 ± 15*	605 ± 27	420 ± 26*	733 ± 14	629 ± 18*	723 ± 14	109 ± 9*

Values are means $\pm$ SE; *n*, no. of mice. SNP, sodium nitroprusside; PE, phenylephrine; MAP, mean arterial pressure; HR, heart rate. *$P < 0.05$ vs. respective baseline.

in part, dependent on the bradycardia. Furthermore, 20 min pretreatment with the adrenergic receptor antagonist prazosin (1 μg/g, ip) in the same animal (i.e., prazosin + atropine) prevented both the increase in blood pressure and the bradycardia in response to chemoreflex activation (MAP, +3 ± 1 vs. +25 ± 3 mmHg, HR, −16 ± 11 vs. −218 ± 30 beats/min, $P < 0.05$). In additional experiments, prazosin alone prevented the increase in blood pressure without affecting the bradycardia and the fall in blood pressure elicited by KCN (increase in MAP: +4 ± 2 vs. +25 ± 3 mmHg, $P < 0.05$), indicating that the pressor component of the response is mediated by an increase in the sympathetic drive to the vasculature. The biphasic response in blood pressure and the bradycardia were recovered 2 h after treatment with the antagonists, as indicated by the cardiovascular responses to a repeat intravenous bolus injection of KCN. Saline, which was used as vehicle control, produced negligible changes in blood pressure (+5 ± 1 mmHg) and HR (9 ± 8 beats/min).

Cardiovascular responses to classical drug-induced baroreflex activation vs. spontaneous baroreflex analysis. Alterations in baroreflex gain assessed by classical vasoactive drug-evoked activation of the baroreflex vs. the sequence method analysis of spontaneous baroreflex were compared in the same animal in all studies. In Table 1, baseline blood pressure and HR values are shown, along with the cardiovascular changes evoked by drug-induced activation of the baroreflex. Representative tracings of the changes in blood pressure and HR produced by intravenous bolus injection of PE or SNP are shown in Fig. 2, *A* and *B*. Summary data from five mice presented in Fig. 3 show that intravenous bolus injection of PE produced an increase in MAP (+35 ± 2 mmHg, $P < 0.05$) with a sig-

nificant reflex bradycardia (−185 ± 17 beats/min, $P < 0.05$), translating into a baroreflex gain of −5.2 ± 0.5 beats·min^{-1}·mmHg^{-1}. This was comparable to the value of the baroreflex gain measured using the sequence method, whereby the gain of baseline "up" sequences (i.e., sequences in which both MAP and the following R-R interval increased) was examined (−3.5 ± 0.3 beats·min^{-1}·mmHg^{-1}). In addition, SNP (iv) produced a decrease in MAP (−57 ± 4 mmHg, $P < 0.05$) with a reflex tachycardia (+143 ± 14 beats/min, $P < 0.05$), resulting in a baroreflex gain of −2.6 ± 0.3 beats·min^{-1}·mmHg^{-1}. This was comparable to the value of the baroreflex gain measured using the sequence method, whereby the baroreflex gain of baseline "down" sequences (i.e., sequences in which both MAP and the following R-R interval decreased) was examined (−2.5 ± 0.7 beats·min^{-1}·mmHg^{-1}).

As shown in Fig. 4*A*, the reflex bradycardia evoked by the increase in blood pressure produced by PE (+46 ± 2 mmHg, $P < 0.05$) was reduced significantly (−94 ± 14 beats/min, $P < 0.05$) by 20-min pretreatment with atropine, indicating that there was an attenuation of the baroreflex gain by cholinergic receptor blockade (−2.6 ± 0.4 beats·min^{-1}·mmHg^{-1}, $P < 0.05$). Alternatively, the bradycardia evoked by the increase in blood pressure produced by PE (+48 ± 3 mmHg, $P < 0.05$) was significantly potentiated (−514 ± 20 beats/min, $P < 0.05$) by 20-min pretreatment with propranolol, demonstrating a significant increase in the baroreflex gain by β-adrenergic receptor blockade (−10.8 ± 0.5 beats·min^{-1}·mmHg^{-1}, $P < 0.05$). Using the sequence method, the baroreflex gain of baseline up sequences was similarly inhibited by intraperitoneal treatment with atropine (−1.7 ± 0.4 beats·min^{-1}·mmHg^{-1}, $P <$
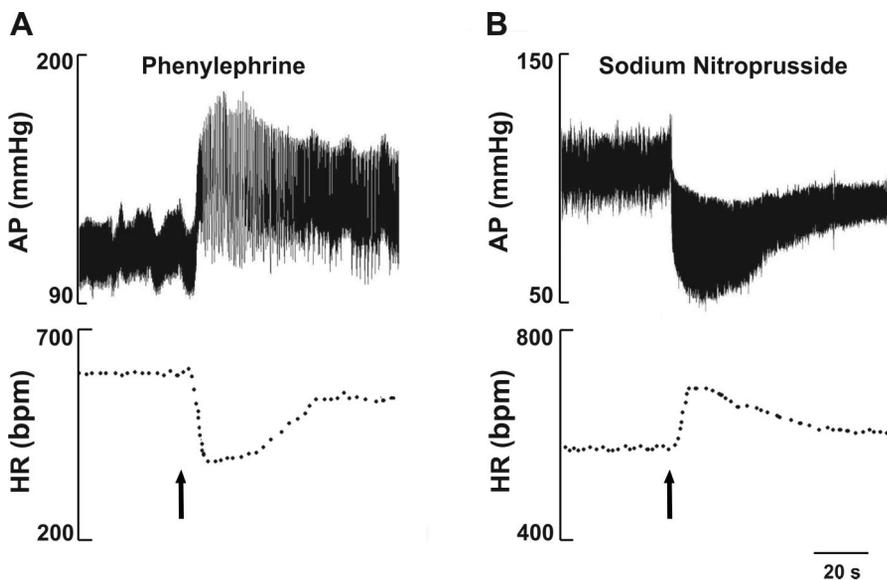

Fig. 2. Representative tracings of the changes in blood pressure and HR produced by iv bolus injection of phenylephrine (PE) or sodium nitroprusside (SNP) in a conscious, telemetered C57BL/6 mouse. Tracings illustrate the typical systemic cardiovascular pressor response and bradycardia produced by iv PE (*A*) or typical systemic depressor response and tachycardia produced by iv SNP (*B*). For all tracings, arterial pressure (AP) was measured via telemetry before and immediately after drug delivery (2,000 Hz, consecutive 2-s sampling interval). Arrows indicate when PE and SNP were administered.
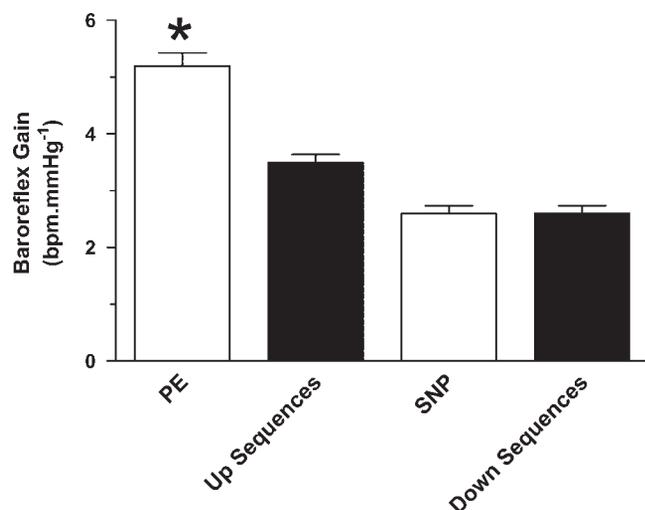
Fig. 3. Comparison of classical vasoactive drug-evoked activation of the baroreflex vs. spontaneous baroreflex activity as analyzed by the sequence method. Baroreflex gain assessed by the iv bolus injection of PE and SNP (open bars) compared with the baroreflex gain assessed by spontaneous baroreflex analysis (solid bars) using the sequence method represented as "up" and "down" sequences ($n = 5$, *$P < 0.05$).

0.05) and potentiated by intraperitoneal pretreatment with propranolol (-6.7 ± 0.7 beats·min^{-1}·mmHg^{-1}, $P < 0.05$). To better compare the drug-induced baroreflex method with the spontaneous baroreflex method given the differences in the absolute values obtained as described above, the effects of autonomic blockade on baroreflex gain were expressed as the percentage of reduction or enhancement of the baroreflex gain produced by atropine or propranolol, respectively, as shown in Fig. 4B. The reduction in the baroreflex gain in response to PE injection produced by pretreatment with atropine was similar to the reduction in the baroreflex gain obtained by examining the up sequences after the administration of atropine (-50 ± 8 and $-48 \pm 10\%$, respectively, not significant). As seen for cholinergic receptor blockade, similar results were found for the increase in baroreflex gain produced after the administration of propranolol using both methods ($+108 \pm 10$ and $+91 \pm 20\%$, respectively, not significant).

DISCUSSION

The present studies characterized the cardiovascular responses to cyanide-induced activation of peripheral chemoreceptors in conscious mice using telemetry. Our findings indicate that activation of this reflex elicits a biphasic change in blood pressure and bradycardia. The initial rise in blood pressure is mediated by an increase in sympathetic drive to the vasculature, whereas the subsequent fall in blood pressure occurs, at least in part, as a consequence of the bradycardia produced by an increase in parasympathetic drive to the heart. In addition, these studies compared two different methods of evaluating baroreflex gain in mice using telemetry. We demonstrate that both drug-induced and spontaneous baroreflex activity using the sequence method are valuable approaches. The predictive power of the spontaneous baroreflex in the context of long-term noninvasive studies with telemetry is certainly a benefit, and analysis of the full range of the reflex using the "gold standard" drug-induced method to examine the

sensitivity of the baroreflex is also important. As such, we suggest that these methods can and should be used complimentarily to each other.

The peripheral chemoreflex is an important mechanism in oxygen sensing during hypoxic states such as obstructive sleep apnea (34, 37) and sudden infant death syndrome (15). Although this reflex is not well understood in mice, it has been well characterized in other species (5, 16, 18, 19). Here our results demonstrate that peripheral chemoreflex activation evokes a biphasic response in blood pressure and bradycardia in mice. The first response to KCN-induced activation of peripheral chemoreceptors was a rapid rise in blood pressure. This was attributable to an increase in sympathetic drive to the vasculature since pretreatment with the α-adrenergic receptor antagonist prazosin prevented this component of the response; similar findings have been previously observed in rats (18) and fetal lambs (19). This rapid elevation in blood pressure was immediately followed by a significant hypotension, which appeared to be, at least in part, dependent on the bradycardia since pretreatment with the muscarinic receptor antagonist atropine attenuated both of these responses. In addition, we cannot rule out the effects of cyanide acting directly in vasculature producing additional hypotension and the activation of the baroreflex during the increase in blood pressure resulting in a transient sympathoinhibition. Moreover, although not examined in the present study, it is likely that the observed biphasic
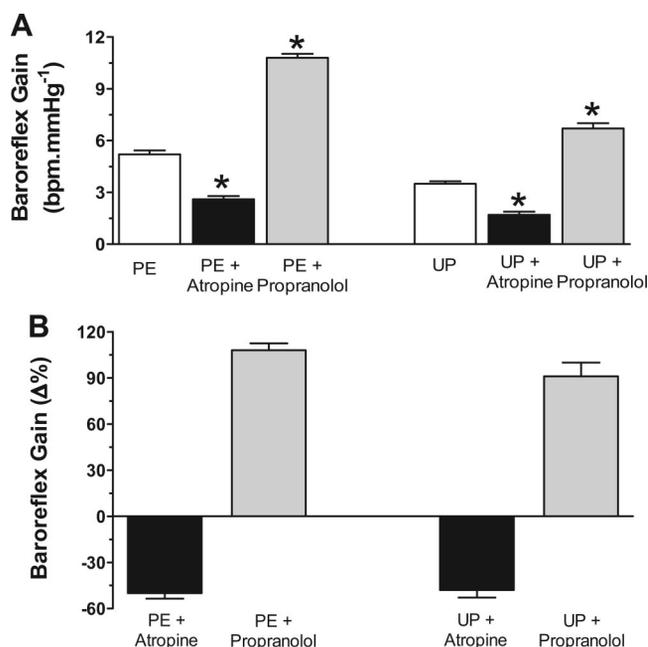

Fig. 4. Comparison of the effects of autonomic blockade during classical drug-induced activation of the baroreflex and spontaneous baroreflex analysis by the sequence method in the same group of mice. A: changes in baroreflex gain evoked by PE injection as well as its reduction after atropine pretreatment and enhancement after propranolol pretreatment were compared with spontaneous changes in baroreflex gain as determined using the sequence method. Data is presented as "up sequences" during baseline as well as following pretreatment with either atropine or propranolol ($n = 5$, *$P < 0.05$ compared with its own control value). B: %change in baroreflex gain. Data show a similar reduction in baroreflex gain produced by PE following pretreatment with atropine and a similar enhancement in baroreflex gain produced by PE following pretreatment with propranolol when using either classical drug-induced activation of the baroreflex or spontaneous baroreflex analysis using the sequence method.

blood pressure response to carotid chemoreceptor activation is strongly influenced by changes in ventilation as previously described in experiments by Rutherford and Vatner (35). In these studies, carotid chemoreceptor stimulation in conscious dogs with spontaneous respiration resulted in a biphasic vascular response, whereby pulmonary inflation reflexes attenuated the initial vasoconstrictor response to carotid chemoreceptor stimulation and were responsible for a later period of vasodilation.

Regarding the cardiovascular responses, the pattern of changes in HR in mice is variable among different studies. A previous report by Pearson et al. (30) showed that acute sustained hypoxia (10% O_2 over 30 min) in conscious mice evoked long-lasting tachycardia. Alternatively, Dematteis et al. (13) have reported that acute intermittent episodes of hypoxia (4% O_2) produced no changes in HR. In our experiments, we show that acute activation of the peripheral chemoreflex with KCN produced significant bradycardia as seen in other species (5, 6, 18, 19). This difference might be related to the different stimulus used to activate peripheral chemoreceptors. Of note, peripheral chemoreflex activation triggered by apnea in humans elicits hypertension and bradycardia known as the diving reflex (38). In addition to the direction of the response (i.e., increase or decrease in HR), there is also a difference in the magnitude of this response among studies. For example, Peotta et al. (32) observed a much more reduced bradycardia using the same dose of KCN in anesthetized mice when compared with our findings. This blunted bradycardia could be explained by the depressive effect of anesthesia on cardiovascular reflexes. The biphasic blood pressure response observed in conscious mice in the present studies was different from that observed in conscious rats, fetal lambs, and anesthetized rabbits in which there is only an increase in blood pressure associated with intense bradycardia (5, 6, 16, 18, 19).

Regarding the baroreflex, in the present study, we compared two methods of assessing baroreflex gain in the same conscious mouse using telemetry. First, baroreceptor reflex control of HR was quantified by classic vasoactive drug administration, which serves to either raise or lower systemic arterial pressure (3, 33, 39). Second, high-frequency blood pressure recordings (2,000 Hz) attained by telemetry were analyzed using the spontaneous baroreflex method, which involves computer-based analysis to locate sequences of four or more heart beats in which blood pressure spontaneously increases or decreases with parallel changes in pulse interval (1, 23, 29, 40). We observed comparable values with both methods except for a higher baroreflex gain in PE-treated animals compared with the up sequences examined during spontaneous fluctuations in blood pressure, which could be explained by the fact that PE may have activated nerve fibers related to the cardiopulmonary reflex, which, in turn, could have contributed to the greater baroreflex gain observed (4). Of note, although the values obtained by us regarding the gain of the baroreflex in the drug-induced method were somewhat lower than those reported by others (25–27), they were close to the values obtained during the spontaneous baroreflex analysis. This discrepancy could be attributed to different mathematical approaches to estimate baroreflex sensitivity.

The pros and cons of classical methods for baroreflex assessment have been reviewed elsewhere (22). Unfortunately, neither classic drug-induced baroreflex assessment nor the spontaneous baroreflex method take into account input variables such as diastolic pressure, pulse pressure, stroke volume, and dP/dt, all of which are involved in baroreflex regulation of R-R interval, but instead only consider systolic and MAP. Thus, it is difficult to control all of these variables with either of the described methods. It could be presumed that the cardiac baroreflex provides a buffer solely against short-term disturbances in blood pressure and only under resting conditions when stress levels are low. In contrast, it can be overridden or reset to higher pressures during rapid rises in HR in response to a variety of stimuli such as exercise, the defense reaction, or during chronic pathological conditions such as hypertension (12, 20, 24, 31). As a result, we suggest that these two methods of analyzing baroreflex sensitivity be implemented complimentarily to each other.

Perspectives and Significance

Chronic intermittent activation of peripheral chemoreceptors in patients suffering from sleep obstructive apnea results in impaired autonomic control and affects baroreflex function, which could facilitate hypertension (11, 28, 38). Considering the potential clinical relevance of the interaction between both the chemoreflex and baroreflex, we have assessed both of these reflexes in the same animal model. In addition, we have evaluated two different methods commonly used to analyze the sensitivity of the baroreflex. The use of telemetry for studying these cardiovascular reflexes in an anesthetic- and stress-free environment will undoubtedly contribute to a better understanding of some of the pathological conditions that adversely affect the functioning of these reflexes such as obstructive sleep apnea and hypertension.

REFERENCES

1. **Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G.** Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol Heart Circ Physiol* 254: H377–H383, 1988.
2. **Bertinieri G, di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G.** A new approach to analysis of the arterial baroreflex. *J Hypertens* 3: S79–S81, 1985.
3. **Bissonnette JM, Knopp SJ, Maylie J, Thong T.** Autonomic cardiovascular control in methyl-CpG-binding protein 2 (Mecp2) deficient mice. *Auton Neurosci* 136: 82–89, 2007.
4. **Bowser-Riley F, Cornish M, Kidd C, Zheng F.** Role of vagal afferent C fibres in the bradycardiac response to an increase in arterial blood pressure in ferrets. *Exp Physiol* 75: 859–862, 1990.
5. **Braga VA, Machado BH.** Chemoreflex sympathoexcitation was not altered by the antagonism of glutamate receptors in the commissural nucleus tractus solitarii in the working heart-brainstem preparation of rats. *Exp Physiol* 91: 551–559, 2006.
6. **Braga VA, Soriano RN, Braccialli AL, de Paula PM, Bonagamba LG, Paton JF, Machado BH.** Involvement of L-glutamate and ATP in the neurotransmission of the sympathoexcitatory component of the chemoreflex in the commissural nucleus tractus solitarii of awake rats and in the working heart-brainstem preparation. *J Physiol* 581: 1129–1145, 2007.

7. **Butz GM, Davisson RL.** Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. *Physiol Genomics* 5: 89–97, 2001.

8. **Campbell IL, Chiang CS.** Cytokine involvement in central nervous system disease. Implications from transgenic mice. *Ann NY Acad Sci* 771: 301–312, 1995.

9. **Chapleau MW, Abboud FM.** Neuro-cardiovascular regulation: from molecules to man. *Ann NY Acad Sci* 940: xiii–xxii, 2001.

10. **Chen Y, Joaquim LF, Farah VM, Wichi RB, Fazan R, Jr Salgado HC, Morris M.** Cardiovascular autonomic control in mice lacking angiotensin AT1a receptors. *Am J Physiol Regul Integr Comp Physiol* 288: R1071–R1077, 2005.

11. **Cooper VL, Pearson SB, Bowker CM, Elliott MW, Hainsworth R.** Interaction of chemoreceptor and baroreceptor reflexes by hypoxia and hypercapnia: a mechanism for promoting hypertension in obstructive sleep apnoea. *J Physiol* 568: 677–687, 2005.

12. **De Angelis K, Wichi RB, Jesus WR, Moreira ED, Morris M, Krieger EM, Irigoyen MC.** Exercise training changes autonomic cardiovascular balance in mice. *J Appl Physiol* 96: 2174–2178, 2004.

13. **Dematteis M, Julien C, Guillermet C, Sturm N, Lantuejoul S, Mallaret M, Levy P, Gozal E.** Intermittent hypoxia induces early functional cardiovascular remodeling in mice. *Am J Respir Crit Care Med* 177: 227–235, 2008.

14. **Ebert TJ.** Differential effects of nitrous oxide on baroreflex control of heart rate and peripheral sympathetic nerve activity in humans. *Anesthesiology* 72: 16–22, 1990.

15. **Gauda EB, Cristofalo E, Nunez J.** Peripheral arterial chemoreceptors and sudden infant death syndrome. *Respir Physiol Neurobiol* 157: 162–170, 2007.

16. **Goncalves V, Rocha I, Silva-Carvalho L.** Attenuation of the carotid body chemoreflex during the stimulation of the posterior vermis in the anaesthetised rabbit. *Auton Neurosci* 101: 78–84, 2002.

17. **Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G.** Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension* 31: 68–72, 1998.

18. **Haibara AS, Colombari E, Chianca DA, Jr Bonagamba LG, Machado BH.** NMDA receptors in NTS are involved in bradycardic but not in pressor response of chemoreflex. *Am J Physiol Heart Circ Physiol* 269: H1421–H1427, 1995.

19. **Itskovitz J, Rudolph AM.** Cardiorespiratory response to cyanide of arterial chemoreceptors in fetal lambs. *Am J Physiol Heart Circ Physiol* 252: H916–H922, 1987.

20. **Kramer JM, Aragones A, Waldrop TG.** Reflex cardiovascular responses originating in exercising muscles of mice. *J Appl Physiol* 90: 579–585, 2001.

21. **Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE, Subcommittee of Professional and Public Education of the American Heart Association.** Recommendations for blood pressure measurement in humans and experimental animals Part 2: blood pressure measurement in experimental animals: a statement for professionals from the subcommittee of professional and public education of the American Heart Association council on high blood pressure research. *Hypertension* 45: 299–310, 2005.

22. **La Rovere MT, Pinna GD, Raczak G.** Baroreflex sensitivity: measurement and clinical implications. *Ann Noninvasive Electrocardiol* 13: 191–207, 2008.

23. **Laude D, Baudrie V, Elghozi JL.** Applicability of recent methods used to estimate spontaneous baroreflex sensitivity to resting mice. *Am J Physiol Regul Integr Comp Physiol* 294: R142–R150, 2008.

24. **Martinka P, Fielitz J, Patzak A, Regitz-Zagrosek V, Persson PB, Stauss HM.** Mechanisms of blood pressure variability-induced cardiac hypertrophy and dysfunction in mice with impaired baroreflex. *Am J Physiol Regul Integr Comp Physiol* 288: R767–R776, 2005.

25. **Masuki S, Takeoka M, Taniguchi S, Nose H.** Enhanced baroreflex sensitivity in free-moving calponin knockout mice. *Am J Physiol Heart Circ Physiol* 284: H939–H946, 2003.

26. **Masuki S, Todo T, Nakano Y, Okamura H, Nose H.** Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. *J Physiol* 566: 213–224, 2005.

27. **Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD.** Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. *J Clin Invest* 97: 1047–1055, 1996.

28. **Monahan KD, Leuenberger UA, Ray CA.** Effect of repetitive hypoxic apnoeas on baroreflex function in humans. *J Physiol* 574: 605–613, 2006.

29. **Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, Pedotti A, Zanchetti A, Mancia G.** Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* 12: 214–222, 1988.

30. **Pearson JT, Shirai M, Yokoyama C, Tsuchimochi H, Schwenke DO, Shimouchi A, Kangawa K, Tanabe T.** Alpha2-adrenoreceptor mediated sympathoinhibition of heart rate during acute hypoxia is diminished in conscious prostacyclin synthase deficient mice. *Pflugers Arch* 454: 29–39, 2007.

31. **Peotta VA, Gava AL, Vasquez EC, Meyrelles SS.** Evaluation of baroreflex control of heart rate in renovascular hypertensive mice. *Can J Physiol Pharmacol* 85: 761–766, 2007.

32. **Peotta VA, Vasquez EC, Meyrelles SS.** Cardiovascular neural reflexes in L-NAME-induced hypertension in mice. *Hypertension* 38: 555–559, 2001.

33. **Pickering TG, Gribbin B, Petersen ES, Cunningham DJ, Sleight P.** Effects of autonomic blockade on the baroreflex in man at rest and during exercise. *Circ Res* 30: 177–185, 1972.

34. **Prabhakar NR, Peng YJ, Kumar GK, Pawar A.** Altered carotid body function by intermittent hypoxia in neonates and adults: relevance to recurrent apneas. *Respir Physiol Neurobiol* 157: 148–153, 2007.

35. **Rutherford JD, Vatner SF.** Integrated carotid chemoreceptor and pulmonary inflation reflex control of peripheral vasoactivity in conscious dogs. *Circ Res* 43: 200–208, 1978.

36. **Salgado HC, Barale AR, Castania JA, Machado BH, Chapleau MW, Fazan R Jr.** Baroreflex responses to electrical stimulation of aortic depressor nerve in conscious SHR. *Am J Physiol Heart Circ Physiol* 292: H593–H600, 2007.

37. **Semenza GL, Prabhakar NR.** HIF-1-dependent respiratory, cardiovascular, and redox responses to chronic intermittent hypoxia. *Antioxid Redox Signal* 9: 1391–1396, 2007.

38. **Shamsuzzaman AS, Somers VK.** Cardiorespiratory interactions in neural circulatory control in humans. *Ann NY Acad Sci* 940: 488–499, 2001.

39. **Smyth HS, Sleight P, Pickering GW.** Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res* 24: 109–121, 1969.

40. **Stauss HM, Moffitt JA, Chapleau MW, Abboud FM, Johnson AK.** Baroreceptor reflex sensitivity estimated by the sequence technique is reliable in rats. *Am J Physiol Heart Circ Physiol* 291: H482–H483, 2006.

41. **Xue B, Pamidimukkala J, Hay M.** Sex differences in the development of angiotensin II-induced hypertension in conscious mice. *Am J Physiol Heart Circ Physiol* 288: H2177–H2184, 2005.