Analysis of afferent, central, and efferent components of the baroreceptor reflex in mice

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Ma, Xiuying, Francois M. Abboud, and Mark W. Chapleau. Analysis of afferent, central and efferent components of the baroreceptor reflex in mice. Am J Physiol Regul Integr Comp Physiol 283: R1033–R1040, 2002—Studies of genetically modified mice provide a powerful approach to investigate consequences of altered gene expression in physiological and pathological states. The goal of the present study was to characterize afferent, central, and efferent components of the baroreceptor reflex in anesthetized Webster 4 mice. Baroreflex and baroreceptor afferent functions were characterized by measuring changes in renal sympathetic nerve activity (RSNA) and aortic depressor nerve activity (ADNA) in response to nitroprusside- and phenylephrine-induced changes in arterial pressure. The data were fit to a sigmoidal logistic function curve. Baroreflex diastolic pressure threshold (Pth), the pressure at 50% inhibition of RSNA (Pmid), and baroreflex gain (maximum slope) averaged 74 ± 5 mmHg, 101 ± 3 mmHg, and 2.30 ± 0.54%/mmHg, respectively (n = 6). The Pth, Pmid, and gain for the diastolic pressure-ADNA relation (baroreceptor afferents) were similar to that observed for the overall reflex averaging 79 ± 9 mmHg, 101 ± 4 mmHg, and 2.92 ± 0.53%/mmHg, respectively (n = 5). The central nervous system mediation of the baroreflex and the chronotropic responsiveness of the heart to vagal efferent activity were independently assessed by recording responses to electrical stimulation of the left ADN and the peripheral end of the right vagus nerve, respectively. Both ADN and vagal efferent stimulation induced frequency-dependent decreases in heart rate and arterial pressure. The heart rate response to ADN stimulation was nearly abolished in mice anesthetized with pentobarbital sodium (n = 4) compared with mice anesthetized with ketamine-acepromazine (n = 4), whereas the response to vagal efferent stimulation was equivalent under both types of anesthesia. Application of these techniques to studies of genetically manipulated mice can be used to identify molecular mechanisms of baroreflex function and to localize altered function to afferent, central, or efferent sites. pressoreceptors; blood pressure; aortic depressor nerve; sympathetic nerve activity; functional genomics

THE ARTERIAL BARORECEPTOR reflex is a major regulator of arterial pressure and cardiovascular function and has been studied extensively in numerous animal species and humans (3, 39). Despite the many advances made toward understanding the baroreceptor reflex, very little is known concerning the identity and function of molecules essential for sensory afferent, central, and efferent components of the reflex. For example, only recently have studies begun to elucidate the nature of the mechanoelectrical transducing ion channel on baroreceptor nerve terminals (9).

The recent explosion of gene discovery and the development of techniques to genetically modify intact animals provide new opportunities for discovery of novel mechanisms. Mice are particularly amenable to genetic manipulation, and their use in physiological studies is rapidly increasing (14, 26, 27). Recently, investigators have begun to study baroreflex function in mice; the majority of studies have examined reflex control of heart rate, and a few of these studies have provided quantitative measurements of baroreflex sensitivity (gain) in conscious and anesthetized mice (25, 30, 32, 34, 38). Studies of baroreflex control of heart rate are limited in that they do not provide information on the sensitivity for control of sympathetic nerve activity and vascular resistance (18, 19, 33). Furthermore, measurements of overall reflex function cannot discriminate effects on afferent, central nervous system (CNS), and efferent components of the reflex. Changes in one component of the reflex, e.g., effector organ responsiveness, may compensate for and obscure changes in another component of the reflex (6, 11, 12).

The major goals of the present study were 1) to characterize baroreceptor afferent sensitivity and reflex function in mice through direct electrophysiologically recording of aortic depressor nerve activity (ADNA) and renal sympathetic nerve activity (RSNA) and 2) to independently assess the CNS mediation of the reflex and the chronotropic responsiveness of the heart to efferent vagal activity by recording responses to electrical stimulation of ADN afferents and vagus nerve efferents, respectively.

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MATERIALS AND METHODS

Studies were performed on 36 male mice (Webster 4 strain, 25–35 g). The mice were anesthetized with either pentobarbital sodium (60 μg/g ip) or ketamine (91 μg/g ip) and acepromazine (1.8 μg/g ip). Supplemental doses of anesthetics were administered as needed to prevent eye blink and withdrawal reflexes and fluctuations in arterial blood pressure. Body temperature was maintained using a heating pad. The left femoral artery was cannulated with polyethylene tubing (PE-10 connected with PE-50). Arterial pressure was measured with a pressure transducer (COBE, CDX-III), and heart rate was derived from the arterial pressure pulse using a cardiotachometer (Beckman, 9857B). Both femoral veins were cannulated with polyethylene catheters (PE-10) for administration of drugs. A cerebral midline incision was performed and the trachea was cannulated with polyethylene tubing (PE-90) to facilitate ventilation in the spontaneously breathing mice. All of the procedures carried out on the mice were approved by the University of Iowa Animal Care and Use Committee and followed the guidelines of the American Physiological Society.

Recording of RSNA. The left kidney was exposed retroperitoneally via a flank incision in mice anesthetized with pentobarbital sodium. A sympathetic nerve leading to the left kidney was identified between the renal artery and vein using a dissecting microscope. The nerve was isolated from surrounding connective tissue and placed on miniaturized bipolar platinum electrodes (0.12-mm outer diameter). The nerve and electrode were encased in Wacker silicone gel. Nerve activity was amplified using a high-impedance probe and a Grass band-pass amplifier (HIP 511J, 300 Hz-3 kHz). The neurogram was displayed on a dual-beam storage oscilloscope (Tektronix 5113) and listened to through an audio speaker. The frequency of spikes that exceeded a selected threshold voltage just above noise level was counted consecutively in 0.5-s bins using a nerve traffic analyzer (University of Iowa Bioengineering, 706C) (20, 21, 31, 33). Correct placement of the threshold level was confirmed by the elimination of counted RSNA during phenylephrine (PE)-induced increases in arterial pressure and after the death of the mice following the experiment. The phasic arterial pressure, mean arterial pressure, heart rate, and the ratemeter output of RSNA (spikes/s) were recorded on a chart recorder (Gould). In some experiments, the neurogram was recorded using a MacLab computerized data-acquisition system.

Recording of baroreceptor activity from the ADN. The left ADN was identified in the cervical region using a dissecting microscope in mice anesthetized with pentobarbital sodium. The nerve was isolated from surrounding connective tissue and placed on miniaturized bipolar platinum electrodes (0.12-mm outer diameter). The ADNA was recorded using the same procedures as for recording RSNA. The ADN was successfully identified in 18 of 28 mice by the characteristic discharge of activity in phase with the arterial pressure pulse (Fig. 1A). In the majority of mice (16 of 18), the left ADN existed as a separate nerve traversing the cervical region between the left common carotid artery and trachea before joining the superior laryngeal nerve. In two mice, the ADN traveled with the cervical vagus nerve or sympathetic trunk before projecting to the superior laryngeal nerve. Thirteen of the 18 preparations enabled functional studies to be performed (ADN recording, n = 5; ADN stimulation, n = 8). Five of the experiments failed because of either anesthesia-induced hypotension or a poor signal-to-noise ratio in the nerve recording.

Measurement of responses to electrical stimulation of ADN. The left ADN was isolated and placed on a bipolar platinum electrode using the same procedures as described in the preceding section. The nerve was then crushed at a point caudal to the electrode. The nerve was stimulated with rectangular 10-V, 2-ms duration pulses that were delivered to the electrode at varying frequencies from a stimulator (Grass, model S44) through an isolation unit (Grass, SIU 5).

Measurement of responses to electrical stimulation of vagal efferent nerves. The right vagus nerve was isolated from surrounding connective tissue and sectioned. The cut peripheral end of the vagus nerve was placed on a bipolar platinum electrode and stimulated electrically as described for the ADN in the preceding section.

Experimental protocols. After completion of the surgical procedures, the mice were allowed to stabilize for a period of 20–30 min before beginning the protocols. Four groups of experiments were performed.

Baroreflex control of RSNA (n = 6). Baroreflex control of RSNA was evaluated by recording reflex changes in RSNA in response to changes in arterial pressure induced by a single intravenous injection of sodium nitroprusside (SNP; 1–5 μg/g in 2–10 μl of saline) immediately followed by an injection of PE (4–20 μg/g in 2–10 μl of saline) in mice anesthetized with pentobarbital sodium. The baroreflex was characterized by analysis of data collected during the PE-induced rise in arterial pressure beginning at the nadir of the SNP-induced fall in pressure.

Afferent baroreceptor sensitivity (n = 5). The afferent component of the baroreflex reflex was evaluated by recording ADNA during SNP- and PE-induced changes in arterial pressure following the same protocol as described for the baroreflex studies.

Central component of baroreflex (pentobarbital sodium anesthesia, n = 4; ketamine-acepromazine anesthesia, n = 4). The central component of the baroreflex was characterized by measuring the reflex changes in mean arterial pressure and heart rate in response to electrical stimulation of the left ADN. The stimulus was composed of rectangular pulses (10 V, 2-ms duration) delivered at 2, 5, 10, and 15 Hz. Responses to each frequency of stimulation were measured at least twice in each experiment with the order of changes in frequency reversed. Each stimulus period was maintained for 10–20 s with recovery intervals of ~3 to 5 min. The protocol was performed in mice anesthetized with pentobarbital sodium (n = 4) and in a separate group of mice anesthetized with ketamine and acepromazine (n = 4). The stimulation-induced changes in heart rate and arterial pressure were abolished after crushing the ADN cranial to the electrode, confirming that the responses were reflex in nature.

Efferent component of baroreflex control of heart rate (pentobarbital sodium anesthesia, n = 5; ketamine-acepromazine anesthesia, n = 4). The chronotropic responsiveness of the heart to increased vagal efferent activity was evaluated by measuring the heart rate response to electrical stimulation of the peripheral end of the cut right vagus nerve. The nerve was stimulated for periods of 10–20 s with rectangular pulses (10 V, 2-ms duration) of varying frequency (2, 5, 10, and 15 Hz). The protocol was performed in mice anesthetized with pentobarbital sodium (n = 5) and in a separate group of mice anesthetized with ketamine and acepromazine (n = 4).

Data analysis. The ratemeter output of ADNA and RSNA (spikes/s) was measured manually from the pen-recorder traces at 0.8-s intervals. Levels of ADNA and RSNA were normalized as a percentage of the maximum level of activity recorded during PE and SNP administration, respectively. The relationship between diastolic arterial pressure and
nerve activity was determined by fitting the data to a sigmoidal logistic function (28). The logistic function for control of RSNA conformed to the mathematical expression $Y = P_1/\left(1 + \exp[P_2 \times (X - P_3)]\right) + P_4$, where $X =$ diastolic arterial pressure, $Y =$ RSNA (%max), $P_1 =$ maximum – minimum RSNA (range), $P_2 =$ slope coefficient, $P_3 =$ diastolic arterial pressure at 50% of the RSNA range ($P_{\text{mid}}$), and $P_4 =$ minimum RSNA. The maximum slope (gain) was calculated as $P_1 \times P_2/4$. The diastolic threshold ($P_{\text{th}}$) and saturation ($P_{\text{sat}}$) pressures were calculated from the third derivative of the logistic function. The same general equation was used for analysis of ADNA where $Y =$ ADNA (%max), $P_4 =$ maximum ADNA, and $P_1$ (ADNA range) was expressed as a negative value. Approximately 15–25 data points measured over 12–20 s were used to construct the function curves. Curve parameters for the baroreceptor function curve (diastolic pressure-ADNA) and the reflex function curve (diastolic pressure-RSNA) were compared using the unpaired $t$-test (GB-Stat 6.0 software). Peak changes in mean arterial pressure and heart rate were measured in response to graded electrical stimulation of baroreceptor afferents in the ADN and vagal efferent nerves. The effects of stimulation frequency and anesthesia (pentobarbital sodium vs. ketamine) were analyzed by ANOVA and Newman-Keuls post hoc test (GB-Stat 6.0 software). Differences were considered significant when $P < 0.05$. Data are presented as means ± SE.

RESULTS

Baseline arterial pressure and heart rate in anesthetized mice. The baseline level of mean arterial pressure averaged 89 ± 2 and 86 ± 4 mmHg in pentobarbital sodium ($n = 10$)- and ketamine-acepromazine ($n = 6$)-anesthetized mice, respectively ($P =$ NS). Baseline heart rate was significantly influenced by the type of anesthesia averaging 514 ± 17 beats/min under pentobarbital sodium anesthesia ($n = 10$) and 477 ± 9 beats/min under ketamine-acepromazine anesthesia ($n = 6$).

Baroreflex control of RSNA. RSNA recorded under baseline conditions exhibited synchronized bursts of activity as has been observed in other species (7, 13, 29, 42, 46, 48) (Fig. 1B). The baroreflex was characterized by measuring RSNA over a wide range of arterial pressure induced by intravenous injections of SNP and PE ($n = 6$). SNP reduced mean arterial pressure to $47 \pm 7$ mmHg and increased RSNA. Subsequent injection of PE produced a ramp increase in mean arterial pressure reaching a maximum of $160 \pm 11$ mmHg and inhibited RSNA (Fig. 2). The RSNA and diastolic arterial pressure data measured during the ramp increase

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in pressure were plotted and fit to a logistic sigmoidal function that defined baroreflex parameters in each mouse studied (Fig. 3, left). The values of $P_{th}$, $P_{mid}$, $P_{sat}$, range, and maximum gain of the baroreflex function curve are shown in Table 1.

**Afferent component of baroreflex.** The afferent component of the baroreflex was characterized by measuring ADNA over a wide range of arterial pressure elicited by intravenous injections of SNP and PE ($n = 5$). The ADNA occurred in bursts in phase with the arterial pressure pulse characteristic of baroreceptor afferents (Fig. 1A), was decreased in response to SNP-induced hypotension, and was increased in response to the PE-induced increase in arterial pressure (Fig. 4). The relationship between ADNA and diastolic arterial pressure was sigmoidal (Fig. 2, right). The values of $P_{th}$, $P_{mid}$, $P_{sat}$, range, and maximum gain for the afferent baroreceptor function curve did not differ significantly from the parameters for the reflex RSNA function curve (Table 1).

**Central component of baroreflex.** Electrical stimulation of the ADN evoked frequency-dependent decreases in arterial pressure and heart rate (Figs. 5 and 6). The magnitude of the reflex decrease in arterial pressure was similar in pentobarbital sodium ($n = 4$)- and ketamine-acepromazine ($n = 4$)-anesthetized mice (Fig. 6). In contrast, the reflex decrease in heart rate was significant in ketamine-acepromazine-anesthetized mice but was essentially nonexistent in pentobarbital sodium-anesthetized mice (Fig. 6).

**Efferent component of baroreflex.** Electrical stimulation of the right vagus nerve evoked frequency-dependent decreases in heart rate (Fig. 7). The magnitude of the bradycardia was not significantly different in pentobarbital sodium ($n = 5$)- vs. ketamine-acepromazine ($n = 4$)-anesthetized mice (Fig. 7). Thus, pentobarbital sodium nearly abolished the bradycardic response to ADN stimulation (Fig. 6) without altering the bradycardic response to stimulation of vagal efferents (Fig. 7).

**DISCUSSION**

The present study provides a quantitative assessment of afferent, central, and efferent components of the arterial baroreflex in anesthetized Webster 4 mice. The combination of direct electrophysiological recording of ADNA and RSNA with measurement of cardiovascular responses to electrical stimulation of baroreflex are shown in Table 1.

### Table 1. Parameters defining the baroreflex (RSNA) function curve and the baroreceptor afferent (ADNA) function curve in pentobarbital sodium-anesthetized mice

<table>
<thead>
<tr>
<th>parameter</th>
<th>RSNA Function Curve</th>
<th>ADNA Function Curve</th>
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<tbody>
<tr>
<td>$P_{th}$</td>
<td>$0.5 \pm 2.9$ mmHg</td>
<td>$39 \pm 3$ mmHg</td>
</tr>
<tr>
<td>$P_{mid}$</td>
<td>$85 \pm 6$ mmHg</td>
<td>$74 \pm 9$ mmHg</td>
</tr>
<tr>
<td>$P_{sat}$</td>
<td>$101 \pm 3$ mmHg</td>
<td>$125 \pm 6$ mmHg</td>
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Data are expressed as means ± SE. The baroreflex function curve parameters were obtained from the logistic function relating normalized renal sympathetic nerve activity (RSNA; %) to diastolic arterial pressure. The baroreceptor afferent function curve parameters were obtained from the logistic function relating normalized aortic depressor nerve activity (ADNA; %) to diastolic arterial pressure. $P_{th}$, diastolic pressure threshold; $P_{mid}$, saturation pressure; $P_{sat}$, pressure at 50% of RSNA or ADNA range.
ceptor afferents and vagal efferents provides a powerful approach to localize the site (e.g., afferent, central, or efferent) responsible for changes in reflex function in pathological states and in genetically modified mice. To illustrate the approach, we demonstrated that pentobarbital sodium anesthesia selectively impairs the central mediation of baroreflex control of heart rate in mice. Baroreflex control of RSNA and arterial pressure as well as the chronotropic responsiveness of the heart to vagal efferent activity are relatively preserved during pentobarbital sodium anesthesia in Webster 4 mice.

**Baroreflex in mice vs. other species.** The baroreceptor reflex has been studied extensively and shown to be of major importance in the regulation of arterial pressure and cardiovascular function in a wide variety of species including humans (3, 39). Recent studies have provided quantitative assessment of baroreflex sensitivity for control of heart rate in conscious and anesthetized mice (25, 30, 32, 34, 38). A few laboratories including our own have recorded RSNA in anesthetized mice (30, 31, 48). The results indicate that baroreflex function is qualitatively similar in mice and other species.

Although differences in methods of analyzing baroreflex function and differences in baseline heart rate and autonomic tone make it difficult to quantitatively compare baroreflex sensitivity between species, some differences are evident. Baroreflex sensitivity for control of heart rate (beats·min⁻¹·mmHg⁻¹) appears to be higher in mice (25, 30, 32, 34, 38) than in rats (13, 22,

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**Fig. 4.** Original recordings showing arterial pressure and ADNA responses to sequential intravenous injections of SNP (2 μg/g) and PE (20 μg/g) in a pentobarbital sodium-anesthetized mouse. A: continuous recordings. B: segments of the traces at an expanded time scale to better illustrate the bursting pattern of ADNA in phase with the arterial pressure pulse at normal and high arterial pressures and the loss of the typical pulse-related activity at low pressure.

**Fig. 5.** Original recordings showing reflex decreases in arterial pressure and heart rate in response to graded electrical stimulation of the left aortic depressor nerve (ADN) in a mouse anesthetized with ketamine and acepromazine. Stimulus parameters were 10-V, 2-ms pulses at 2, 5, and 15 Hz for periods of 10 s.
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In addition to measuring responses to ADN stimulation, insight into the central mediation of the baroreflex can be gained through measurements of ADNA and sympathetic nerve activity over a range of pressure and analysis of the input-output relationship (4, 6, 7, 24). Although our failure to record ADNA and RSNA simultaneously prevents a precise analysis, the finding of similar pressure thresholds and maximum gains for the afferent and reflex function curves suggests a one-to-one coupling of changes in afferent to efferent nerve activity with a “central gain” close to 1 (change in RSNA/change in ADNA) under the conditions of our experiments. Calculated values of central baroreflex gain in previous studies using this type of analysis have ranged from 0.9 to 2.4 (4, 6, 7). The variability between studies may be attributed, in part, to differences in the number of intact cardiovascular afferents (carotid sinus, aortic depressor, and vagus nerves), in the rapidity and magnitude of changes in pressure used to drive changes in baroreceptor afferent activity, and in factors modulating the central mediation of the reflex.

In summary, the results of this study demonstrate the feasibility of assessing afferent, central, and efferent components of the baroreflex in mice. Application of these approaches to genetically modified mice promises to advance our knowledge of the fundamental cellular and molecular mechanisms mediating baroreflex control of the circulation in normal and pathological states.

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