ANALYSIS OF AFFERENT, CENTRAL AND EFFERENT COMPONENTS OF
THE BARORECEPTOR REFLEX IN MICE

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ABSTRACT

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 (P$_{th}$), the pressure at 50% inhibition of RSNA (P$_{mid}$), and baroreflex gain (maximum slope) averaged 74 ± 5 mmHg, 101 ± 3 mmHg, and 2.30 ± 0.54%/mmHg, respectively (n=6). The P$_{th}$, P$_{mid}$, 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 (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 localize altered function to afferent, central, or efferent sites.

Key words: pressoreceptors, blood pressure, aortic depressor nerve, sympathetic nerve activity, functional genomics
INTRODUCTION

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 provides 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 electrophysiological 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.
MATERIALS AND METHODS

Studies were performed on 36 male mice (Webster 4 strain, 25-35 g). The mice were anesthetized with either sodium pentobarbital (60 µg/g, i.p.) or ketamine (91 µg/g, i.p.) and acepromazine (1.8 µg/g, i.p.). 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 cervical 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 Renal Sympathetic Nerve Activity

The left kidney was exposed retroperitoneally via a flank incision in mice anesthetized with sodium pentobarbital. 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 second 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-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, Inc). In some experiments the neurogram was recorded using a MacLab computerized data acquisition system.

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

The left ADN was identified in the cervical region using a dissecting microscope in mice anesthetized with sodium pentobarbital. 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 (see above). The ADN was successfully identified in 18 of 28 mice by the characteristic discharge of activity in phase with the arterial pressure pulse (Figure 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, see below). 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 volt, 2 ms duration pulses which 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 minutes 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 phenylephrine (PE, 4-20 µg/g in 2-10 µL of saline) in mice anesthetized with pentobarbital. 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 baroreceptor 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 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 volts, 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 seconds with recovery intervals of approximately 3 to 5 minutes. The protocol was performed in mice anesthetized with pentobarbital (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 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 seconds with rectangular pulses (10 volts, 2 ms duration) of varying frequency (2, 5, 10, and 15 Hz). The protocol was performed in mice anesthetized with pentobarbital (n=5) and 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 second 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 $Y = \frac{P_1}{1 + \exp[P_2 (X-P_3)]} + P_4$ where $X$ = diastolic arterial pressure, $Y$ = RSNA (%max), $P_1$ = maximum minus minimum RSNA (range), $P_2$ = slope coefficient, $P_3$ = diastolic arterial pressure at 50% of the RSNA range ($P_{mid}$), and $P_4$ = minimum RSNA. The maximum slope (gain) was calculated as $P_1 \times P_2/4$. The diastolic threshold ($P_{th}$) and saturation ($P_{sat}$) 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 seconds 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 vs. ketamine) were analyzed by ANOVA and Newman Keuls posthoc test (GB-Stat 6.0 software). Differences were considered significant when P<0.05. The data are presented as the mean ± standard error (SE).

RESULTS

Baseline Arterial Pressure and Heart Rate in Anesthetized Mice

The baseline level of mean arterial pressure averaged 89 ± 2 mmHg and 86 ± 4 mmHg in pentobarbital (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 anesthesia (n=10) and 477 ± 9 beats/min under ketamine/acepromazine anesthesia (n=6).

Baroreflex Control of Renal Sympathetic Nerve Activity

RSNA recorded under baseline conditions exhibited synchronized bursts of activity as has been observed in other species (7, 13, 29, 42, 46, 48) (Figure 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 ± 7 mmHg and increased RSNA. Subsequent
injection of PE produced a ramp increase in mean arterial pressure reaching a maximum of 160 ± 11 mmHg and inhibited RSNA (Figure 2). The RSNA and diastolic arterial pressure data measured during the ramp increase in pressure were plotted and fit to a logistic sigmoidal function that defined baroreflex parameters in each mouse studied (Figure 3, left panel). 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 (Figure 1A), was decreased in response to SNP-induced hypotension, and was increased in response to the PE-induced increase in arterial pressure (Figure 4). The relationship between ADNA and diastolic arterial pressure was sigmoidal (Figure 2, right panel). 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 (Figures 5 and 6). The magnitude of the reflex decrease in arterial pressure was similar in pentobarbital (n=4) and ketamine/acepromazine (n=4) anesthetized mice (Figure 6). In contrast, the reflex decrease in heart rate was significant in ketamine/acepromazine-anesthetized mice but was essentially nonexistent in pentobarbital-anesthetized mice (Figure 6).
Efferent Component of Baroreflex

Electrical stimulation of the right vagus nerve evoked frequency-dependent decreases in heart rate (Figure 7). The magnitude of the bradycardia was not significantly different in pentobarbital (n=5) vs. ketamine/acepromazine (n=4) anesthetized mice (Figure 7). Thus, pentobarbital nearly abolished the bradycardic response to ADN stimulation (Figure 6) without altering the bradycardic response to stimulation of vagal efferents (Figure 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 electrophysiologic recording of ADNA and RSNA with measurement of cardiovascular responses to electrical stimulation of baroreceptor 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 sodium pentobarbital 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 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/mmHg) appears to be higher in mice (25, 30, 32, 34, 38) than in rats (13, 22, 23, 30, 35). Ling et al. (1998) directly compared baroreflex function in urethane-anesthetized mice (129 Sv/J x ICR strain) and anesthetized Sprague-Dawley rats under similar conditions and observed significantly higher baroreflex sensitivity for control of both heart rate and normalized RSNA in mice (30). Our finding of a relatively high baroreflex-RSNA gain in pentobarbital-anesthetized mice (Webster 4 strain) (2.3 ± 0.5 %/mmHg), a value higher than that observed in anesthetized rats (5, 7, 47), supports the conclusion reached by Ling et al. (30) that baroreflex sensitivity may be higher in mice. Baroreflex sensitivity appears to be slightly lower in mice and rats than in rabbits (29, 42, 46). The physiological significance of differences in baroreflex sensitivity between species is unclear.

The results discussed in the previous paragraph are based on analysis of RSNA normalized as a % change from baseline or a % of maximum activity. It is worthwhile to point out the importance of normalizing measurements of activity recorded from multiple nerve fibers when comparing results obtained from separate groups of animals. The absolute level of activity measured in µV or spikes/s is dependent on the recording conditions, the number and size of the nerve fibers, and the proximity of the active fibers to the electrode. The reduced number and diameter of sympathetic nerve fibers innervating target organs, including the kidneys, in mice (8, 10, 40) likely account for the decreased absolute levels of baseline and maximum RSNA recorded in mice compared with larger species (20, 21, 30, 31, 33). The method of normalization of nerve activity is also important; the same RSNA data
will translate to significantly higher values of baroreflex range and gain when RSNA is expressed as a % change from baseline than when it is expressed as a % of maximum activity.

To our knowledge this is the first full report of ADNA recording in mice. We observed a baroreceptor sensitivity of 2.9 ± 0.5 %/mmHg in pentobarbital-anesthetized Webster 4 mice. In a preliminary report, Moreira et al. reported a gain of 1.67 ± 0.22 %/mmHg in pentobarbital-anesthetized DBA/2 mice (37). It is unclear if the difference in gains in these studies is significant and whether it may reflect differences in methods and/or a difference between the two strains of mice. Values of arterial pressure-ADNA gain range from ~1.2 to 3 %/mmHg in rats and rabbits (7, 15, 17, 36).

Similar to in rats, the ADN in mice appears to contain primarily baroreceptor afferents, with little or no indication of chemoreceptor activity. We observed residual ADNA at low arterial pressure after injection of nitroprusside but it was unclear whether this activity was baroreceptor or chemoreceptor in nature (Figures 3 and 4). Electrical stimulation of the ADN always evoked a baroreflex pattern of response (decreased arterial pressure). A chemoreflex pattern of increased pressure was never observed. Biscoe and Pallot (1982) demonstrated that carotid sinus denervation abolishes oxygen-induced inhibition of ventilation in mice suggesting that the majority of chemoreceptor afferents travel in the carotid sinus nerves in mice (2).

**Importance of Assessing Afferent, Central and Efferent Baroreflex Components**

The redundancy in mechanisms regulating arterial pressure and the tremendous capacity of the nervous system to compensate for alterations in function encourages an experimental approach that can independently assess afferent, central, and efferent components of the reflex. For example, previous studies have shown that baroreflex control of sympathetic activity may be preserved despite a significant decrease in baroreceptor afferent sensitivity in hypertension and heart failure (6, 18, 44). Enhanced CNS mediation of the reflex may compensate for decreased baroreceptor sensitivity (6).
Alternatively, impaired CNS mediation of the reflex may contribute to reflex dysfunction, for example, in hypertension and with aging (16, 20). The responsiveness of the heart and blood vessels to efferent autonomic outflow is an additional important determinant of baroreflex sensitivity. Changes in end-organ responsiveness may contribute to altered reflex function, or alternatively, may compensate for and obscure an impairment in afferent or central mediation of the reflex. For example, decreased baroreflex control of parasympathetic activity and heart rate is accompanied by an increased heart rate response to vagal efferent nerve activity in hypertension (11) and with aging (12). The ability to separately assess afferent, central, and efferent components of the baroreflex will be particularly important in studies of genetically-modified mice where one would expect compensatory changes in function to occur.

To illustrate the usefulness of this approach we evaluated effects of sodium pentobarbital vs. ketamine anesthesia on central and efferent components of the baroreflex. We found that electrical stimulation of baroreceptor afferents in the ADN evoked reflex decreases in heart rate and arterial pressure in ketamine-anesthetized mice (Figures 5 and 6). In contrast, the reflex decrease in heart rate was nearly abolished in pentobarbital-anesthetized mice despite preservation of the reflex decrease in arterial pressure (Figure 6). The results suggest that pentobarbital selectively impairs reflex control of heart rate and does not exert a major effect on reflex control of peripheral sympathetic nerve activity or vascular resistance in mice. The latter suggestion was confirmed by our finding of a high baroreflex-RSNA gain (2.3 ± 0.5 %/mmHg) in pentobarbital-anesthetized mice.

Impaired heart rate responses to ADN stimulation could conceivably result from an impaired CNS mediation of the reflex and/or a reduced cardiac response to reflex changes in parasympathetic and sympathetic nerve activity. Electrical stimulation of vagal efferents decreased heart rate equivalently in pentobarbital and ketamine-anesthetized mice (Figure 7), strongly suggesting that the site of action of pentobarbital was within the CNS. Inhibition of baroreflex control of heart rate by
pentobarbital has been demonstrated in other species (23, 41, 43, 45). Ketamine/acepromazine or urethane (30, 38) may be preferable over pentobarbital or inactin (1) as anesthetic agents for studies of reflex control of heart rate in mice. Although barbiturates such as pentobarbital are generally not recommended for studies of heart rate control, they may be appropriate for studies of reflex control of sympathetic nerve activity in both mice (present study) and rats (7).

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.
ACKNOWLEDGEMENTS

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

<table>
<thead>
<tr>
<th></th>
<th>Baroreflex Function Curve</th>
<th>Baroreceptor Afferent Function Curve</th>
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<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Maximum gain (%/mmHg)</td>
<td>2.3±0.5</td>
<td>2.9±0.5</td>
</tr>
<tr>
<td>Range (%)</td>
<td>85±3</td>
<td>93±8</td>
</tr>
<tr>
<td>Diastolic P_{th} (mmHg)</td>
<td>74±5</td>
<td>79±9</td>
</tr>
<tr>
<td>Diastolic P_{sat} (mmHg)</td>
<td>125±6</td>
<td>120±3</td>
</tr>
<tr>
<td>Diastolic P_{mid} (mmHg)</td>
<td>101±3</td>
<td>101±4</td>
</tr>
</tbody>
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The baroreflex function curve parameters were obtained from the logistic function relating normalized RSNA (%) to diastolic arterial pressure. The baroreceptor afferent function curve parameters were obtained from the logistic function relating normalized ADNA (%) to diastolic arterial pressure. RSNA, renal sympathetic nerve activity; ADNA, aortic depressor nerve activity. Data are expressed as mean ± SE.
FIGURE LEGENDS

Figure 1. Original recordings of arterial pressure and aortic depressor nerve activity (ADNA, Panel A) and renal sympathetic nerve activity (RSNA, Panel B) from two pentobarbital-anesthetized mice under resting baseline conditions. Note that bursts of ADNA occur in phase with the arterial pressure pulse and that RSNA exhibits characteristic synchronized bursts of activity as observed in other species.

Figure 2. Original recordings showing arterial pressure and renal sympathetic nerve activity (RSNA) responses to sequential intravenous injections of sodium nitroprusside (SNP, 1.3 µg/g) and phenylephrine (PE, 20 µg/g) in a pentobarbital-anesthetized mouse.

Figure 3. Baroreflex and baroreceptor afferent function curves derived from responses to intravenous injections of nitroprusside and phenylephrine. **Left:** Arterial pressure-renal sympathetic nerve activity (RSNA) relationship. Data from individual experiment. **Right:** Arterial pressure-aortic depressor nerve activity (ADNA) relationship. Data from individual experiment. Data were fit to a sigmoidal logistic function curve (see Methods).

Figure 4. Original recordings showing arterial pressure and aortic depressor nerve activity (ADNA) responses to sequential intravenous injections of sodium nitroprusside (SNP, 2 µg/g) and phenylephrine (PE, 20 µg/g) in a pentobarbital-anesthetized mouse. Continuous recordings are shown in Panel A. Panel B shows 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.
Figure 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 volt, 2 ms pulses at 2, 5, and 15 Hz for periods of 10 seconds.

Figure 6. Reflex decreases in mean arterial pressure (MAP, left) and heart rate (HR, right) in response to aortic depressor nerve (ADN) stimulation in mice anesthetized with pentobarbital (n=4) or ketamine/acepromazine (n=4). Data points represent means ± SE. * indicates a significant decrease from baseline, P<0.05. † indicates significant difference of responses in ketamine/acepromazine vs. pentobarbital-anesthetized mice, P<0.05. Baseline arterial pressure averaged 88 ± 1 and 86 ± 4 mmHg in pentobarbital and ketamine/acepromazine-anesthetized mice, respectively. Corresponding measurements of baseline heart rate averaged 519 ± 26 and 477 ± 9 beats/min in the two groups of mice.

Figure 7. Decreases in heart rate (HR) in response to stimulation of right vagus nerve efferents in mice anesthetized with pentobarbital (n=5) or ketamine/acepromazine (n=4). Data points represent means ± SE. * indicates a significant decrease from baseline in both groups of mice, P<0.05. Baseline heart rate averaged 494 ± 16 and 495 ± 14 beats/min in pentobarbital and ketamine/acepromazine-anesthetized mice, respectively.
Figure 1

**A**

Arterial Pressure (mm Hg)

ADNA (µV)

**B**

Arterial Pressure (mm Hg)

RSNA (µV)

0.2 s
Figure 2
Figure 3
Figure 4

A

Arterial Pressure (mm Hg)

ADNA (µV)

10 s

B

Arterial Pressure (mm Hg)

ADNA (µV)

0.2 s

Baseline

SNP

PE
Figure 6

![Graph showing changes in MAP and HR with ADN stimulation for different drugs.](image-url)
Figure 7

Vagus Stimulation (Hz)

Δ HR (beats/min)

0 5 10 15

-360

-270

-180

-90

0

Ketamine

Pentobarbital

*