Frequency-dependent baroreflex modulation of blood pressure and heart rate variability in conscious mice

Rubens Fazan, Jr., Mauro de Oliveira, Valdo José Dias da Silva, Luis Fernando Joaquim, Nicola Montano, Alberto Porta, Mark W. Chapleau, and Helio C. Salgado

1Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo; 2Department of Biological Sciences, School of Medicine of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil; 3Department of Clinical Sciences, Internal Medicine II, L. Sacco Hospital, University of Milan, Italy; and 4Departments of Internal Medicine and Physiology and Biophysics, University of Iowa and Veterans Affairs Medical Center, Iowa City, Iowa

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School of Medicine of Ribeirão Preto, University of São Paulo. Experiments were performed on male C57Bl/6J mice (28 ± 3 g). The mice were housed individually with free access to food and water and were maintained on a 10:12-h light-dark cycle. At the end of the experiments, the mice were killed with an intravenous overdose of pentobarbital sodium. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985; Office of Science and Health Reports, DRR/NIH, Bethesda, MD, 20892].

SAD. Mice were anesthetized with pentobarbital sodium (50 µg/g body wt) given intraperitoneally. SAD was performed as described by Krieger (22) for rats and adapted to mice. Briefly, each animal was placed in a supine position, and a ventral midline incision was made in the neck, exposing the carotid bifurcation. The superior cervical ganglion and superior laryngeal nerve were visualized under a surgical microscope, isolated, and removed. The adventitia and associated connective tissue were stripped from the carotid sinus region including the adjacent internal, external, occipital, and common carotid arteries. After this procedure was completed bilaterally, the incision was sutured closed. Sham-operated mice underwent a similar surgical procedure that involved exposure of the carotid bifurcation without damaging the innervation. All animals were allowed to recover for 24 h before carrying out the experiments. Successful baroreceptor denervation in SAD mice was confirmed by the minimal HR responses to increases and decreases in systolic AP evoked by intravenously administered phenylephrine (1 ng/g body wt) and nitroprusside (2 ng/g body wt), respectively (Fig. 1).

Blood pressure and HR recordings. Immediately after the SAD or sham surgery, polyethylene (PE-10) catheters were inserted in the left carotid artery and right jugular vein and exteriorized at the back of the mice. On the following day, mice were taken to the recording room at least 30 min before the beginning of the experiment, and a quiet environment was maintained to minimize stress. Despite the fact that insertion of a catheter could cause stress lasting for >24 h, on the day of the experiments, the mice were active and did not exhibit any visible sign of distress. The arterial catheter was connected to a pressure transducer (model P23 Gb; Statham) by means of a swivel (Instech Laboratories, Plymouth Meeting, PA) that allowed unrestrained movement of the mice without occlusion of the arterial line. Pulsatile AP was continuously sampled (4 kHz) using an IBM computer equipped with an analog-to-digital interface (Di220; Dataq, Akron, OH). The temperature inside the recording room was kept between 22 and 24°C. The files were stored, and the data were analyzed at a later time.

Experimental protocol. All experiments were carried out with the conscious, freely moving mice in individual cages, between 8:00 and 11:00 A.M. A total of 20 sham-operated and 21 SAD mice were studied. After basal pulsatile AP was recorded for ~30 min, one group of sham (n = 10) and one group of SAD (n = 6) mice received methyl atropine (1 µg·kg⁻¹·h⁻¹·iv), whereas separate groups of sham (n = 6) and SAD (n = 6) mice received propranolol (3 µg·g⁻¹·10 µl⁻¹·iv). AP was recorded for 30 min after drug injection.

Data analysis. Pulsatile AP recordings were analyzed by customized computer software designed to detect inflection points of a periodic wave. A graphic interface on the analysis software allowed visual inspection and manual editing of erroneously detected events. The beat-by-beat time series of systolic AP was generated. In addition, the time series of PI were obtained by the intervals between consecutive maximum values of the first derivative of the arterial pulse waveforms. All time series were obtained for each period of the experiment, i.e., before (basal) and after the administration of methyl atropine or propranolol.

From each recording period, the time series of PI and systolic AP were divided into contiguous segments of 350 beats, overlapped by one-half. After the calculations of mean and variance of each segment, they were submitted to a model-based autoregressive spectral analysis, and the power of the oscillatory components was quantified in the following two frequency bands: LF (0.1–1 Hz) and HF (1–5 Hz). Oscillations slower than 0.1 Hz were not quantified in this study. In one group of 10 mice (randomly chosen), we also generate series of diastolic pressure that were submitted to the same analysis protocol. The results of AP variability calculated from systolic or diastolic pressure were similar (data not shown).

To assess the sensitivity of the baroreflex for control of HR, a mathematical model based on bivariate (cross) autoregressive spectral analysis was applied to both systolic AP and PI variabilities, as described elsewhere (10, 37). With this approach, we computed the signal coherence, which describes the linear relationship between the variability in systolic AP and PI at each frequency and has values between 0 (no relationship) and 1 (maximum relationship), and we also computed the phase shift estimating the delay between the two signals at LF and HF bands. In line with previous studies (3, 33), baroreflex sensitivity was expressed as the square root of the ratio between PI and systolic AP powers when the coherence exceeded 0.5.

Statistical analysis. Results are presented as means ± SE. Baseline values of PI and systolic AP, as well as variance, and LF and HF power of PI and systolic AP, were compared between sham-operated and SAD mice using the nonparametric Mann-Whitney test. The same variables were compared before and after administration of methyl atropine or propranolol within each experimental group (sham and SAD) using the nonparametric Wilcoxon signed rank test. Differences were considered statistically significant at P < 0.05.

RESULTS

Systolic AP and PI variability in control vs. SAD mice. Representative tracings of AP and PI of one sham-operated and one SAD mouse over a prolonged time period (10 min) are
shown in Fig. 2. Basal values of systolic AP and PI, and their respective variances for control and SAD mice, are shown in Fig. 3 and Tables 1 and 2. Average systolic AP and PI were not significantly different in SAD and control mice. In contrast, systolic AP variability (variance) was markedly enhanced and PI variance significantly reduced in SAD mice. Inspection of the systolic AP and PI spectra reveals that SAD did not affect systolic AP and PI variability uniformly at all frequencies. Representative spectra of PI and systolic AP from one control and one SAD mouse are shown in Fig. 3. Two oscillatory rhythms at distinct frequencies are evident for both systolic AP and PI. The LF oscillation of these parameters ranged from 0.43 to 0.62 Hz, whereas the HF oscillation occurred within the respiratory frequency range of the mice (2.8–3.2 Hz). HF of systolic AP variability was significantly greater in SAD compared with control mice, whereas LF of systolic AP variability did not differ between the groups (Fig. 3). Conversely, LF PI variability was markedly reduced in SAD mice, whereas HF PI variability did not differ between the groups (Fig. 3).

Coherence and phase relationship between AP and PI oscillations. The LF oscillations in systolic AP and PI were coherent in control mice, with changes in systolic AP leading to changes in PI, but were not coherent in SAD mice. Representative coherence plots obtained by autoregressive cross spectral analysis between systolic AP and PI under basal conditions for one control mouse and one SAD mouse are shown in Fig. 4. Coherence values over the LF range exceeded 0.5 (0.68 ± 0.05) in 19 of 20 sham-operated control mice. The negative phase (−1.41 ± 0.06 radians) observed within the LF range indicates that systolic AP oscillations preceded PI oscillation. The phase lag averaged 591 ± 24 ms. The spontaneous baroreflex sensitivity, calculated from the square root of the ratio between PI and systolic AP powers, averaged 1.92 ± 0.14 ms/mmHg in these 19 control mice.

In contrast, as expected, none of the SAD mice exhibited coherence values >0.5 in the LF range (average = 0.08 ± 0.03). The lack of coherence between LF fluctuations in systolic AP and PI along with the minimal HR responses to

Fig. 2. Representative tracings of arterial pressure and pulse interval from one sham-operated and one SAD mouse over a time period of 10 min.

Fig. 3. Bar graphs showing basal values of systolic pressure (A), pulse interval (B), and their respective variance of sham-operated (open bars) and SAD (hatched bars) mice. *P < 0.05 compared with sham-operated mice.
Table 1. Mean values of pulse interval, and respective variance, LF, and HF powers before and after methyl atropine or propranolol, in both sham-operated and sinoaortic-denervated mice

<table>
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<tr>
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<th>Sham Operated</th>
<th>Sinoaortic Denervated</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td><strong>Atropine</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean, ms</td>
<td>113±5</td>
<td>99±3*</td>
</tr>
<tr>
<td>Variance, ms²</td>
<td>20.6±5.8</td>
<td>3.4±0.9*</td>
</tr>
<tr>
<td>LF, ms²</td>
<td>12.1±4.8</td>
<td>0.1±0.0*</td>
</tr>
<tr>
<td>LF nu, ms²</td>
<td>50.2±11.0</td>
<td>1.6±0.3*</td>
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<tr>
<td>HF, ms²</td>
<td>7.6±1.6</td>
<td>3.1±0.8*</td>
</tr>
<tr>
<td>HF nu, ms³</td>
<td>49.8±11.0</td>
<td>98.4±0.3*</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.82±0.48</td>
<td>0.02±0.00*</td>
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<tr>
<td><strong>Propranolol</strong></td>
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<tr>
<td>Mean, ms</td>
<td>112±4</td>
<td>135±5*</td>
</tr>
<tr>
<td>Variance, ms²</td>
<td>10.2±2.5</td>
<td>6.7±2.2*</td>
</tr>
<tr>
<td>LF, ms²</td>
<td>2.8±1.3</td>
<td>0.4±0.2*</td>
</tr>
<tr>
<td>LF nu, ms²</td>
<td>25.0±9.8</td>
<td>7.3±2.5*</td>
</tr>
<tr>
<td>HF, ms²</td>
<td>5.9±1.5</td>
<td>5.9±2.0</td>
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<tr>
<td>HF nu, ms³</td>
<td>75.0±9.8</td>
<td>92.7±2.5*</td>
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<tr>
<td>LF/HF</td>
<td>0.49±0.04</td>
<td>0.08±0.03</td>
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Values are means ± SE. LF, low frequency; HF, high frequency; nu, normalized units. *P < 0.05 compared with before administration of the autonomic blocker.

Table 2. Mean values of systolic arterial pressure, and respective variance, LF, and HF powers before and after atropine or propranolol, in both sham-operated and sinoaortic-denervated mice

<table>
<thead>
<tr>
<th></th>
<th>Sham Operated</th>
<th>Sinoaortic Denervated</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td><strong>Atropine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mmHg</td>
<td>133±7</td>
<td>133±6</td>
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<tr>
<td>Variance, mmHg²</td>
<td>9.8±1.4</td>
<td>11.8±1.8</td>
</tr>
<tr>
<td>LF, mmHg²</td>
<td>5.6±1.4</td>
<td>7.7±1.9</td>
</tr>
<tr>
<td>HF, mmHg²</td>
<td>3.1±0.3</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td><strong>Propranolol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mmHg</td>
<td>129±4</td>
<td>132±10</td>
</tr>
<tr>
<td>Variance, mmHg²</td>
<td>7.8±1.3</td>
<td>5.6±0.7</td>
</tr>
<tr>
<td>LF, mmHg²</td>
<td>5.3±1.4</td>
<td>3.5±0.7</td>
</tr>
<tr>
<td>HF, mmHg²</td>
<td>1.7±0.2</td>
<td>1.8±0.5</td>
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Values are means ± SE. *P < 0.05 compared with before administration of the autonomic blocker.

DISCUSSION

Although the importance of the baroreceptor reflex in buffering AP variability is universally accepted, the observation that its effectiveness varies depending on the frequency of the AP fluctuations is not widely recognized (7, 11, 17, 26, 44). To assess the baroreflex influence on AP variability at different frequencies in mice, we quantified systolic AP and PI variability using spectral analysis in control sham-operated and SAD mice, both before and after administration of methyl atropine and propranolol. The results indicate that 1) baroreflex modulation of PI contributes to LF, but not HF, PI variability in conscious mice; 2) the baroreflex modulation of PI at LF is mediated by both sympathetic and parasympathetic nervous systems; and 3) the baroreflex limits HF of systolic AP variability in mice despite the lack of baroreflex control of PI in this frequency range.

To our knowledge, this is the first study to examine the effects of acute (24-h) SAD on AP and PI in conscious mice. In our study, the resting mean level of AP was not significantly different in SAD mice compared with sham-operated mice 1 day postsurgery. This finding is in line with previous observations from conscious freely moving SAD animals that exhibited an exaggerated lability of AP without an increase in the average AP (2, 7, 9, 11, 17, 26, 31).

The absence of hypertension 1 day after SAD cannot be attributed to incomplete denervation. Baroreceptor denervation was confirmed by the marked attenuation of PI responses to phenylephrine- and nitroprusside-induced changes in AP (Fig. 1), the loss of coherence between LF oscillations in AP and PI, and the large increase in total AP variability (variance).

Baroreflex influence on PI and systolic AP variability. Calculations of “spontaneous baroreflex sensitivity” from naturally occurring spontaneous fluctuations in systolic AP and PI are increasingly being used to estimate baroreflex sensitivity in humans and animals, including mice (3, 10, 18, 26, 33, 37). Of course, oscillations of AP and HR need not necessarily involve the baroreflex, particularly in conscious animals and humans where a variety of central and nonbaroreflex mechanisms can potentially influence PI. Our finding that LF oscillations in systolic AP and PI are coherent in intact mice with changes in SAD mice, propranolol increased average PI to an even greater extent than in control mice (47.6 ± 2.6 vs. 22.5 ± 2.8 ms, P < 0.05) but did not influence any measure of PI variability (n = 6; Table 1 and Fig. 5).

Neither the mean level of AP nor its variability was affected by methyl atropine or propranolol in control mice (Table 2). In SAD mice, atropine did not affect mean AP or its variability (Table 2). In contrast, propranolol significantly decreased total AP variance and the corresponding LF and HF power spectral densities in SAD mice (Table 2).
systolic AP leading to changes in PI confirms previous studies (18) and suggests that the changes in PI are driven, at least in part, by changes in baroreceptor activity. The marked decrease in LF PI variability and loss of coherence between LF oscillations between systolic AP and PI after SAD strengthens the interpretation that this measure of spontaneous baroreflex sensitivity is valid in mice. Similar results were obtained in conscious rats (7). Moreover, the remarkable reduction of the LF component of PI variability demonstrated the important contribution of the baroreflex in the power of the slow oscillation of HR.

In contrast, although HF oscillations in systolic AP and PI are coherent, the absence of an appropriate phase lag between systolic AP and PI suggests that the changes in PI are not mediated by the baroreflex. As observed in rats (7), the failure of SAD to disrupt the coherence between HF of systolic AP and PI oscillations strongly suggests that oscillations in systolic AP and PI at this frequency should not be used to calculate baroreflex sensitivity in mice.

One might expect that the engagement of baroreflex control of PI (HR) at LF would buffer LF oscillations in systolic AP, predicting that LF of systolic AP variability would increase after SAD. To the contrary, LF of systolic AP variability did not differ in SAD vs. intact mice (Table 2 and Fig. 4). This finding, and the fact that methyl atropine reduces LF of PI variability without affecting LF of systolic AP variability (Tables 1 and 2 and Ref. 18), suggests that baroreflex-mediated changes in HR do not exert a major effect on AP.

In previous studies of rats and cats, SAD paradoxically decreased LF AP variability (7, 26). It has been proposed that a resonance phenomenon in the arterial baroreflex loop may actually cause AP variability within the LF range (5, 7, 10, 11). A time delay in the response of negative feedback systems generates oscillations when the response delay is such that the output becomes in phase with the input, thereby creating a positive feedback (5). The failure to observe a decrease in LF systolic AP variability after SAD in our study suggests that the resonance phenomenon may not be a major contributor to LF oscillations in AP in mice.

We observed that SAD increased HF of systolic AP variability in mice without significantly altering HF PI variability (Fig. 4). HF AP variability corresponding to the respiratory rate has been reported to be increased in rats (17) and unchanged in rats and cats (7, 11, 26) after SAD. The finding of increased systolic AP variability in mice suggests that the baroreflex normally buffers these HF fluctuations in AP. Nevertheless,
sympathetic-mediated changes in vascular resistance generally occur too slowly to modulate AP at such a high frequency. In rats, the maximum frequency of sympathetic-mediated oscillations in vascular resistance is \( \approx 1.0 \) Hz (15, 38, 39). The possibility that neurovascular transmission occurs much more rapidly in mice than in larger species has not been investigated to our knowledge.

Other potential consequences of SAD, e.g., changes in locomotor activity or respiration, also need to be considered. Increased depth of breathing and the corresponding greater fluctuations in intrathoracic pressure could potentially enhance HF oscillations in AP (19, 32). SAD did not significantly change respiratory frequency or tidal volume in conscious rats (28). Interestingly, SAD rats exhibited occasional deep breaths at 60- to 100-s intervals associated with marked transient reductions in AP (28). Thus respiratory changes may contribute to total AP variability but are unlikely to influence HF AP oscillations in SAD rats. Future studies are needed to assess the potential contribution of changes in respiration to HF of systolic AP variability in mice.

Parasympathetic vs. sympathetic control of HR. The relative roles of the parasympathetic vs. sympathetic nervous systems in control of HR (i.e., PI) in awake mice remain controversial. In our study, the resting mean level of PI was significantly decreased by methyl atropine (\(-14\) ms) and increased by propranolol (\(+23\) ms), indicating that both parasympathetic and sympathetic activity tonically modulate PI under the conditions of these experiments, with a sympathetic predominance. The propranolol-induced increase in PI equates to a decrease in HR of 92 beats/min, which is comparable to that observed in previous studies in mice (14, 16, 21, 27, 36, 41). In contrast to the consistent finding of cardiosympathetic tone at rest, the contribution of vagal tone to resting HR has varied considerably among previous studies, with reports of minimal (<10 beats/min; see Refs. 14, 18, and 21), moderate (20–60 beats/min; see Ref. 16), and substantial (>70 beats/min; see Refs. 34, 36, 41, and the present study) HR responses to atropine. The reasons for the variable HR responses to atropine are not clear but may involve differences in recording conditions, genetic background of the mice, and time postsurgery, along with other factors.

The variable and often modest increases in mean HR in response to methyl atropine have led to an underappreciation of the importance of parasympathetic nerve activity in mice. Methyl atropine markedly decreased total, LF, and HF PI variability in our experiments, confirming the results of previous studies (14, 18, 21, 34). In contrast, propranolol produced a much more modest decrease in total and LF PI variability and did not affect HF oscillations in PI. Other investigators have noted variable decreases (16) and increases (14, 18, 21, 27) in PI variability after \( \beta \)-receptor blockade in mice. The results, taken together, suggest that the parasympathetic nervous system plays the predominant role in the acute modulation of PI in conscious mice under resting conditions. Its contribution to the HF oscillations in PI accompanying respiration, i.e., the respiratory sinus arrhythmia, is well documented in a variety of animal species and humans (7, 25, 32). Parasympathetic modulation contributes to LF PI variability in other species, including humans, but the contribution is generally relatively small.

Consequently, the ratio of the normalized spectral powers of the LF and HF components of PI variability (LF/HF) provides a measure of the relative contribution of sympathetic and parasympathetic modulation of HR variability (25, 29, 45). As expected, in our experiments, propranolol decreased the normalized LF-to-HF ratio, appropriately reflecting the shift in sympathovagal balance. On the other hand, the marked decline in both absolute and normalized LF PI variability after methyl atropine resulted in a decrease in the LF-to-HF ratio, an effect opposite to that predicted. These results obtained in mice differ
CARDIOVASCULAR VARIABILITY IN ACUTE SAD MICE

from those obtained in rats (7, 12, 19, 37), dogs (1, 32), and humans (25, 30). The marked decrease in LF PI variability after methyl atropine may indeed reflect blockade of HR responses to fluctuations in vagal efferent nerve activity. Alternatively, the decrease in PI variability may be an indirect consequence of the very high mean HR in the mouse after administration of methyl atropine (>600 beats/min). The LF-to-HF ratio fails to accurately reflect sympathovagal balance in other states associated with very high HR, such as exercise and heart failure (33, 42).

The finding that drug-induced increases in AP, known to elicit baroreflex-mediated activation of parasympathetic activity and inhibition of sympathetic activity, increase LF PI variability in mice (14, 21) argues in favor of the hypothesis that LF PI oscillations are driven primarily by fluctuations in parasympathetic nerve activity. Our finding that SAD completely eliminates parasympathetic modulation of baseline mean PI and total LF and HF PI variability is consistent with this hypothesis. Interestingly, the sympathetic modulation of PI variability at LF was abolished after SAD, despite an increase in tonic sympathetic drive to the heart. This result leaves open the possibility that the sympathetic nervous system may also contribute to baroreflex-mediated LF oscillations in PI in conscious mice.

One might expect that the decrease in parasympathetic tone and increase in sympathetic tone after SAD would translate into an elevated baseline HR. To the contrary, HR was not significantly different in control and SAD mice, suggesting that SAD decreases the intrinsic HR in mice. A reduction of intrinsic HR resulting from SAD was previously observed in rats (43).

In control mice, the methyl atropine- and propranolol-induced changes in PI are not secondary to changes in systolic AP. Neither autonomic blocker altered any measure of AP variability in intact mice. In contrast, after SAD, propranolol significantly reduced systolic AP variance and both LF and HF oscillations in systolic AP (Table 2). The mechanism of these changes is unclear. Propranolol did not influence AP variability in SAD rats (46).

ACKNOWLEDGMENTS

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REFERENCES


