

Clonidine Improves Spontaneous Baroreflex Sensitivity in Conscious Mice Through Parasympathetic Activation

Jens Tank, Jens Jordan, André Diedrich, Michael Obst, Ralph Plehm, Friedrich C. Luft, Volkmar Gross

Abstract— α -2 Adrenoceptors are important in baroreflex regulation. We tested the impact of α -2 adrenoceptors on heart rate variability (HRV) and spontaneous baroreflex sensitivity (BRS) in conscious mice with telemetry (TA11PA-C20). Baseline beat-to-beat measurements (2 hours between 8:00 AM to 12:00 PM) were compared with measurements after intraperitoneal α -2 adrenoceptor blockade (yohimbine 2 mg/kg) and α -2 adrenoceptor stimulation (clonidine 1, 10, and 50 mg/kg). Blood pressure (BP) was $128 \pm 6/87 \pm 6$ mm Hg and heart rate (HR) was 548 ± 18 bpm at baseline. BRS, calculated with the cross-spectral method, was 1.2 ± 0.1 ms/mm Hg at baseline. BP increased $20 \pm 2/13 \pm 2$ mm Hg with yohimbine. HR increased by 158 ± 23 bpm. BRS did not change. BP decreased $16 \pm 7/5 \pm 4$ mm Hg with 1 mg/kg of clonidine and did not change with a higher dose. HR decreased with clonidine (176 ± 28 , 351 ± 21 , 310 ± 29 bpm during 1, 10, and 50 mg/kg of clonidine, $P < 0.01$). HRV (total power = 4629 ± 465 , 7002 ± 440 , and 6452 ± 341 ms² during 1, 10, and 50 mg/kg of clonidine, $P < 0.01$) and BRS were profoundly increased with clonidine (14 ± 1 , 13 ± 1 , and 10 ± 1 ms/mm Hg, $P < 0.01$). The effects of clonidine were abolished with atropine (2 mg/kg plus 50 mg/kg of clonidine) but not with metoprolol (4 mg/kg plus 50 mg/kg of clonidine). These data suggest that α -2 adrenoceptors exert a regulatory influence on autonomic cardiovascular control and baroreflex function. The effect of clonidine on baroreflex HR regulation is mediated by the parasympathetic nervous system. These murine data fit well with recent human observations regarding parasympathetic activation via α -2 adrenoceptors. (*Hypertension*. 2004;43:1042-1047.)

Key Words: antihypertensive agents ■ blood pressure ■ heart rate ■ baroreflex

The arterial baroreflex acts as an effective buffer of short-term blood pressure (BP) fluctuations and prevents excessive BP swings.¹ Adrenergic mechanisms in the brain have a major contribution to baroreflex function. α -2 Adrenoceptors may be particularly important in central nervous baroreflex regulation, both in animals and in humans.²⁻⁵ Nonselective stimulation of α -2 adrenoceptors with clonidine elicited a depressor effect and enhanced baroreflex heart rate (HR) gain in human studies,² in rabbits,⁶ and in dogs.⁷ However, other studies in rats⁸ and in humans⁹ suggested that clonidine does not improve baroreflex HR gain. Species differences and different study protocols including anesthetic states may contribute to this controversy. We and others have recently shown the possibility to assess standard time and frequency domain measures of heart rate variability (HRV) and baroreflex sensitivity (BRS) testing by telemetric techniques in conscious mice.¹⁰⁻¹³ Therefore, the mouse is a well-suited animal model to further elucidate the functional role of α -2 adrenoceptors in more detail.¹⁴⁻¹⁶ The model may be particularly relevant given the large number of genetically modified mouse strains. Data on the effects of α -2 adrenoceptor manipulation on HRV and BRS in freely moving mice

are still lacking. The aim of this study was to test the effects of the α -2 adrenoceptor blocker, yohimbine, and of the α -2 adrenoceptor agonist, clonidine, on spontaneous HRV and BRS in freely moving wild-type mice. We tested the contribution of sympathetic and parasympathetic responses to these changes using a combination of physiological and pharmacological methods.

Methods

Animals

Experiments were performed on adult male mice (129SvJ) bred in our animal facility. The animals were allowed free access to standard chow (0.25% sodium; SNIFF Spezialitäten GmbH, Soest, Germany) and drinking water ad libitum. Thirteen mice were instrumented as described. All drug protocols were performed on at least 5 mice in each experiment. Some mice received more than 1 drug. At least 48 hours transpired between experiments. The protocol was approved by the local council on animal care and corresponds to requirements of the American Physiological Society.

Surgery

Mice were anesthetized with isoflurane (CuraMed Pharma-GmbH, Karlsruhe, Germany). The catheter of the TA11PA-C20 BP device (Data Sciences International, St. Paul, Minn) was placed into the

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right femoral artery and advanced into the abdominal aorta below the branching of the renal vessels. The transmitter body was placed into a subcutaneous pouch formed along of the flank of the animal.

Protocol

The mice were synchronized to a light–dark schedule of 12–12 hours, with lights on at 06:00 hours. First recordings were started at least 1 week after surgery, when the mice had regained their circadian BP and HR rhythm, and the surgery and anesthesia-dependent initial changes were followed by stable values. Baseline measurements were compared with measurements after intraperitoneal injection of the α -2 adrenoreceptor blocker, yohimbine (2 mg/kg), and of the α -2 adrenoreceptor agonist, clonidine (1, 10, and 50 mg/kg). In addition, the mice were given metoprolol (4 mg/kg), atropine (2 mg/kg), clonidine (50 mg/kg) plus atropine (2 mg/kg), or clonidine (50 mg/kg) plus metoprolol (4 mg/kg) on separate days. All drugs were administered by intraperitoneal injections; 0.9% NaCl solution was used as vehicle. Intraperitoneal injection of adequate volumes of 0.9% NaCl had no sustained cardiovascular effect.

Ancillary Experiments

In 3 adult male 129SvJ mice, we measured the respiratory rate (breaths/min) directly in a plethysmographic chamber.

Data Acquisition and Analysis

The data from the TA11PA-C20 device were transmitted via radiofrequency signals to a receiver below the home cage and thereafter collected (Dataquest software, sample rate 1 kHz). BP waveforms were stored 1 hour before and 1 hour after intraperitoneal injections, between 8:00 AM and 12:00 PM. Beat-by-beat BP and HR values were derived from these continuously recorded data.

Spectral Analysis and Baroreflex Sensitivity

The baroreceptor HR reflex was calculated using the sequence method over the whole 2-hour period.^{13,17,18} Briefly, the spontaneous BRS was calculated as the slope of the linear regression lines between the systolic blood pressure (SBP) and the subsequent R-R intervals with a delay of 1 heart beat using the sequence technique. Sequences with at least 3 intervals, 0.5 mm Hg BP changes, and 5-ms R-R interval changes were analyzed. BRS was calculated as the mean value of all slopes obtained.

HRV was analyzed in the time and frequency domain. As time domain method, we used the square root of the mean squared differences of successive intervals, which reflects short-term variations of HR. The power spectra of SBP and R-R interval time series (segment length 512 beats, resampling with 12 Hz) and the cross-spectra^{10–13,19} were calculated using fast Fourier transformation. The baroreflex gain was determined as mean value of the transfer function in low-frequency (LF) and high-frequency (HF) bands. The frequency bands were adapted for analysis in mice considering the ranges of HR and breathing frequencies (very LF=0.015 to 0.25 Hz, LF=0.25 to 1.0 Hz, HF=1.0 to 6.0 Hz). BRS was considered significant if the coherence in the analyzed frequency band was >0.8. The data analysis was performed by use of PVwave software (VisualNumerics, Houston, Tex). Five representative 512-beats intervals were chosen according to the following criteria: (1) steady-state conditions; (2) no large sudden changes of BP; and (3) no artifacts. Data from these intervals were averaged.

Statistics

All data are expressed as mean \pm SEM. ANOVA testing for repeated measures was used for multiple comparisons between the changes induced by different drugs or doses. The 1-hour measurement during each intervention was compared with the 1-one hour measurement at baseline by the paired *t* test. A value for $P < 0.05$ was considered significant.

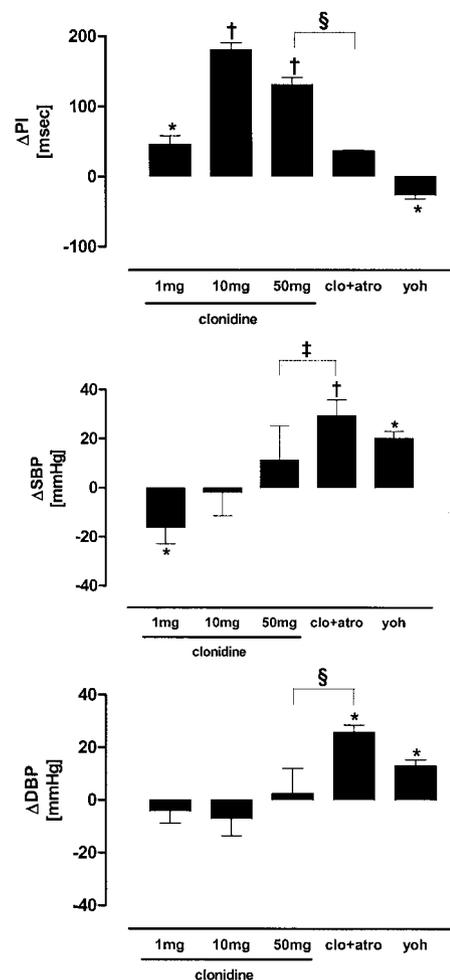


Figure 1. Changes of pulse interval (Δ PI, top), systolic blood pressure (Δ SBP, middle), and diastolic blood pressure (Δ DBP, bottom) during clonidine (1 mg/kg, 10 mg/kg, and 50 mg/kg IP), during clonidine (50 mg/kg) with atropine (2 mg/kg, clo+atro), and during yohimbine (4 mg/kg) versus baseline. Clonidine profoundly increased PI. BP decreased only with low-dose clonidine (*significant changes, $P < 0.05$, † $P < 0.01$, paired *t* test, compared with baseline). The clonidine effects were abolished with atropine (‡significance, $P < 0.05$, § $P < 0.01$, 1-way ANOVA for repeated measures).

Results

BP and HR

BP was $128 \pm 6/87 \pm 6$ mm Hg and HR was 548 ± 18 bpm at baseline. With clonidine 1 mg/kg, HR decreased by 176 ± 28 bpm ($P < 0.01$). The maximum HR decrease (Figure 1, top) was achieved with 10 mg/kg of clonidine (-351 ± 21 bpm, $P < 0.01$). BP decreased $16 \pm 7/5 \pm 4$ mm Hg with 1 mg/kg of clonidine but did not change with higher clonidine doses. SBP (Figure 1, middle) increased markedly when clonidine was administered together with atropine ($P < 0.01$). Diastolic blood pressure (DBP) (Figure 1, bottom) did not change significantly with any clonidine dose and increased during clonidine plus atropine ($P < 0.01$). In contrast, with yohimbine, HR increased 158 ± 23 bpm and BP increased $20 \pm 2/13 \pm 2$ mm Hg. HR and BP did not change significantly with atropine (Δ HR = 22 ± 4 bpm, Δ BP = $3 \pm 1/1 \pm 1$ mm Hg) or with metoprolol (Δ HR = -16 ± 4 bpm, Δ BP = $-7 \pm 2/$

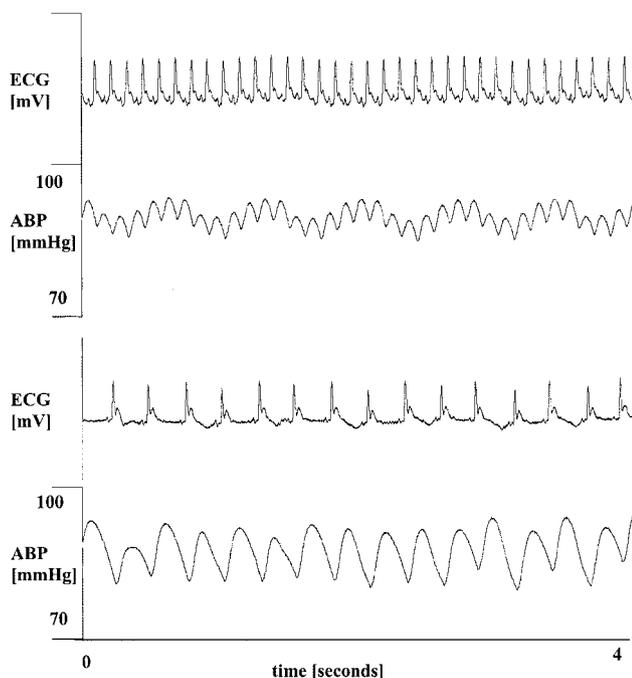


Figure 2. Original tracings of the ECG and arterial blood pressure (ABP) (carotid artery) measured in 1 animal during isoflurane anesthesia (1.8%) at baseline (top) and during clonidine (10 mg/kg, IP, bottom). Note the profound decrease in HR with clonidine (461 bpm versus 231 bpm) and no premature beats.

-6 ± 2 mm Hg). We validated these measurements with ECG and intra-arterial BP recordings in 3 anesthetized mice (Figure 2). In these experiments shown with clonidine 10 mg/kg, we observed that mice remained in sinus rhythm during the experiments (bottom), whereas the HR decreased markedly. Occasional atrioventricular blockade was observed with high-dose clonidine (50 mg/kg) only.

Heart Rate Variability

The increase in pulse interval variability with clonidine was profound (Figure 3 in a representative animal). Baseline pulse interval was narrow (top). With clonidine (50 mg/kg), the pulse interval increased to a range of ≈ 250 to almost 500 ms (middle). Atropine blocked the response completely (bottom). The root mean square of successive differences of pulse intervals was 3 ± 1 ms at baseline and increased with clonidine (105 ± 4 , 161 ± 6 , and 148 ± 8 ms, $P < 0.01$). We used spectral analysis to obtain deeper insight into the frequency components of these HRV changes (Figure 4). The power spectral density increased during clonidine in the low-frequency (top) ($\Delta\text{LF}-\text{pulse interval [PI]} = 2708 \pm 408$, 3831 ± 312 , and 3576 ± 364 ms², $P < 0.01$) and in the high-frequency (middle) band ($\Delta\text{HF}-\text{PI} = 1165 \pm 89$, 2202 ± 170 , and 2212 ± 226 ms², $P < 0.01$) compared with baseline values. Atropine plus clonidine not only abolished the effects of clonidine on HRV but also reduced the LF power. HRV in the LF band and in the HF band decreased during yohimbine ($\Delta\text{HF}-\text{PI} = -1.1 \pm 0.2$, $\Delta\text{LF}-\text{PI} = -1.3 \pm 0.6$ ms², $P < 0.05$). The effects of metoprolol on HRV data were not significant ($\Delta\text{HF}-\text{PI} = -0.2 \pm 0.4$, $\Delta\text{LF}-\text{PI} = -0.7 \pm 0.5$ ms², NS). Metoprolol did not abolish the effects of clonidine on HRV.

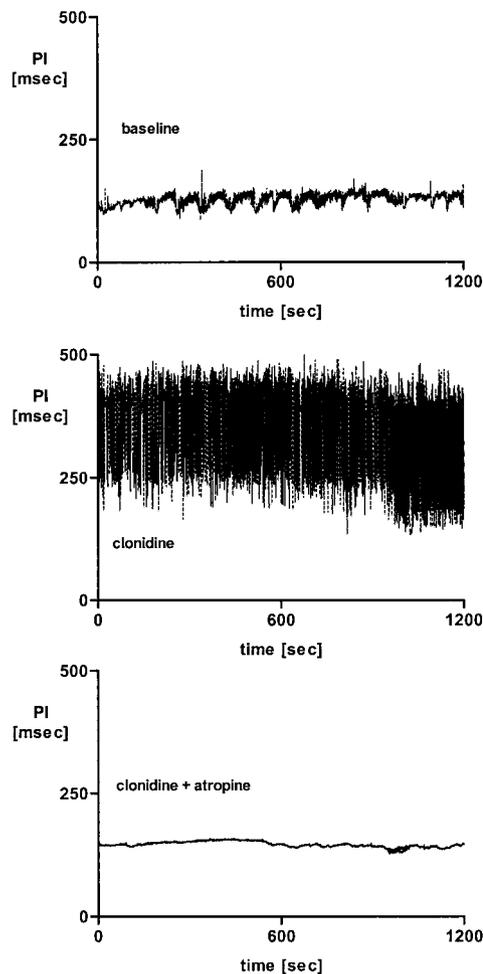


Figure 3. Original tracings of PI measured in 1 animal at baseline (top), after clonidine 50 mg/kg IP (middle), and after clonidine (50 mg/kg) plus atropine (2 mg/kg, bottom). Note the profound increase in HRV with clonidine, which was abolished with atropine.

Interestingly, the LF/HF ratio (bottom), which has been suggested to reflect the sympathovagal balance in humans,²⁰ increased during clonidine ($\Delta\text{LF}/\text{HF} = 1.59 \pm 0.45$, 0.89 ± 0.29 , and 0.72 ± 0.4 , $P < 0.05$). However the LF/HF ratio decreased during metoprolol ($\Delta\text{LF}/\text{HF} = -0.33 \pm 0.15$, $P < 0.05$).

Spontaneous Baroreflex Sensitivity

BRS, calculated by the sequence method for increasing systolic BP, was 0.8 ± 0.2 ms/mm Hg at baseline and was profoundly increased with clonidine (13 ± 3 , 18 ± 2 , and 16 ± 2 ms/mm Hg during 1, 10, and 50 mg/kg doses of the drug, $P < 0.01$). Clonidine plus atropine showed no change in BRS (0.13 ± 0.1 ms/mm Hg). Results obtained by using sequences with a correlation coefficient > 0.85 were similar despite the fact that the number of sequences decreased by $\approx 30\%$. The same result was obtained for sequences of decreasing systolic BP.

BRS calculated using cross-spectral analysis and the mean values of the transfer function in the LF band (BRS-LF, Figure 5) was 1.2 ± 0.1 ms/mm Hg at baseline and increased by 14 ± 1 , 13 ± 1 , and 10 ± 1 ms/mm Hg during 1, 10, and 50

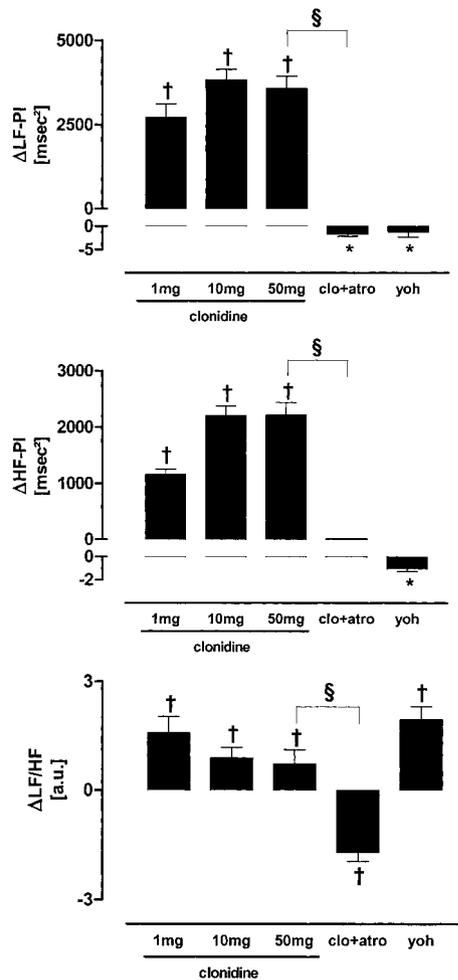


Figure 4. Changes of LF power of HRV (Δ LF-PI, top), HF power (Δ HF-PI, middle), and of LF-to-HF ratio (Δ LF/HF, bottom) during clonidine (1 mg/kg, 10 mg/kg, and 50 mg/kg IP), during clonidine (50 mg/kg) with atropine (2 mg/kg, clo+atro), and during yohimbine (4 mg/kg) versus baseline. Clonidine profoundly increased HRV in the LF and HF ranges. The changes in LF power were more pronounced according to the LF/HF ratio (*significant changes, $P < 0.05$, † $P < 0.01$, paired t test, compared with baseline). The clonidine effects were abolished with atropine (§significance, $P < 0.01$, 1-way ANOVA for repeated measures).

mg/kg of clonidine ($P < 0.01$). The clonidine-induced change in BRS was abolished with atropine (2 mg/kg) but not with metoprolol (4 mg/kg). BRS decreased with yohimbine ($P < 0.05$).

Respiratory Rate

The respiratory rate, as determined in the plethysmography chamber, was 210/min before clonidine. With clonidine (50 mg/kg), breathing rate was 200/min. Thus, clonidine had very little effect on the respiratory rate.

Discussion

We measured the effect of α -2 adrenoreceptor stimulation with clonidine and of α -2 adrenoreceptor blockade with yohimbine on spontaneous HRV and on BRS in freely moving mice. The important finding in our study is that clonidine profoundly augmented HRV and spontaneous BRS. Atropine abolished

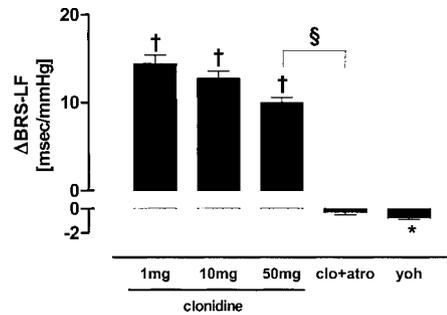


Figure 5. Changes in spontaneous baroreflex sensitivity (Δ BRS-LF) during clonidine (1 mg/kg, 10 mg/kg, and 50 mg/kg IP), during clonidine (50 mg/kg) with atropine (2 mg/kg, clo+atro), and during yohimbine (4 mg/kg) versus baseline. BRS was calculated as the mean value of the transfer function in the LF using cross-spectral analysis. BRS-LF was increased with clonidine (*significant changes, $P < 0.05$, † $P < 0.01$, paired t test, compared with baseline). The clonidine effect was abolished with atropine (§significance, $P < 0.01$, 1-way ANOVA for repeated measures).

the effects of clonidine on HRV and on BRS. In contrast, yohimbine decreased HRV and BRS. Our findings suggest that the clonidine-induced changes in HRV and in BRS are mainly explained by cardiac parasympathetic activation.

Clonidine is a widely used centrally acting antihypertensive drug. At low doses, clonidine acts mainly centrally through stimulation of α -2 adrenoreceptors to produce hypotensive effects. Approximately 90% of mouse brain α -2 adrenoreceptors belong to the α -2A subtype.²¹ Clonidine also decreases norepinephrine release from postganglionic adrenergic neurons through stimulation of presynaptic α -2 adrenoreceptors. The clonidine-induced depressor effect is sometimes preceded by a short initial increase in BP.²² The phenomenon is explained by stimulation of postsynaptic vascular α -2 adrenoreceptors and, perhaps, also α -1 adrenoreceptors.^{23–24} This peripheral effect may dominate over the sympatholytic effect when larger clonidine doses are used. At these doses, BP may not change or may even increase. Our data suggest that in freely moving mice, the central sympatholytic effect of clonidine dominates at doses of 1 mg/kg IP. BP decreases. At higher doses (10, 50 mg/kg), the sympatholytic effect is offset in part by stimulation of peripheral α -1 adrenoreceptors and postsynaptic α -2 adrenoreceptors. These findings may establish a useful dose range for future studies in freely moving mice.

We observed profound changes in HR regulation with clonidine and opposite changes with yohimbine. We used time and frequency domain measures of HRV using telemetric techniques to further elucidate the role of α -2 adrenergic mechanisms in HR regulation. Experience with this technique in the mouse model is limited. Recent pharmacological studies demonstrated that in mice, HRV reflects autonomic cardiovascular control despite the 10-fold higher HR in mice compared with humans.^{10–12,25} However, the rather small changes in HR regulation with atropine and with metoprolol suggest that cardiac sympathetic and cardiac parasympathetic tone at “rest” may be relatively low. Nevertheless, HRV analysis appears to be useful to monitor changes in autonomic HR regulation.

HRV in the time and in the frequency domain increased profoundly with clonidine. The response was obliterated with atropine. Remarkably, the HRV increases were quantitatively comparable to changes induced by direct cholinergic stimulation with carbachol.²⁶ In humans, HRV in the HF range is predominantly mediated by the parasympathetic nervous system. The LF component is under both parasympathetic and sympathetic control. Therefore, some investigators suggested that the LF/HF ratio provides a measure of sympathovagal balance in humans.²⁰ The contribution of parasympathetic and sympathetic activity to each frequency range appears to be different in mice.²⁵ We found a more pronounced increase in LF power than in HF power with clonidine, which led to a paradoxical increase in the LF/HF ratio. Normally, the breathing frequency fluctuates considerably in mice peaking at ≈ 3.5 Hz (ie, not in LF range). We excluded that HRV changes with clonidine are caused by changes in the breathing frequency. Atropine not only abolished the clonidine-induced changes in LF oscillations but also decreased the LF power of HRV. Atropine abolished the clonidine-induced changes in HF oscillations. In contrast, metoprolol had a minimal effect on the clonidine-induced change in HRV. Thus, the results suggest that the LF component of HRV in mice is mainly under parasympathetic control. Furthermore, the LF/HF ratio does not provide a measure of "sympathovagal" balance in mice.

The clonidine-induced changes in HRV were associated with a profound increase in BRS in freely moving mice. The sequence technique may be susceptible to breathing-induced mechanical effects. However, cross-spectral analysis of HR in the LF range showed very similar changes in baroreflex function. The atropine data suggest that these changes in baroreflex HR regulation are mediated by the parasympathetic nervous system.

The mechanisms by which α -2 adrenoceptor stimulation increases parasympathetic HR modulation and BRS are not known. One possible explanation is that a peripheral pressor effect of clonidine caused a compensatory baroreflex mediated increase in parasympathetic activity. However, this mechanism cannot explain the HR response to lower doses of clonidine, which elicited a depressor response. It is also possible that clonidine directly influences vagal nuclei in the brain stem. α -2 adrenoceptors are highly expressed in vagus motor nuclei in animals and in humans.²⁷ Sleight et al concluded from studies in rabbits that bradycardia caused by clonidine may be caused by additional action on the baroreceptors themselves.²

We are not the first to show that the α -2A adrenoceptor subtype is responsible for sympathoinhibition in mice. Mice in which the gene for this adrenoceptor subtype was disrupted had higher systolic BPs and HRs and higher norepinephrine values than did wild-type mice.⁵ When these mice were subjected to reduced renal mass and given saline as drinking water, the gene-deleted mice became hypertensive much faster and exhibited a greater increase in norepinephrine and epinephrine values than control mice.²⁸ In a further study, the group found that the α -2B adrenoceptor subtype gene-deleted mice were particularly resistant to the BP-raising effects of salt.²⁹ These studies underscore the importance of α -2 adre-

noceptors in cardiovascular regulation, particularly under pathological conditions.

Perspectives

Our study suggests that stimulation of α -2 adrenoceptors induces a major increase in HRV and in BRS in freely moving mice through parasympathetic activation. One implication of our study is that clonidine may be a useful experimental tool to study "parasympathetic reserve" in mice. Given the importance of HRV and BRS as a prognostic marker in human cardiovascular disease, similar mechanisms should be explored in humans. Our presented findings caused us to conduct analogous studies in humans and we were interested to find responses similar in kind and in degree.²⁸ Studies in mice with different α -2 adrenoceptor subtype gene deletions are clearly relevant, as earlier studies showed.^{29–30} Finally, our observation that combination of clonidine and atropine resulted in a substantial pressor response suggests that parasympathetic activity can be an important determinant of BP. Recent studies in humans support the idea that this notion has distinct therapeutic implications.³¹

Acknowledgments

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