Heart Rate Variability and Baroreflex Function in AT₂ Receptor-Disrupted Mice

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

Abstract—We adapted telemetry and sequence analysis employed in humans to mice and measured heart rate variability and the spontaneous baroreflex sensitivity in angiotensin II type 2 (AT₂) receptor–deleted (AT₂−/−) and wild-type (AT₂+/+) mice with either deoxycorticosterone acetate (DOCA)-salt hypertension or NO-nitro-L-arginine methylester hydrochloride (L-NAME) hypertension. Mean arterial pressure leveled during the day at 101±1 mm Hg and during the night at 109±1 mm Hg in AT₂ receptor–deleted mice, compared with 98±2 mm Hg (day) and 104±2 mm Hg (night) in wild-type mice. Mean arterial pressure increased in AT₂ receptor–deleted mice with L-NAME to 114±1 mm Hg (day) and 121±1 mm Hg (night), compared with 105±2 mm Hg (day) and 111±2 mm Hg (night), respectively. DOCA-salt also increased day and night blood pressures in AT₂ receptor–deleted mice to a greater degree than in wild-type mice. Heart rate variability in the time and frequency domain was not different between AT₂ receptor–deleted mice and AT₂ receptor–deleted mice at baseline. Systolic blood pressure variability in the low frequency band was lower in AT₂ receptor–deleted mice (0.6±0.1 ms² versus 3.9±1.3 ms²) than in wild-type mice. Baroreceptor-heart rate reflex sensitivity was significantly increased in AT₂ receptor–deleted mice compared with wild-type mice (3.4±0.6 versus 2.1±0.5 ms²/mm Hg). These differences remained after DOCA-salt and L-NAME treatments. We conclude that activation of the AT₂ receptor impairs arterial baroreceptor reflex function, probably by a central action. These data support the existence of an inhibitory central effect of the AT₂ receptor on baroreflex function. (Hypertension. 2002; 40:207-213.)

Key Words: mice ■ receptors, angiotensin II ■ baroreflex ■ L-NAME ■ deoxycorticosterone

Angiotensin II (Ang II)–related effects are mediated by at least 2 receptor isoforms (AT₁ and AT₂). The AT₁ receptor mediates vasoconstriction, aldosterone secretion, regulation of glomerular and renal tubular function, and cardiovascular hypertrophy. Although not yet as clearly delineated as the AT₁ receptor, the AT₂ receptor may be responsible for counterregulating the effects induced by the AT₁ receptor.¹ In accord with this view, disruption of the AT₂ receptor gene– disrupted (AT₂−/−) mice to study blood pressure and continuous spontaneous blood pressure and heart rate fluctuations allows the assessment of baroreflex function without invasive or provocative interventions. We adapted telemetry to AT₂ receptor

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were used to capture interrelationships between parameters in the further in DOCA-salt hypertensive mice. The correlation coefficients were used only values calculated with this delay for further analysis. Because the number of sequences calculated as the slope of the linear regression lines between the SBP and the subsequent RR intervals, with a delay of 0, 1, or 2 heartbeats, found was highest for the delay of 1 heartbeat in both strains, we using the sequence technique. Because the number of sequences and the subsequent RR intervals, with a delay of 0, 1, or 2 heartbeats, calculated as the slope of the linear regression lines between the SBP and the subsequent RR intervals, with a delay of 0, 1, or 2 heartbeats, found was highest for the delay of 1 heartbeat in both strains, we using the sequence technique. Because the number of sequences and the subsequent RR intervals, with a delay of 0, 1, or 2 heartbeats, found was highest for the delay of 1 heartbeat in both strains, we using the sequence technique. Because the number of sequences found was highest for the delay of 1 heartbeat in both strains, we used only values calculated with this delay for further analysis. With at least 3 intervals, 0.5 mm Hg blood pressure changes, and 5 ms RR interval changes were analyzed only if the correlation coefficients were >0.85. BRS was calculated as the mean value of the significant slopes obtained.

Power spectral analysis has provided useful information about the temporal fluctuations of different hemodynamic parameters such as heart rate variability in animal and human studies. Cross spectra were used to capture interrelationships between parameters in the time and frequency domain. Therefore, we calculated the power spectra of SBP and RR interval time series using fast Fourier transformation (segment length 32 s, resampling with 4 Hz, resolution 0.004 Hz, mean values of 3 to 5 segments) and the cross spectra. The baroreflex gain was determined as mean value of the transfer function in the very low, low, and high frequency bands (VLF = 0.015 to 0.250 Hz; LF = 0.250 to 0.600 Hz; HF = 1.0 Hz). BRS was considered significant if the coherence in the analyzed frequency band was >0.8. The frequency bands were adapted for analysis in mice considering the ranges of heart rate and breathing frequencies. The data analysis was performed by use of PV-WAVE software (VisualNumerics). Representative 256 seconds were chosen according to the following criteria: (1) steady state conditions, (2) no large sudden changes of blood pressure, (3) no artifacts, and (4) high heart rate variability. Data are presented as means ± SEM. Differences were tested by ANOVA for repeated measures, the Bonferroni t test, and the Mann Whitney U test. Probability values <0.05 were considered statistically significant.

Results

Figure 1 shows that 4 to 5 days after transmitter placement, heart rate and systolic and diastolic blood pressure again exhibited a normal daytime variation. Therefore, we are confident that blood pressure regulation was not affected by the surgical procedure. Figure 2 shows the spontaneous changes in blood pressure and heart rate used to analyze baroreflex function. Shown is a 2-hour beat-to-beat registration period. The figure illustrates the profound spontaneous oscillations of RR intervals and arterial blood pressure recorded in a representative AT$_2$−/− mouse. The AT$_2$+/+ mice had patterns that were not different. The figure underscores the marked range of baroreflex function, even in resting, unperturbed mice. The lower panel of Figure 2 shows blood pressure and heart rate spectra obtained for 32 seconds in the same mouse. Note the similar characteristic peaks as obtained in humans, only different in the frequency scale. This supports the hypothesis that blood pressure control in mice has major similarities to that in other mammals. We believe that this state of affairs indicates that spontaneous baroreflex sequences represent physiological rather than chance interactions also in mice. Figure 3 shows that the
The number of sequences was highest for the delay of one heartbeat. The sequence number at the 2-heartbeat delay was significantly lower. The strains did not differ in that regard.

Body weight averaged 30 ± 0.8 g in AT_2_−/− mice and 31 ± 0.6 g in AT_2_+/+ mice before surgery. After surgery, the weights decreased by 2 to 3 g. One week later, the mice showed slightly reduced but stable body weights. The body weights increased continuously and averaged 29 ± 0.6 g in AT_2_−/− and 30 ± 0.6 g in AT_2_+/+ mice at the baseline period and 29 ± 0.5 g in AT_2_−/− and 32 ± 1.0 g in AT_2_+/+ mice at the end of the protocol.

In AT_2_−/− mice, the 12-hour mean of MAP leveled during the day 101 ± 1 mm Hg and the night 109 ± 1 mm Hg compared with 98 ± 2 mm Hg (day) and 104 ± 2 mm Hg (night) in AT_2_+/+ mice. The heart rate leveled in AT_2_−/− mice 502 ± 7 (day) and 530 ± 8 beats per minute (bpm, night) compared with 507 ± 7 (day) and 538 ± 7 bpm (night) in AT_2_+/+ mice. Figure 4 shows the changes in MAP and HR after L-NAME and DOCA-salt treatments during day and night. L-NAME increased blood pressure more in AT_2_−/− than in AT_2_+/+ mice, so that MAP leveled in AT_2_−/− mice 114 ± 1 (day) and 121 ± 1 (night) compared with 105 ± 2 mm Hg (day) and 111 ± 2 mm Hg (night), respectively. In the following week with tap water vehicle, all parameters returned to levels measured before L-NAME treatment. The DOCA-salt regime induced an increase in blood pressure that was also more pronounced in AT_2_−/− than in AT_2_+/+ mice. The increases in blood pressure after DOCA-salt and L-NAME treatments were not significantly different, indicating that similar degrees of hypertension were achieved with both regimens. The blood pressure increases after L-NAME and DOCA-salt treatments were accompanied by decreases in heart rate in both strains.

Heart rate variability in the time domain (coefficient of variation, CV) was similar in the two mouse strains and not affected by the interventions with the exception of DOCA-salt mice. CV in DOCA-salt–treated AT_2_−/− mice was 9.7 ± 1.9% compared with 5.6 ± 0.6% in DOCA-salt–treated

Figure 2. Representative 2-hour recording of systolic blood pressure (SBP) and heart rate (RR interval) in a representative AT_2_ receptor −/− mouse at baseline on a beat-to-beat basis. Upper panel: Note the high intra-individual variability of the parameters. The segment for spectral analysis is marked by arrows. The lower panel illustrates the selected 32 seconds of RR intervals and the corresponding power spectra (left), and the selected 32 seconds of systolic blood pressure and the corresponding power spectra (right).

Figure 3. Number of significant baroreflex sequences in AT_2_−/− and AT_2_+/+ mice for a lag period of 0 (solid bar), 1 (hatched bar), and 2 (open bar) heartbeats between systolic blood pressure and the following RR interval. The number of sequences was highest for a lag of one heartbeat. *P<0.05 lag of 1 heart beat versus 0 and 2 heart beats.
AT2+/− mice. Heart rate variability in the frequency domain (power spectral density in the very low [VLF], low [LF], and high frequency [HF] bands) in AT2−/− mice was also similar to that in AT2+/+ mice. However, the coefficient between LF and HF spectral power density, which is presumed to reflect sympathovagal balance in heart rate control, was significantly reduced in L-NAME–treated AT2−/− mice compared with L-NAME–treated AT2+/+ mice (1.3±0.3% compared with 2.7±0.7%).

Systolic blood pressure variability in terms of standard deviation was similar in the 2 strains. Shown in Figure 5 are systolic blood pressure variability in the low frequency band (SBPLF, top), baroreflex sensitivity calculated by cross-spectral analysis in the low frequency band (BRSLF, middle), and baroreflex sensitivity calculated by the sequence technique for upslopes of systolic blood pressure (BRSup, bottom) in control and AT2 receptor−/− mice at baseline (P<0.05). Baroreflex sensitivity was increased in AT2−/− mice, accompanied by reduced systolic blood pressure variability in the LF band compared with controls.

**Discussion**

The important observation in our study was that systolic blood pressure variability was reduced in AT2−/− mice compared with AT2+/+ mice. Moreover, the baroreflex sensitivity calculated by use of the sequence analysis and cross-spectral analysis was increased in AT2 receptor−/− mice compared with AT2+/+ controls. These differences were sustained during the development of secondary hypertension in these mice.
Cardiovascular reflexes play an important role in the regulation of circulation in both physiological and pathophysiologic states. In contrast to baroreflex control of blood pressure or peripheral resistance, which has been reported to be normal in humans with essential hypertension or in experimental models of hypertension,29 baroreflex control of heart rate has been shown to be impaired in hypertension and is associated with the risk of cardiovascular events.20 This state of affairs is the basis for the interest in these parameters. The technique of recording heart rate and arterial pressure on a beat-to-beat basis allowed us to analyze “spontaneous” baroreflex control of heart rate under free-living daily conditions.21

To account for changes in baroreceptor input, we calculated BRS using the sequence technique over 2 hours. We also used cross-spectral analysis as a second method of BRS analysis for selected periods of 32 seconds each. Both methods showed similar results. We are aware that spontaneous BRS reflects only one point of the baroreflex response curve. However, investigations in humans have shown a close correlation between invasive and different noninvasive methods, such as the cross-spectral and the sequence techniques.22 All methods have advantages and limitations. The traditional pharmacological technique (phenylephrine and nitroprusside) can induce changes in the mechanical properties of the arterial wall where the baroreceptors are located. This potential confounder may interfere with physiological baroreceptor stimulation. Therefore, differences between the results obtained by these methods would be expected. We believe that invasive and noninvasive approaches should be regarded as complementary approaches.23 To our knowledge, this study is the first to use blood pressure telemetry to calculate BRS repeatedly under different pathophysiological situations in the mouse.

Functional investigations show that angiotensin II modulates the baroreceptor reflex in the nucleus tractus solitarii (NTS) via the AT1 receptor.24–26 Furthermore, the AT2 receptor has been reported to have a tonic inhibitory effect on the baroreceptor reflex. This effect is believed to have origins within the central nervous system.9,10 Microinjections of Ang II and Ang III into the NTS lead to a tonic inhibition of the baroreceptor reflex. The action of Ang II is confined to the AT1 receptor, whereas the action of Ang III is mediated via both the AT1 and AT2 receptor subtypes.9,10,27 Furthermore, at the site of the rostral nucleus reticularis ventrolateralis, both Ang II and Ang III may promote a tonic suppression of the spontaneous baroreceptor reflex via the AT2 receptor.9 Our study suggests that Ang II attenuates the baroreceptor reflex via the AT2 receptor. The AT2 −/− mice clearly exhibited baroreflex sensitivity that was increased compared with AT2 +/+ mice. Therefore, in wild-type +/+ mice, the net effect of Ang II on both the AT1 and AT2 receptors may have depressed baroreflex activity. Moreover, the more effective baroreflex in AT2 −/− mice may enable these mice to decrease their blood pressure variability because of dampening blood pressure changes. This interpretation is supported in part by the changes of heart rate variability in the time (CV) and frequency domains (LF/HF) in these mice. However, the HRV parameters did not reach statistical significance because of the high inter- and intraindividual variability normally found in free-moving, conscious mice. We were very surprised to observe such a high degree of variability in resting, unperturbed mice.

On the other hand, Ang II acting within the NTS may depress the baroreceptor reflex via release of NO.26 These observations prompted us to investigate baroreceptor function in L-NAME hypertensive mice with blocked NO synthesis and to compare these mice with DOCA-salt hypertensive mice. In DOCA-salt hypertensive mice, the NO system is stimulated.29 Hypertension, induced either by L-NAME or DOCA-salt treatment, increased blood pressure similarly in both strains. The present results are in good agreement with other reports showing that chronic administration of L-NAME causes arterial hypertension in rats30 and mice.31,32 In L-NAME-hypertensive rats, both a decrease and an increase in baroreflex sensitivity have been reported.28,30,33 NO synthesis in mice resulted in an enhancement of baroreflex sensitivity in an earlier study.31 In our study, L-NAME treatment increased blood pressure to a greater degree in AT2 −/− than in controls, suggesting an augmented sensitivity of AT2 −/− mice to L-NAME. The baroreflex sensitivity was not affected by L-NAME in our study. This result contradicts the observation of Peotta et al.,31 who found an increase in baroreflex sensitivity after L-NAME treatment by use of the phenylephrine and sodium nitroprusside technique in urethane-anesthetized mice, which decreases mean arterial blood pressure. The differences may be related to the fact that we measured arterial baroreflex control of heart rate in the conscious state under free-living conditions without pharmacological interventions. This result would support the view that the influence of NO on baroreceptor sensitivity is small under these circumstances.

DOCA-salt also increased blood pressure in both strains. However, again the blood pressure increase in AT2 −/− mice was greater than in AT2 +/+ mice. The mechanisms of DOCA-salt hypertension are not fully understood. However, changes in hormonal and neural pressor mechanisms, including bradykinin, nitric oxide, altered neural Ang II, and vasopressin activity, elevated sympathetic drive, endothelin-related mechanisms, and a distorted baroreflex response, all appear to contribute to DOCA-salt hypertension.34–39 DOCA-salt–treated mice in our study had similar responses as L-NAME–treated mice, even though their NO-related mechanisms were presumably stimulated. The findings suggest that NO-related influences on baroreflex regulation did probably not have a major impact on our findings. We assume that the stronger blood pressure increase to L-NAME and DOCA-salt treatment in AT2 −/− may be caused by the missing AT2-mediated vasodilatory effect normally opposing the AT1-mediated vasoconstriction and may be also accelerated by the upregulated AT1 receptor in AT2 −/− mice.5,40,41 In contrast, the normally synergistic inhibitory central action of the AT1 and AT2 receptors may be responsible for the improved baroreflex function seen under baseline conditions that were not further influenced by L-NAME and DOCA-salt treatment. The approximately 10 to 15 mm Hg increase of mean arterial blood pressure after L-NAME and DOCA-salt treatment may be not sufficient to induce the expected
reduction of the baroreflex-mediated heart rate control caused by hypertension.

Another important observation of our study was the finding that telemetric measurements required a week’s recovery before the normal cardiovascular responses, particularly circadian cycles, were restored. General anesthesia is required for placement of intra-arterial catheters in mice. We suggest that baroreflex and similar measurements in conscious mice should not be performed acutely. A weakness in our study is that we included only a relatively small number of mice (AT2−/− n = 8, AT2+/+ n = 7). However, under the view of telemetric chronic studies, these numbers are relatively high. We elected the intra-abdominal placement of our transmitters to avoid the confounding effects of carotid catheterization in our mice.6 The mortality of abdominal placement is higher, and the technical difficulty involved is greater. However, the amount of data we analyzed in our chronic study was substantial. The animals have a heart rate of 500 to 600 bpm, and we collected data for days at a time. Thus, we were able to measure blood pressure over weeks and to apply repeated-measures analyses.

Perspectives

We suggest that future perspectives provided by our data include research into the central role of the AT2 receptor in baroreflex activation. A tonic suppression effect by AT2 receptors on baroreflex sensitivity has been suggested. However, additional mechanistic studies will be necessary to verify this possibility. Also poorly explained is why AT2 receptor–deficient mice developed a greater increase in blood pressure than did controls and yet exhibited responses suggesting superior baroreflex sensitivity in response to 2 vastly different forms of secondary hypertension. Reconciling these findings in future studies may lead to a new appreciation of different forms of secondary hypertension. Reconciling these findings in future studies may lead to a new appreciation of different forms of secondary hypertension. Reconciling these findings in future studies may lead to a new appreciation of different forms of secondary hypertension. Reconciling these findings in future studies may lead to a new appreciation of different forms of secondary hypertension. Reconciling these findings in future studies may lead to a new appreciation of different forms of secondary hypertension.

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