CARDIOVASCULAR AUTONOMIC CONTROL IN
MICE LACKING ANGIOTENSIN AT1A RECEPTORS

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Studies examined the role of angiotensin (Ang) AT1a receptors in cardiovascular autonomic control by measuring arterial pressure (AP) and heart rate (HR) variability and the effect of autonomic blockade in mice lacking AT1a receptors (AT1a-/-). Using radiotelemetry in conscious AT1a +/- and AT1a -/- mice, we determined: 1) AP and pulse interval (PI) variability in time and frequency (spectral analysis) domains; 2) AP response to $\alpha_1$-adrenergic and ganglionic blockade and 3) intrinsic HR after ganglionic blockade. Pulsatile AP was recorded (5 kHz) for measurement of AP and PI and respective variability. Steady state AP responses to prazosin (1 µg/g, i.p.) and hexamethonium (30 µg/g, i.p.) were also measured. AP was lower in AT1a -/- vs AT1a +/- while HR was not changed. Prazosin and hexamethonium produced greater decreases in MAP in AT1a -/- than in AT1a +/- . The BP difference was marked after ganglionic blockade ($\Delta$ MAP: -44±10 vs -18±2, mmHg, -/- vs +/-). Intrinsic HR was also lower in AT1a -/- mice (431 ± 32 vs 524 ± 22 bpm; -/- vs +/-). Beat-by-beat series of systolic AP (SAP) and PI were submitted to autoregressive spectral estimation with variability quantified in low (LF: 0.1-1 Hz) and high (HF: 1-5 Hz) frequency ranges. AT1a -/- mice showed a reduction in SAP LF variability (4.3±0.8 vs 9.8±1.3 mmHg$^2$) with no change in HF (3.3±0.6 vs 2.7±0.2 mmHg$^2$). There was a reduction in PI variability of AT1a -/- in both LF (18.7±3.7 vs 32.1±4.2 ms$^2$) and HF (17.7±1.9 vs 40.3±7.3 ms$^2$) ranges. The association of lower AP and PI variability in AT1a -/- mice with enhanced AP response to $\alpha_1$-adrenergic and ganglionic blockade suggests that removal of the Ang AT1a receptor produces autonomic imbalance. This is seen as enhanced sympathetic drive to compensate for the lack of Ang signaling.
INTRODUCTION

Genetically manipulated animal models have been extensively used to study the physiological role of the renin-angiotensin system (RAS) in the control of arterial pressure (AP), cardiac function and fluid homeostasis (2; 12; 13). Despite some drawbacks, the deletion or over-expression of a specific gene for any component of the RAS provides a unique way to understand the role of the RAS not only in cardiovascular development and function, but also in pathological processes. It is widely accepted that the RAS is important in the maintenance of normal AP levels. It contributes to the development of hypertension not only by direct vasoconstrictor actions of angiotensin II (Ang II), but also via Ang II effects on the central nervous system (CNS) to increase sympathetic drive to the heart and vasculature (53). Recent studies in AT1a receptor gene deletion mice have demonstrated the importance of these receptors in the maintenance of body fluid homeostasis (8; 14; 37; 45), blood pressure (11; 22; 36) and endocrine function (34; 35; 38). Support for a cardiovascular role of the complementary AT1b receptor was provided by data which showed that losartan lowered AP in AT1a -/- mice (36); that hypotension was enhanced in the combined AT1a/AT1b gene deletion model (39) and that AT1b receptors produce vasoconstriction in resistance vessels (50).

Interactions between the RAS and autonomic nervous system have been well documented (53). For example, Ang II increases sympathetic nerve activity, stimulates norepinephrine release and directly activates sympathetic ganglionic cells, effects which may be mediated by AT1 receptors (6; 27; 28; 42; 47). Studies using Ang AT1 receptor and converting enzyme antagonists suggest that the RAS is also involved in autonomic balance (4; 21; 32) For example, Bezerra et al. reported that AT1 receptors were involved in the autonomic changes associated with coarctation hypertension in rats, acting to facilitate sympathetic outflow to
the heart and vasculature (4). In healthy humans, treatment with an AT1 antagonist reduced HR variability, an effect which was thought to be mediated by increased plasma Ang II (21). A critical lack in our knowledge base is related to the role of the AT1a subtype specific receptors in autonomic balance.

The cardiovascular effects of pharmacological blockade of cholinergic/adrenergic receptors and autonomic ganglionic transmission as well as measurement of neural activity and baroreflex sensitivity are useful tools for assessment of autonomic influences on the heart and vasculature (19). However, all of these tests are invasive and difficult to standardize. A recent advance in the study of autonomic function has been the development of methods for the evaluation of pulse interval (PI) and AP variability in time and frequency domains (1; 3; 31; 40). AP and PI fluctuate at regular frequencies, and the magnitude of each can be accurately quantified using power spectral analysis. With simple measurement and processing of AP data under baseline conditions, information on autonomic influences on the heart and vessels is available. These methods have been applied successfully to the mouse model with evaluation of the input of autonomic transmitters (20; 23-25).

Experiments were conducted to evaluate the role of Ang AT1a receptors in the control of cardiovascular autonomic function using high fidelity telemetric AP recordings in conscious Ang AT1a gene deletion mice coupled with autoregressive spectral analysis and autonomic blockade. To our knowledge this is the first study to examine Ang AT1a subtype specific involvement in autonomic function. The aims are to determine: 1) AP and HR variability in time and frequency domains; 2) spontaneous baroreflex sensitivity; 3) AP response to \(\alpha_1\) adrenergic blockade with prazosin and 4) AP and HR response to total ganglionic blockade with hexamethonium.
MATERIAL AND METHODS

**Animals:** Male Ang AT1a receptor knockout mice (AT1a -/-, n = 26) and wild-type controls (AT1a +/-, n = 25) were used in the present study. The founder animals were developed by Ito et al (22) and the original breeders were obtained from T. Coffman (Duke University, Durham, NC). F2 generation mice were produced from crosses of (129 x C57BL/6J) F1 AT1a +/- parents. Experimental animals had the same genetic and environmental background. Genotypes were determined using DNA from tail extracts and PCR methods. The animals (~26 g) were housed individually at 22°C on a 12 h dark-light cycle. They were fed a standard pellet diet (Harlan-Teklad #8640, 0.4% sodium by weight) with tap water *ad libitum*. The Laboratory Animal Care and Use Committee of Wright State University approved all experimental protocols.

**Surgical Procedures:** Under ketamine-xylazine mixture anesthesia (120:20 mg/kg, im), radiotelemetric catheters (model TA11PA-C20, Data Sciences Intl, St. Paul, MN) were inserted into the carotid artery. The methods have been described in detail in a previous publication (17). Briefly, the left common carotid artery was isolated, ligated (~ 3 mm below the carotid bifurcation), occluded and the catheter inserted into the artery. The telemetric transmitter probe was positioned subcutaneously on the right flank. Mice were returned to their home cages which were placed on top of the telemetric receivers.

**Cardiovascular Recording:** Recordings were carried out after the mice had fully recovered from surgery (5 to 7 days). On the day of the experiment, the telemetric probe was magnetically activated at least 1h before initiation of recording (~ 08.00h). Pulsatile arterial
pressure (PAP) was continuously sampled (5 kHz) for 1 hr using an IBM/PC interfaced with the telemetry system. Only animals with a PAP amplitude of > 30 mmHg were included in the study (Example in Figure 1).

**Experimental Protocols:** AP, HR and their respective variabilities, as well as spontaneous baroreflex sensitivity, was measured in AT1a-/- and AT1a+/+ (n = 13-14/group) using a AP recording (continuous 1 hr) made under low stress conditions. Other groups (AT1a-/- and AT1a+/+) were used to test the effect of prazosin (1 µg/g; Sigma-Aldrich Inc.; 7/group) and hexamethonium chloride (30 µg/g; Sigma-Aldrich Inc.; 5/group). AP recordings were made 30-60 min after i.p. injection.

**Data Analysis:** PAP files of the entire 1 hr recording were analyzed by software (CODAS, Dataq Instruments Inc, Akron, OH) that detects beat-by-beat values of systolic AP. PI series were generated from the intervals between successive systolic AP values. The variance (σ²) of the PI and systolic AP series was calculated (time domain). The variability of PI and SAP was also evaluated in the frequency domain using an autoregressive spectral estimation according to methods described elsewhere (16; 31). Briefly, PI and systolic AP series were divided into 300 beat segments, overlapped by 50% and the spectra of each segment were calculated via the Levinson-Durbin recursion, with the model order chosen according to Akaike’s criterion. The oscillatory components found by spectral analysis were quantified in low (LF: 0.1-1.0 Hz) and high (HF: 1.0-5.0 Hz) frequency bands. The coherence (k²) between the oscillatory components of PI and systolic AP in LF range was estimated by bivariate autoregressive spectral analysis (3). Coherence is a measurement of the statistical link between the variability of two series at any given frequency and is expressed as a number between 0 (total lack of relationship) and 1 (maximum relationship). Only values > 0.5 were
considered significant (3; 44). When the oscillations of systolic AP and PI in LF range were found coherent, the α-index was calculated by the square root of the ratio between spectral density of PI and the corresponding spectral density of systolic AP, as first described by Robbe et al. (43). The presence of a significant coherence between PI and systolic AP oscillation in the LF range and the α-index are both an expression of the spontaneous baroreflex control of HR (3; 16; 20).

Spontaneous baroreflex sensitivity was calculated in each recording period by means of the sequence analysis technique, as described elsewhere (20; 26). Briefly, we used software that automatically detected sequences of three or more consecutive beats in time series of systolic AP and PI, with delays of about 0 to 4 beats determined by cross-correlation between the AP and PI series. These sequences were characterized by either a progressive rise in AP and lengthening of PI (+PI/+SBP sequences), or by a progressive decrease in SAP and shortening PI (-PI/-SBP sequences) with linear correlation higher than 0.8. The mean individual slope of significant SBP/PI relationship obtained by averaging all slopes computed within the test period was calculated and taken as a measure of spontaneous baroreflex sensitivity.

To determine the AP response to α1-adrenergic and ganglionic blockade, MAP was calculated from the AP signals. The steady state response to prazosin or hexamethonium was determined (10-40 min after ip injection) and shown as the change from baseline levels (ΔMAP). Since hexamethonium abolishes both vagal and sympathetic influences to the heart [47], we considered the intrinsic pacemaker rate to be the HR value achieved after hexamethonium injection.

Statistical Analysis: Results are expressed as mean ± SEM. Basal values of SAP and PI were
compared between groups by Student t-test. All parameters of variability in time ($\sigma^2$) or frequency domain, as well as baroreflex indices and MAP responses to administration of prazosin and hexamethionium, were compared between groups by the non-parametric Mann-Whitney on rank test. Differences were considered statistically significant if $P<0.05$.

RESULTS

Figure 1 shows representative PAP tracings of one animal from each group studied. The AT1a -/- mouse exhibits hypotension as compared to its AT1a +/+ control. The reduction in mean AP variability in the AT1a -/- mouse is clearly seen by comparing the mean AP tracings (white line). Group data shows that AT1a -/- mice have significantly reduced MAP as compared to AT1a +/+ controls ($82 \pm 3$ vs. $99 \pm 3$ mmHg; AT1a-/- vs AT1a+/+; $p<0.001$). HR was similar in groups ($475 \pm 11$ vs. $487 \pm 10$ bpm; AT1a-/- vs AT1a +/+; $p = 0.42$).

Table 1 shows group data for baseline PI and SAP, as well as their respective variances. PI is not different between groups, while PI variance is significantly lower in AT1a -/- as compared to AT1a +/+ mice. AT1a -/- also showed lower levels of SAP and its variance.

Representative time series and respective autoregressive spectra for PI and SAP from each group are shown in Figure 2. Both time series presented visible variability, which could be discriminated in two distinct oscillatory components by spectral analysis. PI spectra showed LF (0.43 - 0.45 Hz) and HF (2.69 - 2.81 Hz) peaks, which were not different between groups. Frequencies of SAP oscillations were similar to those found for the PI spectra (data not shown). Figure 3 shows the group data for LF and HF power for PI and SAP oscillations. There were significantly lower PI and SAP fluctuations in the LF range for the AT1a -/-
group (more than 2 fold). For the HF component of variability, AT1a-/- mice showed reduced power for PI but not SAP (Figure 3).

Spontaneous baroreflex sensitivity, calculated by the α-index, was similar in AT1a-/- and AT1a +/+ mice (3.5 ± 0.4 vs. 3.1 ± 0.4 ms/mmHg, AT1a-/- vs AT1a+/+; p = 0.79). Results were also similar when baroreflex function (4.1± 0.5 vs. 3.2 ± 0.3 ms/mmHg, AT1a-/- vs AT1a+/+; p = 0.15 ) was calculated using the sequence analysis method (Figure 4).

α₁-adrenergic blockade with prazosin produced a greater depressor response in AT1a-/- mice (Δ24 ± 3 vs 9 ± 2 mmHg; AT1a-/- vs AT1a+/+; p < 0.05; Figure 5). Ganglionic blockade with hexamethonium also elicited a greater decrease in MAP in AT1a-/- (Δ44 ± 10 mmHg) as compared to AT1a +/+ (18 ± 2 mmHg, Figure 5). The bradycardia elicited by hexamethonium was more pronounced in AT1a-/- mice (431 ± 32 vs 524 ± 22 bpm; AT1a-/- vs AT1a+/, p < 0.05; Figure 6). This provides evidence for a reduced intrinsic HR (HR achieved after ganglionic blockade) in mice lacking the AT1a receptor.

DISCUSSION

These results provide new information on the role of the Ang AT1a receptor in the control of cardiovascular autonomic function. Using two different methods, autoregressive analysis of telemetric BP recordings and pharmacological blockade, we demonstrated the autonomic imbalance presented by mice lacking AT1a receptors. In AT1a-/- mice, there was a marked reduction (~ 2 fold) in PI and SAP variability in the LF range. Blockade of α₁-adrenergic receptors or ganglionic transmission produced an enhanced AP fall in AT1a-/- mice. Moreover, the intrinsic HR, observed after ganglionic blockade, was lower in AT1a-/- mice as compared to the controls. Data suggest that increased sympathetic input acts to
compensate for the lack of Ang receptor signaling.

There is evidence supporting a role for AT1a receptors in the maintenance of BP. In the original publication describing the knockout model, Ito et al. reported that AT1a -/- mice showed a reduction in SAP of about 24 mmHg (22). In the present study, MAP was reduced 17 mm Hg in AT1a-/- mice. However, even though AT1a -/- mice are hypotensive, the BP levels are not as low as one might predict, given the importance of vascular Ang receptors. It has been suggested that AT1a deficiency results in compensation by other systems. A feedback action is seen in the absence of AT1a receptors, a situation in which Ang II secretion is stimulated (8). In rodents, this Ang II could interact with other Ang receptors to take over some of the functions of AT1a receptors (33; 36; 50; 52). This is supported by results showing an additional depressor response in AT1a -/- mice treated with AT1 blockers (36). Zhu et al (52) also suggested that AT1a and non-AT1a receptors share common signal transduction pathways, since cultured smooth muscle cells from AT1a-/- mice showed the same intracellular calcium changes in response to the pharmacological stimulation with Ang II. In the peripheral vasculature (aorta and femoral artery), there was evidence for a predominance of AT1b subtypes as well as an Ang II-induced vasoconstriction in AT1a-/- mice (50). Finally, in the brainstem dorsal vagal complex, AT1b receptors were higher in AT1a-/- mice and were upregulated in response to dietary salt (9; 10).

While there is much information on the role of AT1a receptors in arterial pressure maintenance, there is less information on their role in autonomic function. One of the best ways to answer this question is to take advantage of an animal model which lacks the Ang receptor signaling pathway. To our knowledge, this is the first study to examine autonomic status in AT1a -/- mice, by determining AP and HR variability and their spectral components.
HR and AP variability are used to estimate autonomic modulation of the cardiovascular system in both clinical (1; 3; 31) and experimental settings (16; 20; 23; 24; 41; 44). Overall variability indices of HR and AP are strongly correlated with autonomic modulation of the heart and vessels, while spectral analysis of the intrinsic rhythms provides reliable information on sympathetic and parasympathetic modulation (1; 3; 16; 20; 23). There is evidence that LF oscillations of HR variability are a marker of sympathetic modulation, while HF oscillations are widely recognized as a marker of vagal modulation of the sinus node (1; 31). In addition, spectral analysis applied to spontaneous AP fluctuations has revealed, in humans and rats, that slow rhythms are modulated by sympathetic drive to the heart and vasculature which is also related to baroreflex activity (3; 7; 15; 31; 44).

There is less information on autonomic modulation of cardiovascular function in mice than in humans or other species. Nevertheless, based on available reports, data in mice show that HR and AP variability are influenced by sympathetic and parasympathetic input (18; 20; 23; 25). Studies using spectral analysis in mice have revealed slow oscillations between 0.08 and 1.0 Hz and the higher respiratory frequency of 2.5 to 3.5 Hz, both for HR or AP series (17; 20; 24; 25). There are also reports that autonomic blockers modify the power of LF oscillations either in HR or AP series. Janssen et al. reported that both ganglionic and α-adrenergic blockade decreased MAP variability; muscarinic blockade had no effect on MAP fluctuations, but decreased those of PI (23). We found similar results although PI was reduced after atropine in contrast to a lack of change in the Janssen study (18). The present results showed lower variability in SAP and HR in both time and frequency domains, suggesting changes in autonomic balance in AT1a-/- mice.

Studies have shown that LF for AP is associated with sympathetic control of the vascular
tone as demonstrated by means of acute pharmacological interventions or stress exposure (17; 18; 25). In AT1a -/-, chronic hypotension was associated with a reduced LF for AP. On face value, this might indicate a reduction in sympathetic input. However, studies in humans of varying ages and gender showed no correlation between sympathetic activity and LF power for AP (48). Likewise, we found that chronic stress in rats was associated with increased blood pressure, but reduced LF power for AP (17). Reports have also showed reduced LF oscillations of HR in situations of high sympathetic tone (46; 49). Therefore, the finding of reduced LF variability of SAP and PI in the present study supports the idea of altered sympathetic modulation of the cardiovascular system. However, based only on the spectral analytical data, one cannot determine the degree of the sympathetic tone. For HF oscillations of PI, there was also a reduction in the AT1a-/-, suggesting an impairment in cardiac vagal modulation in these mice.

There is a body of evidence suggesting that the RAS interacts with the sympathetic nervous system in the control of circulation (42; 53). This interaction could involve a RAS stimulatory effect on sympathetic activity at the level of the CNS, sympathetic ganglia or adrenergic nerve terminals (42). The mechanisms of the RAS stimulatory effects on sympathetic nerve activity involves Ang actions mainly through its AT1 receptors (51). In mice, evidence points to a significant stimulatory effect of Ang II on the sympathetic nerve transmission. Ma et al. (27) reported that intravenous Ang II infusion induced continuous low-amplitude discharges in renal sympathetic nerve activity (SNA). These authors stated that Ang-induced sympathetic activation was still present after ganglionic blockade, but was abolished by the Ang AT1 receptor antagonist, losartan. Further studies showed that Ang II increased, by means of AT1 receptors, cytosolic calcium influx in postganglionic sympathetic neurons (28).
Although data from Ma et al. (27, 28) suggest that Ang II signaling is critical in modulating sympathetic neural activity and might predict a reduction in SNA after disruption of AT1a receptors, our results point to another direction. Both, α1-adrenergic antagonist or autonomic ganglion blockade elicited a fall in AP that was much more pronounced in AT1a -/- mice as compared to their counterparts. Although the AP response to prazosin in the control group was not as great as in other studies (23, 25), the decrease in AP observed 30 to 60 minutes after ganglionic blockade in this group was similar to that found by Janssen et al. in male Swiss mice (23). In addition, hexamethonium also elicited a significantly greater bradycardia in AT1a-/- mice, supporting the idea that these animals have a low intrinsic HR. This finding is in agreement with data which show that Ang II regulates intrinsic HR. An intravenous infusion of Ang II elicited an increase in intrinsic HR, as did endogenous overactivity of the RAS, observed during the onset of 1K1C renal hypertension (29; 30). In the coarctation model of hypertension, there was also an increase in HR which was mediated by Ang AT1 receptors (4). Taken together, these findings suggest that removal of Ang AT1a input should increase sympathetic drive. Indeed, the lack of Ang AT1 signaling in the gene deletion mice leads to sympathetic compensation in order to maintain BP for adequate tissue perfusion. Conversely, genetically normal mice may use the AT1a receptor system to counterbalance any change in adrenergic drive to the vasculature.

With regard to cardiac baroreflex, there is also evidence for interactions between the RAS and reflex control (5; 20; 42). To our knowledge, this is the first study to evaluate the influence of AT1a receptors on baroreflex sensitivity. Surprisingly, there were no differences in spontaneous baroreflex sensitivity in AT1a-/- mice, studied either by sequence or cross-spectral (α-index) analysis. Since central Ang II impairs baroreflex function through AT1 receptors (5; 20; 42), one would expect that baroreflexes would be enhanced in mice lacking
AT1a receptors. Rather, a study by Gross and colleagues (20) suggested that AT2 receptors are important in baroreflex control. In AT2 -/- mice, the baroreflex index was increased, suggesting that AT2 receptors impaired cardiac baroreflex by means of central actions on the autonomic nervous system.

In conclusion, data suggest that Ang AT1a receptors are required for maintaining normal autonomic control of AP and HR. The increased fall in pressure elicited by either $\alpha_1$-adrenergic or autonomic ganglion blockade, as well as the greater effect of autonomic ganglionic blockade on HR, strongly suggests that an increased sympathetic drive to the vasculature may compensate for the lack of Ang AT1a signaling in AT1a-/- mice. In addition, our results suggest that Ang AT1a receptors may be not required for maintaining normal baroreflex function. Nevertheless, it should be pointed out that a limited analysis of the baroreflex was carried out in the present study, and probably the spontaneous baroreflex might be different if conditions such as basal AP and the magnitude of AP fluctuations were similar between the studied groups.
ACKNOWLEDGEMENTS

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Table 1. Baseline values of pulse interval (PI) and systolic arterial pressure (SAP) of both groups (AT1a +/+ and AT1a -/-), and their respective indices of variability in the time domain (variance). Data are mean ± SEM. * P<0.05 vs AT1a +/+ mice.

<table>
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<tr>
<th>Group</th>
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<td>PI, ms</td>
<td>133 ± 3</td>
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<td>8.0 ± 1.0 *</td>
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FIGURE LEGENDS

**Figure 1.** Telemetric recordings of pulsatile AP (mmHg) from a representative mouse of each group. Upper panel is AT1a +/+ mouse and lower panel is AT1a -/- mouse. White lines represent the mean arterial pressure.

**Figure 2.** Left and middle panels show time series of pulse interval (PI, ms) and systolic arterial pressure (SAP) from a representative mouse of each group (AT1a +/+ and AT1a -/-). Their respective spectra are shown in the right panel.

**Figure 3.** Power spectral density of pulse interval (PI, ms²) and systolic arterial pressure (SAP, mmHg²) for low (LF, 0.1-1.0 Hz) and high (HF, 1.0-5.0 Hz) frequency components of variability, calculated by means of autoregressive spectral estimation. Solid bars correspond to AT1a +/+ and dashed bars to AT1a -/- mice. Data show mean ± SEM. * P<0.05 vs. AT1a +/+; # P<0.01 vs. AT1a +/+.

**Figure 4.** Baroreflex sensitivity calculated using sequence analysis (left panel) and cross-spectral analysis (α-index, right panel) in AT1a +/+ and AT1a -/- mice. Open circles are individual values.

**Figure 5.** Decrease in mean arterial pressure (ΔMAP) observed after i.p. injection of either prasozin or hexamethonium in AT1a +/+ and AT1a -/- mice. * P<0.05 compared to AT1a +/+.

**Figure 6.** Heart rate (bpm) measured before (basal; open bars) and after (intrinsic; dashed
bars) i.p. injection of hexamethonium. * P<0.05 compared to Basal; ** P<0.05 compared to intrinsic heart rate of AT1a +/- group.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Reference List


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