Chronic AT1 Receptor Blockade Alters Aortic Nerve Activity in Hypertension

Claudia M. dos Santos, Edson D. Moreira, Eduardo M. Krieger, Lisete C. Michelini

Abstract—In the chronic phase of coarctation hypertension (CH) we have shown both reduction in baroreceptor sensitivity (Hypertension. 1992;19[suppl II]:II-198–II-201.) and normalization of the depressed baroreceptor reflex control of heart rate, even with the persistence of hypertension in losartan-treated animals (Am J Physiol. 1995;269:H812-H818). In the present study we analyzed the effects of angiotensin II blockade on afferent aortic nerve activity of CH and sham-operated groups treated chronically with vehicle or losartan (10 mg/kg per day PO). CH was induced by subdiaphragmatic aortic coarctation, and the treatments lasted 8 days (4 control and 4 experimental days). Aortic pressure (conscious rats) and aortic nerve activity simultaneous to pressure (anesthetized rats) were recorded on the fourth day of the experimental period. Losartan-treated rats showed reduced tail pressure (104±3 versus 117±3 mm Hg in the vehicle group). In both groups, aortic coarctation caused a significant increase in pressure (25% and 28%, respectively) and a depression of the aortic nerve activity/pulse pressure relationship when compared with sham-operated coarcted animals. In the physiological range of pulse pressure changes, the depression was significantly smaller after losartan treatment (3.30±0.33 versus 2.18±0.37%/mm Hg in the losartan- and vehicle-treated CH groups, respectively, versus 5.05±0.33%/mm Hg in the sham-operated vehicle-treated group). Angiotensin type 1 (AT1) receptor blockade was also accompanied by reduced variability of the afferent discharge. The data suggested that apart from its pressure effect, angiotensin II acts at AT1 receptors to decrease the sensitivity of aortic afferents during physiological (±10 mm Hg) increases and decreases in pressure. Thus, angiotensin II may contribute to reductions of baroreceptor gain in chronic hypertension. (Hypertension. 1998;31:973-977.)

Key Words: baroreceptors ■ angiotensin II ■ receptors, angiotensin ■ blood pressure ■ losartan ■ hypertension, coarctation

The presence of an endogenous renin-angiotensin system in brain areas involved in cardiovascular regulation has been confirmed by several techniques.1–3 Stimulation of high-affinity Ang II receptors4–6 leads to a set of coordinated autonomic responses, yielding increases in blood pressure.5–8 An important central action of Ang II is to modulate the baroreceptor reflex control of heart rate9–14 and sympathetic tone,9,15–18 determining for a given increase in blood pressure small compensatory reflex responses, thus contributing to the maintenance of hypertension.

In normotensive freely moving rats, we showed that subpressor doses of Ang II administered either into the nucleus tractus solitarii or into the fourth cerebral ventricle caused a marked blunting of the reflex bradycardia, with the bradycardic response being significantly improved by the blockade of endogenous Ang II with saralasin into the nucleus tractus solitarii.19 We also showed that rats made hypertensive by subdiaphragmatic aortic constriction presented a significant depression of baroreceptor reflex control of heart rate,20 which was completely normalized by chronic treatment with losartan even with the persistence of hypertension.21 These observations indicate a specific effect of Ang II on the reflex control of heart rate other than that exerted on the blood pressure levels. These results, taken together with both the description of Ang II binding sites in all elements of the vagal afferent system (peripheral and central processes, nodose ganglion, brain stem nuclei containing terminal projections22,23) and the observation of changes in Ang II receptor density in the solitarii-vagal complex after sinoaortic denervation,24 prompted us to study whether Ang II modulates the afferent discharge of baroreceptors to the central nervous system. Therefore, the main objective of the present work was to determine the effects of chronic blockade of AT1-Ang II receptors on the aortic nerve activity of coarcted hypertensive and normotensive control rats during the control period and during loading/unloading of aortic baroreceptors.

Methods

Male Wistar rats aged 3 to 4 months and weighing 200 to 300 g were used. During the study period, the rats were housed in individual cages on a 12-hour light/dark schedule and allowed free access to...
To quantify the whole aortic nerve activity, the nerve traffic was obtained from the activity measured during each experimental situation. Control value. Complete curves and physiological range protocols were used as controls. CH, which induces hypertension of the upper part of systemic circulation was produced by partial subdiaphragmatic aortic constriction, according to a previously described technique.\textsuperscript{25,26} Briefly, under ether anesthesia, the rats were subjected to a median laparotomy, and the abdominal aorta was isolated just below the diaphragm, near the exit of the superior mesenteric artery. A cotton thread was used to constrict the aorta, the extent of narrowing being limited by a hypodermic needle (0.7 to 0.8 mm OD), according to the rat's body weight. The needle was removed and the abdomen sutured. SHAM rats were subjected to the same surgical procedures, except for the narrowing of the abdominal aorta. All rats received 60,000 IU penicillin (Pentabiotic Veterinário, Fontoura-Wyeth).

Arterial pressure was measured by the tail-cuff technique using a Programmed Electro Sphygmomanometer (model PE-300, Narco Bio-Systems) for 2 weeks before treatments were started and after 3 to 4 days of losartan or vehicle therapy, immediately before CH or SHAM surgery. Pressure was measured directly with a Gould-Statham P23 Db transducer in conscious rats on day 4 of CH or SHAM. The right common carotid artery and jugular vein were cannulated at the end of day 3 to allow complete recovery from ether anesthesia. On day 4 after measurement of baseline pressure for 20 to 30 minutes, the rat was anesthetized with pentobarbital sodium (40 mg/kg IV), had a second artery (left brachial) cannulated, and was prepared for recording of aortic nerve activity. The procedure used to record whole nerve activity of aortic baroreceptors was similar to that used in previous studies.\textsuperscript{26,27} Aortic fibers of the left isolated aortic nerve or as a branch isolated from the left recurrent laryngeal nerve in the lower part of the neck were used. To assure the stability of the neural recording, a flexible, thin 5-mm gold electrode (0.05 mm in diameter) covered by a vinyl tube (0.50×0.20 mm) was placed around the nerve and carefully insulated with silicone rubber (Wacker Sil-Gel 604, Wacker Co). Arterial pressure (brachial) and aortic nerve activity were continuously monitored on a oscilloscope (5115 Storage Tektronix) and recorded on-line with a computer. The experimental protocol consisted of measurements of aortic nerve activity/pressure relationship at baseline pressure (control) and during rapid changes in pressure (10 to 15 seconds) induced by blood withdrawal (≈0.08 mL/kg) and blood reinfusion (the volume withdrawn plus 0.04 to 0.05 mL/kg of blood from a donor rat, via the right carotid artery). The systolic pressure threshold (SP\textsubscript{th}) at which the aortic baroreceptors initiated firing, the saturation point (100% discharge), and the interval of pressure/nerve activity (range) were determined several times in each rat. To avoid influence of hysteresis, only the values obtained when pressure was changed from low to high levels were used to construct the aortic nerve function curves. To obtain the afferent activity/pressure relationship at the physiological range of pressure fluctuations (±10 mm Hg from control value). Complete curves and physiological range protocols were randomized. The data presented are the average of two to three consistent measurements made during each experimental situation. To quantify the whole aortic nerve activity, the nerve traffic was filtered through a bandpass of 100 to 3000 Hz, amplified, full-wave rectified, and integrated with a time constant of 3.9 milliseconds. The integrator output provides the nerve activity for each cardiac cycle. Pressure and nerve activity were sampled at 2000 Hz and subjected to analog-to-digital conversion (Codas, DataQ Instruments).

Blood Pressure Changes

In the control period, losartan-treated rats (4 days) were already hypertensive when compared with vehicle-treated animals (104±3 versus 117±3 mm Hg, tail pressure determinations, inset in Fig 1). Direct measurements of pressure in conscious rats (Fig 1) showed that CH\textsubscript{LOS} rats were hypertensive on day 4 of coarctation (a 28% increase; mean arterial pressure, 151±4 versus 118±3 mm Hg in SHAM\textsubscript{LOS}). In the chronic losartan-treated groups, baseline pressure was attenuated from the beginning, but CH\textsubscript{LOS} rats (mean arterial pressure, 130±4 mm Hg) were still hypertensive (a 25% increase) when compared with the SHAM\textsubscript{LOS} group (mean arterial pressure, 104±3 mm Hg).

Baroreceptor Function Curves

Pentobarbital anesthesia did not change the pressure levels presented by the four groups in the conscious state. The absolute afferent discharge of aortic nerve at the control period of CH and SHAM, treated with vehicle or losartan, is presented in the Table. For all groups, SP\textsubscript{th} values were similar to the respective baseline diastolic pressure.

Fig 2 illustrates and the Table summarizes the normalized afferent discharge/systolic pressure relationships for the four
Values of Aortic Nerve Activity and Arterial Pressure During the Control Period and Loading/Unloading of Aortic Baroreceptors in Anesthetized Hypertensive (CH) and Normotensive (SHAM) Rats Treated With Vehicle or Losartan

<table>
<thead>
<tr>
<th>Control values</th>
<th>SHAM(_{\text{VEH}})</th>
<th>CH(_{\text{VEH}})</th>
<th>SHAM(_{\text{LOS}})</th>
<th>CH(_{\text{LOS}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>AoN, mW/cycle</td>
<td>34 ± 6</td>
<td>37 ± 15</td>
<td>42 ± 7</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>AP, mm Hg</td>
<td>137 ± 2/110 ± 3 (124 ± 2)</td>
<td>169 ± 5/134 ± 8* (150 ± 6)*</td>
<td>126 ± 2/94 ± 4† (110 ± 3)†</td>
<td>140 ± 4†/108 ± 2† (124 ± 2)*†</td>
</tr>
</tbody>
</table>

Aortic Nerve Sensitivity in the Physiological Range

When the analysis of aortic nerve activity was restricted to a more physiological range of pressure changes (±10 mm Hg from the respective baseline control pressure), a different pattern was observed. In this range, a greater depression was observed in the gain of CH\(_{\text{VEH}}\) when compared with SHAM\(_{\text{VEH}}\) (−57%, from 5.05 ± 0.33 to 2.18 ± 0.37%/mm Hg; see Fig 3, left panel). In the losartan-treated group, CH still depressed the gain (−34%, from 4.97 ± 0.33 to 3.30 ± 0.33%/mm Hg), but the depression was significantly smaller than that of CH\(_{\text{VEH}}\). As observed in the right panel of Fig 3, aortic nerve activity was corrected during increases but not pressure decreases from the basal value. The gain of SHAM\(_{\text{VEH}}\), CH\(_{\text{VEH}}\), SHAM\(_{\text{LOS}}\), and CH\(_{\text{LOS}}\) afferents plotted against the respective baseline pressure (inset in Fig 3) showed a significant depression after CH in both CH\(_{\text{VEH}}\) and CH\(_{\text{LOS}}\), but in the absence of Ang II effects, the reduction in the gain was significantly smaller than that observed in the CH\(_{\text{VEH}}\) group (3.30 ± 0.33 versus 2.18 ± 0.37%/mm Hg, Table). The inset also discriminates between the different effects of chronic groups of rats. Hypertension displaced the SP\(_{\text{a}}\) to hypertensive levels (+8%) and reduced the slope of the curves, indicating afferent gain, which extended over a broader pressure interval (on average the range was increased by 66% CH\(_{\text{VEH}}\) versus SHAM\(_{\text{VEH}}\)). The aortic nerve activity curves of CH\(_{\text{VEH}}\) also showed greater variability. Treatment with losartan did not change the afferent activity curve of SHAM animals (Fig 2, lower panel left) but only displaced the SP\(_{\text{a}}\) to the left (from 109 ± 4 to 96 ± 4 mm Hg, Table) because of its pressure-lowering effect. Establishment of hypertension in losartan-treated rats also displaced the SP\(_{\text{a}}\) to hypertensive levels (96 ± 4 to 105 ± 4 mm Hg), but the individual variability was smaller than that of the CH\(_{\text{VEH}}\) group (Fig 2, lower panel right). The hypertension-induced decrease in the slope of the curve was still present in the CH\(_{\text{LOS}}\) group, but the depression (−25%) was less pronounced than that observed in the CH\(_{\text{VEH}}\) group (−36%, Table). This difference, however, was not significant because of the great variability of the CH\(_{\text{VEH}}\) group.

Figure 2. Normalized aortic nerve activity/systolic pressure curves of normotensive (SHAM, left) and hypertensive (CH, right) rats treated with vehicle (upper panels) or losartan (lower panels). Curves were obtained on day 4 after coarctation or sham surgery.

Figure 3. Percent changes of aortic nerve activity in the physiological range of pressure changes of coarcted (CH) and sham-coarcted rats (SHAM) treated chronically with vehicle or losartan. Relationships were determined on the 4th day after surgery. C represents the control pressure of each group. Inset: Reduction in the slope of the afferent discharge (gain) induced by coarctation in vehicle- and losartan-treated groups. Significance at P<.05: *vs SHAM; †vs VEH; ‡CH\(_{\text{LOS}}\) vs SHAM\(_{\text{LOS}}\).
Losartan treatment: (1) the pressure-lowering effect demonstrated by the displacement of the points to the left, according to the reduction of baseline blood pressure, and (2) the partial restoration of afferent gain during establishment of hypertension, since the line representing the magnitude of gain reduction was significantly smaller in the presence of losartan. On the other hand, losartan did not change the sensitivity of the afferent discharge in SHAM rats.

Discussion

The present new set of data demonstrates that Ang II, activated by CH, impairs the aortic nerve afferent activity/pressure relationship, depressing the gain in the physiological range of pressure fluctuations and increasing the variability of the afferent discharge. The depression of aortic nerve sensitivity after coarctation is significantly smaller in animals receiving chronic AT, receptor blockade. This effect occurs simultaneously with the pressure-lowering effect of losartan.

In previous studies on baroreceptor reflex control of heart rate during the development of hypertension, we demonstrated activation of the renin-angiotensin system in the chronic phase of CH,29 depression of both reflex bradycardia and reflex tachycardia by the increased levels of endogenous Ang II,20,21 and complete normalization of the baroreceptor reflex control of heart rate by chronic treatment with losartan.21 In this model of hypertension, we also described that the sensitivity of aortic baroreceptors was reduced, with a blunted afferent activity/pressure relationship occurring during the chronic phase.27 What we show here is that part of the depression of baroreceptor reflex control of heart rate could be attributed to the effects of Ang II on the afferent signaling of pressure levels by aortic baroreceptors. The presence of Ang II binding sites in the central and peripheral processes of the vagus nerve, in the nodose ganglion, and in the brain stem nuclei containing terminal projections,22,23 together with the demonstration of decreased receptor density in the nucleus tractus solitarii after sinoaortic denervation,24 suggested that Ang II could modulate the transmission of neuronal inputs from the periphery to the central nervous system. Our data, showing that the sensitivity of afferent discharge in losartan-treated CH rats is markedly increased relative to their sham-coarcted controls, confirm the previous suggestion and demonstrate that this effect is mediated by AT, receptors.

Both AT, and AT, receptor subtypes have been mapped in the central nervous system of the rat and of humans,4,6 but most of the functional Ang II effects are mediated by the G protein–coupled AT, receptor. In addition, brain stem structures involved in cardiovascular control such the nucleus tractus solitarii and dorsal motor nucleus of the vagus, as well as the area postrema, have been shown to contain AT,Ang II receptors exclusively.4,4 The AT,-mediated Ang II–depressing effect on the baroreflex is very complex, involving, as shown here, changes in baroreceptor afferent activity. Previously, it has been shown that Ang II also has an inhibitory effect on vagal efferents2,11 and attenuates sympathetic activity.9,15 It is possible that both the depression of reflex bradycardia by Ang II administration into the nucleus tractus solitarii of conscious19 or anesthetized rats20,28 and the improvement of the bradycardic response by endogenous block-ade of Ang II at this level11,19 are partially due to the inhibitory effect of Ang II on aortic nerve afferent discharge. We cannot exclude possible concomitant changes in the carotid sinus afferent activity (not measured in the present study).

It should be stressed that the modulatory effect of Ang II on aortic nerve activity is not exclusively dependent on its blood pressure effects. Indeed, losartan produced an equivalent percent decrease of blood pressure in hypertensive and control animals (CH, −14%, SHAM, −12% in the conscious state [Fig 1]: CH, −17%, SHAM, −11% after anesthesia [Table]). In contrast, gain increased and SPa decreased only in the CH,LOS group. It is true that levels of hypertension differed between CH,VEH and CH,LOS, but there are no data in the literature correlating decrease in baroreceptor afferent gain with the severity of hypertension. In previous studies,26,27 we showed a comparable reduction of gain after 2 or 6 days of moderate hypertension (CH) and after 2 months of severe renal hypertension. In addition, the observation that hypertension-induced reduction of gain was significantly smaller in losartan-treated than in the vehicle-treated group indicates a specific effect of Ang II on the baroreceptor afferent discharge that occurs simultaneously with the effect on blood pressure. Dissociation between pressure level/plasma renin activity28 and between plasma renin activity/lower brain stem angiotensinogen mRNA level10 has been demonstrated in rats made hypertensive by ligation of the aorta between the renal arteries. In accordance, we have previously shown that the modulatory effect of Ang II on baroreceptor reflex control of heart rate was not dependent on the pressure level, since supressor doses of Ang II administered into the nucleus tractus solitarii of normotensive rats depressed the reflex bradycardia19 and CH rats treated chronically with losartan showed normalization of bradycardic response even in the persistence of hypertension.21

In a recent report, Collister et al10 showed that chronic treatment with losartan was able to lower arterial pressure of normotensive rats on a normal sodium diet. Accordingly, a similar daily dose of losartan caused a reduction of pressure levels in CH and SHAM groups, which was of similar magnitude and maintained throughout the experiment. Indicative of a chronic effect of losartan on blood pressure levels was the observation that aortic afferent discharge was reset to the existing pressure in all groups studied, as revealed by the similarity between SPa and baseline diastolic pressure. The losartan-induced reduction in pressure emphasizes the importance of endogenous Ang II levels in the maintenance of basal vasoconstriction and in the determination of baseline blood pressure in both groups. In the CH groups, the huge constriction of the upper abdominal aorta, imposing a large resistance to blood circulation (mechanical factor3) determined the additional increase in pressure. The mechanical factor was not altered by losartan treatment because mean arterial pressure of CH,VEH and CH,LOS were similarly elevated when compared with the respective SHAM groups (+28% and +25%, respectively).

Another finding of the present study is that chronic losartan treatment did not change the magnitude of control afferent discharge (in millivolts per cardiac cycle) of CH or SHAM groups. Accordingly, losartan has been shown not to alter the
baseline firing pattern of Ang II–sensitive neurons in the rat medial nucleus tractus solitarii, but only to reverse the increased firing and to block the excitation induced by Ang II administration.\textsuperscript{32}

In summary, the present data showed that Ang II, stimulated after CH and acting on AT\textsubscript{1} receptor, depresses the gain of aortic nerve afferents during increases and decreases in pressure, thus contributing to the deficient signaling of pressure levels by aortic baroreceptors in the chronic phase of hypertension. Chronic treatment with losartan, in addition to having a blood pressure–lowering effect, enhances the sensitivity of aortic nerve discharge during loading and unloading of receptors in the physiological range of pressure changes.

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