Time Course of Changes in Sigmoidal-Fitting Baroreceptor Curves in One-Kidney, One Clip Hypertensive Rats

Margareth R. Moyses, Antonio M. Cabral, Nazare Bissoli, Elisardo C. Vasquez

Abstract The present study examined the time course of changes in baroreceptor reflex function by means of sigmoidal curve-fitting analysis in conscious, unrestrained renovascular one-kidney, one clip (1K1C) rats at 1, 3, 7, 15, 30, and 60 days after renal artery clipping. The reflex heart rate responses were elicited by alternate intravenous bolus injections of phenylephrine (change, +5 to +50 mm Hg) and sodium nitroprusside (change, -5 to -50 mm Hg). Atropine methyl-nitrate and atenolol were given to evaluate the responses mediated by the cardiac sympathetic or vagal component, respectively. The average baroreceptor reflex gain (sensitivity) decreased progressively (day 1, 3.35±0.3 beats per minute [bpm] per millimeter of mercury), reaching a maximal attenuation in the 30-day 1K1C group (1.83±0.5 bpm/mm Hg) compared with sham rats (approximately 4.60 bpm/mm Hg).

The data showed a decreased vagal activity contributing to the attenuation of the baroreceptor reflex gain only in the 30-day 1K1C group. In contrast, the cardiac sympathetic component of the baroreceptor reflex was significantly decreased in all 1K1C groups (from 2.10±0.4 to 0.50±0.2 bpm/mm Hg) compared with the respective sham groups (from 3.80±0.3 to 3.10±0.4 bpm/mm Hg). These results suggest that a reduced contribution of the sympathetic component to the baroreceptor heart rate reflex may be the main cause of the progressive attenuation of the baroreceptor reflex sensitivity observed in conscious 1K1C hypertensive rats. (Hypertension. 1994;23[suppl I]: I-87-I-92.)

Key Words • hypertension, renovascular • pressoreceptors • blood pressure • heart rate

Methods

Experimental Animals

Experiments were performed in 108 adult male Wistar rats (200 to 300 g) supplied by the breeding unit of Animal Care, Department of Physiological Sciences, Biomedical Center. Rats were housed at a controlled temperature (25°C) and exposed to a 12-hour light/dark cycle each day. Animals were allowed free access to water and food. All experiments were conducted in conformity with the international guiding principles for biomedical research involving animals (CIOMS).

Renovascular Hypertension

With rats under ether anesthesia, renovascular hypertension (Goldblatt 1K1C model) was obtained by placing a preset U-shaped silver clip (0.2 mm internal diameter) around the left renal artery, combined with nephrectomy of the right kidney through a median abdominal incision. Weight-matched controls were sham-operated rats submitted to right-sided nephrectomy. After the procedures, the muscle layers were...
reconnected, and the skin incision was closed. Rats were placed in individual plastic cages with supplies of water and food pellets. The animals were divided into six groups of 1K1C and six of sham rats, with nine rats in each group. They were studied at 1, 3, 7, 15, 30, and 60 days after surgery.

**Hemodynamic Measurements**

Arterial pressure measurements were recorded directly through an indwelling catheter. A polyethylene catheter (PE-50 with tapered end) filled with heparinized saline (40 U/mL) was positioned in the aorta through the left femoral artery with rats under ether anesthesia. Another catheter was inserted into the inferior vena cava through the left femoral vein for administration of drugs. The free ends of the cannulas were tunneled under the skin of the back and exteriorized between the scapulae. Approximately 6 hours later, the arterial catheter was connected to a 1280C pressure transducer by flexible connecting tubing, and hemodynamic measurements were continuously recorded on a polygraph (7754B, Hewlett-Packard Co, Palo Alto, Calif). MAP was obtained by electronic averaging of the arterial pulsatile pressure. HR was computed from the pulsatile signal.

**Baroreceptor Reflex Testing**

Experiments were performed 1, 3, 7, 15, 30, and 60 days after renal artery clipping in conscious, freely moving rats. Alternate intravenous injections of 1 to 50 μL of phenylephrine (0.2 to 10.0 μg/kg; Sigma Chemical Co, St Louis, Mo) and sodium nitroprusside (0.5 to 20.0 μg/kg; Hypofarma, Minas Gerais, Brazil) were given with 50-μL glass syringes (Hamilton Co, Reno, Nev) to produce a series of increases and decreases in MAP (approximately 5 to 50 mm Hg) in each animal. After baroreceptor reflex testing was complete, responses mediated only by cardiac sympathetic nerves were assessed by determining the MAP-HR responses after vagal inhibition with atropine methylnitrate (1 mg/kg IV, Sigma). The adequacy of the blockade was assessed by the bradycardia evoked by a bolus injection of acetylcholine (1 μg/kg). On the following day, the baroreceptor reflex testing was repeated, and the contribution of the cardiac vagal component was assessed by blocking the sympathetic component with the β1-adrenergic receptor antagonist atenolol (1 mg/kg IV, Sigma). Adequate atenolol blockade was assessed by observing the lack of a chronotropic response to an intravenous bolus injection of isoproterenol (0.1 μg/kg). During the barorecep-
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with atropine (see Fig 2). As previously reported, 13 a

expected, 24 hours after renal artery clipping, the

of hypertension 7 days after surgery. A similar pattern

tive sham rats at 1, 3, 7, 15, 30, and 60 days after renal

adjustment for multiple comparisons. Significance was ac-

tor curves (with both sympathetic and vagal components

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itor reflex testing, there was sufficient time between injections to allow both MAP and HR to return to basal values. Peak values of MAP and HR in response to phenylephrine and sodium nitroprusside injections were fitted to a sigmoidal logistic equation. The best-fitting curve method was similar to that used by others16 and based on the Marquardt algorithm.17 All curves were forced to go through the average basal MAP and HR values.

Data Analysis and Statistics

Results are expressed as mean±SEM. The basal MAP and HR and the sigmoidal-fitting baroreceptor curve parameters were analyzed using two-way analysis of variance with repeated measures. Significance of differences between means was determined by calculation of the r statistic with the Tukey adjustment for multiple comparisons. Significance was accepted at a value of P<.05.

Results

The 1K1C rats showed a reduction in growth, as indicated by the decreased (P<.01) body weight at 30 and 60 days after renal artery clipping (Table). As expected, 24 hours after renal artery clipping, the resting MAP was significantly higher in 1K1C than sham rats, and it increased progressively, reaching a plateau of hypertension 7 days after surgery. A similar pattern of resting MAP values was observed after cardiac sympathetic blockade with atenolol or vagal blockade with atropine (see Fig 2). As previously reported,13 a transitory tachycardia was observed in 1K1C rats (7 to 15 days) that was abolished by atenolol.

Fig 1 shows the average sigmoidal-fitting baroreceptor curves (with both sympathetic and vagal components intact), comparing conscious 1K1C rats with the respective sham rats at 1, 3, 7, 15, 30, and 60 days after renal artery clipping. Clear upper and lower plateaus (reflex tachycardia and bradycardia, respectively) were noted at all stages of hypertension in both sham and 1K1C rats. The 1K1C baroreceptor curves were shifted to the right of sham rats, closely following the changes in resting MAP. A progressive attenuation of baroreceptor reflex HR gain was observed in 1K1C rats, reaching a maximal decrease at 30 days (−60%) compared with the 30-day sham group (Table). The upper plateau was significantly attenuated at 30 days, and the lower plateau was significantly attenuated from 7 to 60 days in 1K1C rats compared with sham rats. As a consequence of the attenuation in both plateaus, a significant lower HR range was observed from 3 days on and was drastically reduced at 30 days (49±10 beats per minute [bpm]) in 1K1C rats compared with sham rats (186±5 bpm).

The cardiac sympathetic blocker atenolol did not affect the resting MAP in both 1K1C and sham rats but completely abolished the resting tachycardia observed in the 7- and 15-day 1K1C groups. As expected, atro-

pine did not cause significant changes in MAP and increased the HR of 1K1C and sham rats to similar levels. The cardiac vagal blockade did not revert the attenuated baroreceptor average gain in 1K1C rats (1 to 60 days) compared with sham rats (Figs 2 and 3). On the contrary, under these conditions, the reduction in baroreceptor reflex gain at 30 days in 1K1C rats was even worse compared with the 30-day sham group (0.5±0.2 versus 3.7±0.3 bpm/mm Hg, respectively, P<.01). In contrast, no significant differences in baroreceptor gain between 1K1C and sham groups were observed after the cardiac sympathetic blockade with atenolol, with the exception of the 30-day 1K1C group.

<table>
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<tr>
<th>Parameter</th>
<th>Group</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>15</th>
<th>30</th>
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<tr>
<td>Body weight, g</td>
<td>Sham</td>
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<td>139±3*</td>
<td>157±8*</td>
<td>152±5*</td>
<td>163±7*</td>
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<td>Heart rate, bpm</td>
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<td>401±9*</td>
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<td>Lower plateau, bpm</td>
<td>Sham</td>
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<td>104±8*</td>
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<td>Average gain, bpm/mm Hg</td>
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<td>-2.7±0.2*</td>
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<td>96±1</td>
<td>101±1</td>
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<tr>
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<td>113±6†</td>
<td>127±5*</td>
<td>151±11*</td>
<td>139±5*</td>
<td>181±6*</td>
<td>149±5*</td>
</tr>
</tbody>
</table>

1K1C indicates one-kidney, one clip; bpm, beats per minute; and BPao, mean arterial pressure at the midpoint of heart rate range.

Values are mean±SEM.

*P<.01, †P<.05 compared with sham group.
Before Blockade

Vagal Component

Sympathetic Component

1 day

3 days

7 days

15 days

30 days

60 days

FIG 2. Plots show relation between mean arterial pressure and heart rate obtained before and after β₁-adrenergic blockade with atenolol (vagal component) or after cholinergic blockade with atropine methylnitrate (sympathetic component). Circles are resting values. No significant differences were observed between sham groups at different time points. Top left shows baroreceptor curves of the 1-day sham group; other panels show baroreceptor curves of one-kidney, one clip groups from 1 to 60 days after surgery. bpm, beats per minute.

(1.0±0.3 versus 2.3±0.2 bpm/mm Hg, respectively, \(P<.01\)). The vagal but not the sympathetic blockade completely reverted the alterations in lower and upper plateaus (maximal reflex bradycardia and tachycardia, respectively) observed in 1K1C rats compared with sham rats (Fig 2).

Discussion

The present work is the first to show changes in baroreceptor reflex in conscious renovascular 1K1C hypertensive rats using a sigmoidal logistic function.\(^1\,\,14,\,16\) This method was first used in conscious normotensive rats by Head and McCarty\(^16\) and showed that the relation of HR and MAP followed a sigmoidal rather than a linear function (\(P<.001\)). The aim of this study was not to compare the two methods of study but to compare the baroreceptor reflex function in 1K1C rats 1, 3, 7, 15, 30, and 60 days after renal artery clipping using the sigmoidal-fitting baroreceptor curve analysis.

Our results clearly demonstrate a progressive decrease of the baroreceptor reflex sensitivity, which was already significantly attenuated at 1 day and reached the lowest value at 30 days after renal artery clipping. One could speculate that this reduction in baroreceptor reflex sensitivity was caused or at least significantly influenced by the renin-angiotensin system\(^15,\,19\) and/or
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but not at other times, contrasting with the huge reduction in the sympathetic component of the baroreceptor reflex gain in the 1K1C groups from 1 to 60 days. This sympathetic-mediated reduction in reflex sensitivity was even observed (not surprisingly) in those groups 7 and 15 days after clipping in which we had previously reported an increase in resting cardiac sympathetic tone. Therefore, our results showing that the progressive attenuation of the baroreceptor reflex gain in 1K1C rats was confined to the cardiac sympathetic component (except for the 30-day 1K1C group, in which the vagus was also significantly involved) contrast with studies in other experimental models of hypertension and in clinical hypertension, in which a decreased reflex vagal activity seems to be the most important contribution to the attenuation of baroreceptor reflex gain. In those studies, the depression in vagal HR range and baroreceptor reflex gain were consequences of arterial hypertension. This could be the same for our 30-day 1K1C group, which presented the highest level of hypertension. On the other hand, it was not surprisingly an impairment of the sympathetic component of the baroreceptor reflex that could be a consequence (downregulation) of the high cardiac sympathetic tone, as has been observed in renovascular hypertensive rats, and in clinical hypertension. Additional studies could elucidate the benefit (if any) of a reduced sympathetic component of the baroreceptor reflex HR sensitivity in renovascular 1K1C hypertension. However, it remains a controversial question as to whether the decreased sensitivity of the baroreceptor reflex function is a consequence of hypertension or a causal factor in the development of elevated arterial pressure in humans.

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