Potentiation of the Baroreceptor–Heart Rate Reflex By Sympathectomy in Conscious Rats

Alberto U. Ferrari, Anna Daffonchio, Cristina Franzelli, and Giuseppe Mancia

In both animals and humans, stimuli leading to sympathetic activation are accompanied by an impairment of the baroreceptor–heart rate reflex. To determine whether sympathetic activity normally interferes with this reflex function we examined in conscious Wistar-Kyoto (WKY) rats the effect of chemical sympathectomy by 6-hydroxydopamine on the bradycardic response to baroreceptor stimulation induced by raising blood pressure via intravenous phenylephrine boluses; control rats received vehicle. Spontaneously hypertensive rats were also studied because in these animals there is both a baroreceptor reflex impairment and a sympathetic overactivity. Baroreceptor reflex sensitivity, calculated as the ratio of the peak increase in pulse interval to the peak increase in mean arterial pressure, was 75% greater in sympathectomized WKY rats than in control WKY rats (1.28±0.15 versus 0.73±0.10 msec/mm Hg, mean±SEM; p<0.01). The sympathectomy-induced increase in sensitivity was even larger in spontaneously hypertensive rats (SHR) (1.26±0.12 versus 0.44±0.06 msec/mm Hg in sympathectomized SHR versus control SHR, +186%; p<0.01) so that the impaired baroreceptor reflex sensitivity observed in control SHR as compared with control WKY rats (−40%, p<0.01) was no longer detectable in the sympathectomized groups. To establish whether the sympathectomy-induced potentiation of the reflex was due to an increase in cardiac responsiveness to vagal stimuli, we subjected separate groups of anesthetized, vagotomized SHR and WKY rats to graded electrical stimulation of the right efferent vagus. The bradycardic effects of vagal stimulation, however, were similar in sympathectomized and control animals. It is concluded that 1) sympathetic nerve activity normally exerts an antagonistic effect on the baroreceptor–heart rate reflex, 2) this phenomenon is much more pronounced in SHR than in WKY rats and may contribute to the baroreceptor reflex impairment typical of the former animals, and 3) the sympathectomy-induced potentiation of the reflex does not depend on an increase in cardiac vagal responsiveness. (Hypertension 1991;18:230–235)
age of 10–11 weeks and was studied within the following 7–10 days. Sympathectomy was obtained by 6-OHDA (100–150 mg/kg) administered two to three times over a 1-week period. The drug was injected intraperitoneally except for the final dose, which was given as a slow intravenous infusion. Control rats from either strain received injections of vehicle alone. To protect the animals from the release of catecholamines, the marked rise in blood pressure, and sometimes the fatal pulmonary edema associated with the first dose of 6-OHDA, the α-adrenergic antagonist phentolamine (150 μg/kg) was administered with the first dose of 6-OHDA. The adequacy of sympathectomy was verified by the drastic attenuation of the pressor and tachycardic responses to tyramine (150 μg/kg i.v.) (see below).

Animal Instrumentation

The sympathectomized and control rats were anesthetized with ketamine (80 mg/kg i.p.) and were instrumented with polyethylene cannulas in a femoral artery and a femoral vein. The cannulas were passed subcutaneously, were exteriorized at the interscapular region, and were kept patent by twice daily flushing with heparinized (5 units/ml) saline solution. After surgery the animals were housed in a wide individual cage for a 24–48 hour period. This allowed them to recover and become acclimated to the environment.

Separate groups of sympathectomized and control rats from the WKY rat and SHR strains were also anesthetized with ketamine (80 mg/kg i.p.). Implantation of the intravascular cannulas was followed by an anterior midline incision of the neck and isolation and section of the vagi. The peripheral end of the right vagus was immersed in a pool of mineral oil and placed on a stainless steel electrode connected to a model S88 stimulator (Grass Instruments, Quincy, Mass.). This was done to examine whether sympathectomy affected the baroreceptor reflex by altering the cardiac responsiveness to vagal stimuli (see below).

Protocol and Data Analysis

In each rat, blood pressure was measured by connecting the arterial catheter to a Statham P23De (Gould, Inc., Oxnard, Calif.) transducer and was continuously displayed on a Grass 7D polygraph. Mean arterial pressure was obtained by electronic damping of the pulsatile signal. Heart rate was monitored on a beat-to-beat basis by a tachograph triggered by the blood pressure wave. In the conscious animals equipped to examine the baroreceptor reflex, the cage was maintained in a moderately illuminated and silent room. Animal manipulation and arousal were minimized by connecting the venous catheter with a long thin tubing, which allowed the investigator to be out of the animal’s sight. The pressor and tachycardic responses to tyramine were tested by an intravenous injection of 150 μg/kg of the drug. Then a 30-minute period was allowed to obtain drug washout and hemodynamic recovery. Finally, arterial baroreceptors were stimulated by raising blood pressure via 2–4 intravenous bolus injections of phenylephrine at the doses of 1 and 2 μg/kg. Each injection was separated from the following one by a 3-minute interval. The ratio of the peak increase in pulse interval to the peak increase in mean arterial pressure was calculated for each injection. The average ratio from all injections was defined as the animal’s baroreceptor reflex sensitivity. The baroreceptor–heart rate reflex could be evaluated from the bradycardia induced by phenylephrine because this reflex response depends almost entirely on cardiac vagal drive and is thus not directly interfered with by 6-OHDA. Such an interference does occur, on the other hand, for the sympathetically mediated tachycardia induced by nitroprusside, so that this technique was not feasible for the purpose of our study.

In the anesthetized vagotomized animals, the right vagus was electrically stimulated at the frequency steps of 1, 2, 4, and 8 Hz delivered in random order. Pulse voltage and duration were kept constant (5 V, 2 msec). Each step lasted 10–15 seconds and was separated from the following one by a 3-minute interval. Stimulus-response curves were obtained in each of the four groups by plotting the peak bradycardic response observed during each step against the respective stimulation frequency. In keeping with the aims of this part of the study, consideration was given to the possible differences between the sympathectomized and the control group within each WKY rat and SHR strain, whereas the possible differences between the two strains were neglected.

Statistical Comparisons

Individual data were averaged to obtain mean±SEM. Data from 6-OHDA–treated versus vehicle–treated and from SHR versus WKY rats were compared by the Wilcoxon nonparametric rank test complemented by the Kruskal-Wallis test for multiple comparisons. The statistical significance was set at p<0.05.

Results

At the time of study, the body weight was 254.2±15.3 and 244.5±18.9 g in control and 6-OHDA–treated WKY rats, respectively. The corresponding values in control and 6-OHDA–treated SHR rats were 247±13.7 and 253±19.3 g.

Responses to Tyramine

As shown in Figure 1 and in the group data of Table 1, the pressor response to intravenous tyramine was significantly and markedly less pronounced in the 6-OHDA–treated than in the control vehicle–treated rats. This was the case also for the concomitant tachycardic response. For either response, the attenuation induced by 6-OHDA treatment was similarly pronounced in SHR and WKY rats.
TABLE 1. Pressor and Tachycardic Response to Tyramine in Vehicle-Treated and 6-Hydroxydopamine-Treated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTACT WKY (n = 19)</td>
<td>+29.6±5.9</td>
<td>+31.5±11.1</td>
</tr>
<tr>
<td>SYMPX WKY (n = 18)</td>
<td>+6.1±1.35*</td>
<td>+8.8±2.1*</td>
</tr>
<tr>
<td></td>
<td>(-79.4%)</td>
<td>(-72.1%)</td>
</tr>
<tr>
<td>INTACT SHR (n = 18)</td>
<td>+33.1±3.8</td>
<td>+42.4±7.7</td>
</tr>
<tr>
<td>SYMPX SHR (n = 19)</td>
<td>+6.8±1.5*</td>
<td>+5.9±1.8*</td>
</tr>
<tr>
<td></td>
<td>(-79.6%)</td>
<td>(-86.1%)</td>
</tr>
</tbody>
</table>

Entries are mean±SEM from all animals. Data from the unanesthetized and anesthetized animals were pooled. Figures in parentheses refer to the percent reductions in the response observed in SYMPX vs. INTACT rats. MAP, mean arterial pressure; HR, heart rate; INTACT, vehicle-treated control group; SYMPX, sympathectomized rats.

*p<0.01 vs. INTACT rats of the same strain.

Baroreceptor–Heart Rate Reflex

As shown in Table 2, treatment with 6-OHDA was associated with a moderate reduction of baseline blood pressure in both SHR and WKY rats. Baseline heart rate was slightly higher in sympathectomized than in control rats, but for both the WKY and the SHR strain the difference was not statistically significant.

The effects of sympathectomy on the baroreceptor–heart rate reflex are shown in Figure 2 and in the group data shown in Figure 3 and Table 3. The bradycardic response to baroreceptor stimulation was clearly greater in sympathectomized as compared with control WKY rats with both the 1 µg/kg and the 2 µg/kg phenylephrine dose (Table 3).

FIGURE 1. Original recordings show pressor and tachycardic response to intravenous tyramine in a control (INTACT) and a 6-hydroxydopamine–treated (SYMPATHECTOMY) conscious rat. Note almost complete abolition of both responses in sympathectomized animal.

FIGURE 2. Original recordings illustrate bradycardic response to baroreceptor stimulation by intravenous phenylephrine (PNE) in unanesthetized Wistar-Kyoto rats (top panel) and spontaneously hypertensive (SHR) rats pretreated with vehicle (INTACT) or with 6-hydroxydopamine (SYMPATHECTOMY). Note potentiation of response associated with sympathectomy and much larger extent of this phenomenon in SHR.
was the case also for sympathectomized SHR versus control SHR. In SHR, however, the sympathetically-induced enhancement of baroreceptor reflex sensitivity was much greater than in WKY rats (186% versus 75%). The baroreceptor reflex sensitivity was lower in control SHR as compared with control WKY rats (−40%, \( p<0.01 \)). This difference was no longer detectable after sympathectomy.

**Cardiac Vagal Responsiveness**

Graded electrical stimulation of the right efferent vagus-induced bradycardic responses of the same order of magnitude as those elicited by baroreceptor stimulation. In both SHR and WKY rats, the stimulus-response relation observed in the control animals was not modified by sympathectomy (Figure 4). Absence of any sympathetically-induced change was confirmed by treating the data as linear regressions of the bradycardic response to the stimulus and statistically comparing the slopes of the regression lines obtained in the sympathectomized versus the control rats (data not shown).

**Discussion**

Our study shows that the bradycardic response to arterial baroreceptor stimulation is greater in normotensive conscious rats pretreated with 6-OHDA at a dose capable of producing an extensive destruction of the adrenergic nervous system than in vehicle-treated animals. This demonstrates for the first time that 1) sympathetic activity exerts an antagonistic influence on the baroreceptor–heart rate reflex and 2) this influence occurs not only under a physical or psychological challenge but also during daily life in the absence of any stressful stimuli.

A further new finding of our study is that in SHR, 1) sympathectomy is associated with an increase in baroreceptor–heart rate reflex sensitivity more than twice as large as in WKY rats and 2) the increase is so pronounced as to abolish the baroreceptor reflex impairment characterizing the intact SHR. Thus, the latter phenomenon is entirely accounted for by an enhanced antagonizing influence of the sympathetic nervous system. Of course, this applies to the 12-week-old animals used in our experiments, and it cannot be excluded that in older SHR, structural rather than functional factors perpetuate the baroreceptor reflex impairment.

The mechanisms responsible for the negative effect of the sympathetic nervous system on the baroreceptor–heart rate reflex are not clarified by our study. However, the increase in baroreceptor reflex sensitivity did not depend on the fact that the blood pressure fall induced by sympathectomy moved the reflex toward a steeper portion of the stimulus–response curve because 1) in both SHR and WKY rats the baroreceptor reflex operates well within the linear portion of this curve; 2) the blood pressure fall induced by sympathectomy was not modified by sympathectomy (Figure 4).

**Table 3. Increases in Mean Arterial Pressure and in Pulse Interval and Calculated PI/MAP Ratios Separately Averaged for the 1 and 2 μg/kg Phenylephrine Doses in Four Groups of Rats**

<table>
<thead>
<tr>
<th>Phenylephrine (1 μg/kg)</th>
<th>MAP (mm Hg)</th>
<th>PI (msec)</th>
<th>PI/MAP (msec/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTACT WKY (n=12)</td>
<td>27.3±1.9</td>
<td>19.6±1.9</td>
<td>0.71±0.1</td>
</tr>
<tr>
<td>SYMPX WKY (n=11)</td>
<td>36.1±2.9</td>
<td>41.2±5.7</td>
<td>1.17±0.2*</td>
</tr>
<tr>
<td>INTACT SHR (n=9)†</td>
<td>35.6±2.3</td>
<td>17.1±3.7</td>
<td>0.46±0.1‡</td>
</tr>
<tr>
<td>SYMPX SHR (n=12)†</td>
<td>32.7±3.5</td>
<td>38.9±5.2</td>
<td>1.21±0.1*</td>
</tr>
<tr>
<td>Phenylephrine (2 μg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTACT WKY (n=12)</td>
<td>39.3±2.4</td>
<td>31.3±3.4</td>
<td>0.77±0.1</td>
</tr>
<tr>
<td>SYMPX WKY (n=11)</td>
<td>39.8±3.8</td>
<td>52.4±4.3</td>
<td>1.39±0.2*</td>
</tr>
<tr>
<td>INTACT SHR (n=10)</td>
<td>47.5±2.4</td>
<td>22.7±3.8</td>
<td>0.47±0.1§</td>
</tr>
<tr>
<td>SYMPX SHR (n=13)</td>
<td>43.2±3.6</td>
<td>53.7±5.8</td>
<td>1.28±0.1*</td>
</tr>
</tbody>
</table>

Entries are mean±SEM.

\*\( p<0.01 \) vs. INTACT rats of the same strain.

†In one rat from this group no valid 1 μg/kg test could be obtained.

§\( p<0.01 \) vs. INTACT WKY rats.

MAP, mean arterial pressure; PI, pulse interval; INTACT, vehicle-treated control rats; WKY, Wistar-Kyoto rats; SYMPX, sympathectomized rats; SHR, spontaneously hypertensive rats.
fall induced by sympathectomy was of small and similar extent in SHR and WKY rats, whereas the baroreceptor reflex potentiation was marked in both strains and much greater in the latter than in the former; and 3) in all groups of rats the ratio between the reflex increase in pulse interval and the phenylephrine-induced blood pressure rise was similar for the smaller and larger blood pressure change induced by the lesser and greater dose of phenylephrine, documenting that the baroreceptor stimuli did not approach reflex saturation in either intact or sympathectomized animals.

It can also be excluded that sympathectomy increased the sinus node responsiveness to the baroreceptor modulation of cardiac autonomic drive because the bradycardic response to electrical stimulation of vagal efferent fibers (i.e., the fibers largely responsible for the reflex cardiac effects) was superimposable in the vehicle-treated and sympathectomized rats. Therefore, the baroreceptor reflex potentiation could only originate from central or afferent mechanisms, or both.

A central mechanism might appear to be unlikely because 1) there is no evidence that peripherally administered 6-OHDA destroys catecholaminergic neurons in the central nervous system, and 2) when delivered directly into the brain, this substance impairs rather than augments the reflex responses mediated by arterial baroreceptors. It can be argued, however, that in the absence of sympathetic nerves, blood pressure may have been supported by angiotensin II and vasopressin (i.e., by two substances affecting the central integration of the baroreceptor–heart rate reflex). Vasopressin does in fact markedly increase this reflex function, possibly explaining our results.

These can also be explained by underlying afferent mechanisms; indeed, α-adrenergic blockade has been reported to increase arterial distensibility, thereby augmenting the responsiveness of baroreceptors to their physiological stimulus. Such a mechanism is also compatible with the evidence that the impairment of aortic baroreceptors characterizing SHR as compared with WKY rats mainly depends on an abnormally high aortic stiffness, which might have been reduced by sympathectomy to a larger extent in the former than in the latter animals.

Our data have physiological and pathophysiological implications. It has recently been observed that the sensitivity of the baroreceptor–heart rate reflex undergoes major moment-to-moment changes when assessed in unrestrained animals and in ambulant humans. In light of the present data, we can speculate that these changes depend on moment-to-moment changes in sympathetic tone. We can also speculate that the impairment of the baroreceptor–heart rate reflex associated with essential hypertension is due to the increase in sympathetic tone believed to characterize this condition. The same may apply to the baroreceptor reflex impairment associated with aging, congestive heart failure, and acute myocardial infarction because these conditions are also characterized by sympathetic overactivity. It may thus be that an exaggerated sympathetic activity represents a pathogenetic factor contributing to baroreceptor reflex impairment in different cardiovascular diseases.

References

KEY WORDS • baroreceptors • phenylephrine • experimental hypertension • sympathetomy • hydroxydopamines • rat studies
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