Cardiac Parasympathetic Hyperresponsiveness in Spontaneously Hypertensive Rats

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The bradycardic response to baroreceptor stimulation is impaired in human and experimental hypertension. Because this bradycardia mainly depends on the vagus, this may reflect a reduced cardiac parasympathetic responsiveness, which would parallel the reduced cardiac adrenergic responsiveness observed in hypertension. To test this hypothesis, 12-week-old spontaneously hypertensive rats (n = 12) and normotensive Wistar-Kyoto rats (n = 11) were anesthetized with ketamine and underwent bilateral vagotomy. Cardiac parasympathetic responsiveness was assessed from the bradycardia induced by 1) graded electrical stimulation of the right efferent vagus (1–16 Hz) and 2) graded intravenous injections of methacholine (1–8 μg · kg⁻¹). The slope of the linear regression between the bradycardic response and the applied stimulus was taken as the measure of cardiac parasympathetic responsiveness. To identify the onset of possible alterations in cardiac parasympathetic responsiveness in hypertension, the study was extended to younger (8-week-old) spontaneously hypertensive (n = 11) and Wistar-Kyoto (n = 13) rats. With vagal stimulation, cardiac parasympathetic responsiveness was greater in 12-week-old spontaneously hypertensive rats than in 12-week-old Wistar-Kyoto rats (24.8±5.4 versus 10.1±1.2 beats per minute per hertz, mean±SEM, p<0.035). This was also the case with methacholine (18.8±3.5 versus 13.1±4.4 beats per minute per microgram per kilogram, p<0.045). In contrast, cardiac parasympathetic responsiveness was similar, with both vagal stimulation and methacholine, when tested in the younger spontaneously hypertensive and Wistar-Kyoto groups. Thus, in established (but not in early) hypertension cardiac parasympathetic responsiveness is not reduced but rather augmented, suggesting that factors other than an end-organ responsiveness are responsible for the impaired baroreceptor–heart rate reflex. The enhanced cardiac parasympathetic responsiveness in spontaneously hypertensive rats may be due to upregulation of cardiac muscarinic receptors in response to the chronically reduced vagal tone that characterizes hypertension. (Hypertension 1992;19:653–657)

Key WORDS • sinoatrial node • baroreceptor reflexes • receptors, muscarinic • methacholine chloride • vagus nerve • spontaneously hypertensive rats

Many studies have documented that the bradycardia induced by stimulating arterial baroreceptors is impaired in hypertension and that this is the case both in humans and in animal models of chronic blood pressure elevation.¹⁻³ Although the mechanisms underlying this impairment are poorly defined, one possibility is that since the bradycardia mainly depends on vagal activation⁴ a diminished responsiveness of the heart to parasympathetic stimuli is involved. This is suggested by the observation that cardiac responsiveness to adrenergic stimuli is indeed reduced in hypertension.⁵⁻⁶ We therefore set out to test this hypothesis by evaluating cardiac parasympathetic responsiveness in normotensive and hypertensive rats. The experimental model of hypertensive hypertension selected for the study was the spontaneously hypertensive rat.

Methods

Spontaneously hypertensive rats and normotensive control Wistar-Kyoto rats aged 12 weeks were purchased from Charles River Inc., Calco, Italy. At the time of the study mean±SEM body weight was 288±11 g in the hypertensive (n = 12) and 285±6 g in the normotensive (n = 11) groups. Each rat was anesthetized by a ketamine HCl injection at the dose of 80 mg/kg i.p., and the anesthesia was maintained throughout the surgical preparation and the experimental protocol by small supplements of the anesthetic agent as needed. Polyethylene catheters were inserted into a femoral artery for blood pressure recording and into a femoral vein for drug administration. The neck was opened via a ventral midline incision, and the vagal trunks were bilaterally isolated and sectioned. The distal end of the right vagus was placed on a stainless steel electrode immersed in a pool of mineral oil and connected with a model S88 stimulator (Grass Instrument Co., Quincy, Mass.). After 30 minutes of equilibration cardiac parasympathetic responsiveness was tested by two different techniques, namely, by 1) electrical vagal stimuli via separate, randomly applied steps having a duration of 15–20 seconds, a pulse energy of 5 V and 2 msec, and a frequency doubling from 1 up to 16 Hz and 2) intravenous bolus injections of methacholine at the randomly applied doses of 1, 2, 4, and 8 μg/kg, each injection being separated by an interval of 3–5 minutes from the following one. Blood pressure was measured by con-
VAGAL STIMULATION

Figure 1. Line plots show mean (±SEM) bradycardic responses to graded electrical vagal stimulation (top panel) and to graded methacholine injection (bottom panel) in 8-week-old Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Calculated regression slopes (not shown) were not significantly different in the former as compared with the latter group with either the electrical or the pharmacological stimulus. HR, heart rate; b/min, beats per minute.

Results

Baseline Hemodynamic Data

In 8-week-old spontaneously hypertensive rats the average mean arterial pressure and heart rate were 139±8.5 mm Hg and 360±10.0 beats per minute, respectively. The corresponding values in 8-week-old Wistar-Kyoto rats were 125±5.2 mm Hg and 389±11.9 beats per minute. The differences with the spontaneously hypertensive rats were not statistically significant.

In the 12-week-old spontaneously hypertensive rats, mean arterial pressure averaged 179±8.1 mm Hg and heart rate 391±13.0 beats per minute. The corresponding values for the 12-week-old Wistar-Kyoto rats were 137±5.0 mm Hg and 368±11.2 beats per minute. The difference in the blood pressure of the spontaneously hypertensive rats was statistically significant (p<0.01).

Cardiac Parasympathetic Responsiveness

In the 8-week-old Wistar-Kyoto and spontaneously hypertensive rats, vagal stimulation at increasing frequencies and methacholine injection at increasing doses caused a progressive fall in heart rate. With the former stimulus the slope was 11.3±4.0 beats per minute per hertz in Wistar-Kyoto and 11.4±1.6 beats per minute per hertz in spontaneously hypertensive rats. The cor-

necting the arterial catheter to a Statham P23Dc transducer; heart rate was obtained beat to beat by tachographic conversion of the pulsatile blood pressure signal. All data were continuously displayed on a Grass 7D ink-writing recorder. All the procedures followed were in accordance with institutional guidelines.

To investigate the onset of the possible alterations in cardiac parasympathetic responsiveness in hypertension, the experiments were performed in an identical fashion in younger (8-week-old) Wistar-Kyoto (n=11) and spontaneously hypertensive (n=13) rats. The body weights of these animals were 201±5 and 219±8 g, respectively.

Data obtained by electrical vagal stimulation and methacholine injection were analyzed separately. Because the bradycardic response was linearly related to the frequency of electrical stimulation or the dose injected, the results were examined both as responses to individual stimuli and as regression lines, the slopes observed in each rat being taken as the measure of the animal's cardiac parasympathetic responsiveness. The slopes of individual animals were averaged, and the means observed in hypertensive and normotensive rats of the same age were compared by the Wilcoxon nonparametric rank test. A value of p<0.05 was taken as the level of statistical significance.
responding values for the latter stimulus were 12.7±4.2 beats per minute per microgram per kilogram in Wistar-Kyoto and 13.0±2.6 beats per minute per microgram per kilogram in spontaneously hypertensive rats. The differences between the two groups were never statistically significant. These data are shown in Figure 1.

In the 12-week-old spontaneously hypertensive rats the bradycardic responses were markedly and significantly greater than in the 12-week-old Wistar-Kyoto rats. As shown in the examples of Figure 2 and in the mean data of Figure 3, this applied both to the bradycardia elicited by vagal stimulation and to that elicited by methacholine injection.

**Discussion**

Our experiments demonstrate that in the spontaneously hypertensive rat the bradycardic response to electrical or pharmacological parasympathetic stimuli is not impaired. On the contrary, this response is increased in these animals and the increase is so large that cardiac parasympathetic responsiveness is approximately twice that of normotensive control rats.

Before commenting on the implications of our findings, we will discuss a potential technical problem related to the use of methacholine. Because this agent has a powerful vasodilator effect, the blood pressure fall that follows its intravenous injection may produce a reflex tachycardic influence that would counter to an unmeasurable extent the direct bradycardic influence of the drug; this is, however, unlikely to have had any major relevance in our experiments because 1) in rats, the tachycardic response to baroreceptor deactivation is predominantly mediated by the vagus with only a minor contribution by the sympathetics and 2) the baroreceptor reflex tachycardia has a rather slow time course, whereas the methacholine-induced bradycardia develops and peaks in a matter of 3–5 seconds (see examples in Figure 2).

The finding of a cardiac parasympathetic hyperresponsiveness in hypertension allows several important points to be discussed. The first point is that the impairment of the vagally mediated bradycardic response to baroreceptor stimulation that characterizes human and experimental hypertension, including the spontaneously hypertensive rat, cannot originate from a reduced ability of the heart to respond to vagal impulses. It must originate from alterations of other portions of the baroreceptor reflex arc, and these alterations must be so pronounced as to override the increased parasympathetic responsiveness that facilitates rather than opposes the baroreceptor–heart rate reflex.

The second point is that in hypertension the cardiac parasympathetic hyperresponsiveness stands in striking contrast to the previously documented cardiac hyporesponsiveness to β-adrenergic stimuli. Thus, hypertension does not uniformly affect autonomic cardiac functions, and indeed some components of sympathetic and parasympathetic cardiac control are affected by this condition in an opposite direction.

The third point is that, if present in humans, the hypertension-related cardiac parasympathetic hyperresponsiveness may have clinical importance. This phenomenon may be implicated in the occurrence of a vasovagal reaction, i.e., a rare but dreaded event.
brought about by administration of several antihypertensive drugs (α-blockers, angiotensin converting enzyme inhibitors, and β-blockers). It may more frequently cause an undesired bradycardia during treatment with vagomimetic agents such as digitalis. It may also favor untoward falls in heart rate and cardiac output when parasympathetic agents are administered for noncardiovascular conditions such as constipation or ileus. Whether this is indeed the case in the clinical setting should be tested by comparison studies in hypertensive and normotensive patients.

Our current study was not designed to investigate the mechanisms responsible for the increased cardiac parasympathetic responsiveness of the spontaneously hypertensive rats. However, the observation that the parasympathetic hyperresponsiveness was evident both with a technique using a circulating muscarinic agent (methacholine injection) and with a technique releasing acetylcholine from the nerve terminals (electrical vagal stimulation) suggests that the hyperresponsiveness involved not only extrasynaptic muscarinic receptors but also intrasynaptic muscarinic receptors normally stimulated by the rapidly inactivated acetylcholine secreted into the synaptic cleft. Furthermore, the observation that the cardiac parasympathetic hyperresponsiveness can be detected in the 12-week-old but not in the 8-week-old hypertensive rats indicates that muscarinic receptor hyperresponsiveness is not a congenital feature of this hypertension model but that it develops during the third month of life, when the rats attain steadily elevated blood pressure values. Interestingly, there is evidence that in this phase vagal tone is reduced, which allows speculation that cardiac parasympathetic hyperresponsiveness develops as a compensatory upregulation of cardiac muscarinic receptors. This is in line with the observation that in another condition in which vagal tone is reduced, i.e., aging in the normotensive Sprague-Dawley rat, cardiac parasympathetic responsiveness is equally increased. However, ligand or gene expression studies will have to be carried out to prove this hypothesis.

In summary, our current study provides evidence that cardiac parasympathetic responsiveness is enhanced and not attenuated in spontaneously hypertensive rats.
and that this enhancement is detectable only in the established hypertensive stage. The data also suggest that the enhancement involves vagally innervated muscarinic receptors and may be explained by a muscarinic receptor upregulation secondary to a chronic reduction in vagal tone. Therefore the impairment of the vagally mediated baroreceptor reflex bradycardia characterizing spontaneously hypertensive rats must not depend on end-organ hyporesponsiveness but on marked alterations of other portions of the reflex arc.

References
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