Regulation of Sympathetic Activity in SHR/Coote and Sato

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Summary. The influence that the pressure-sensitive receptors in the cardiovascular system have on renal nerve activity and on heart rate was compared in normotensive rats (NTR) and spontaneously hypertensive rats (SHR). The cardiovascular receptors were stimulated by raising the blood pressure (BP) with intravenous phenylephrine. The duration of silence in the record of renal sympathetic nerve activity produced by a number of different rises in BP was measured. We found that the pressure that was just able to produce a silence in the nerve activity (threshold pressure) was higher in the SHR (170 mm Hg) than in the NTR (130 mm Hg). Also, comparable rises in BP above the threshold pressure in the SHR and NTR were less effective in the SHR in producing a complete inhibition of sympathetic nerve activity as judged by the short duration of inhibition. In contrast, we found that the changes in heart rate produced by rises in BP above threshold pressure were similar in NTR and SHR although the threshold pressure was somewhat higher in the latter. It was, therefore, concluded that the cardiovascular pressure receptors, apart from being reset to operate at a higher pressure level in the SHR and NTR, are less able to inhibit ongoing sympathetic activity than in the NTR. It is suggested that this is most likely due to a higher sympathetic activity in the SHR.

Peripheral factors localized to the blood vessels, such as changes in the reactivity of smooth muscle to transmitter release or a decrease in the wall to lumen ratio of the vessels may contribute to the development of hypertension in the spontaneously hypertensive rat. However, there is also evidence that an increase in sympathetic activity plays an important role in the development of hypertension. This is somewhat surprising because it has long been thought that the activity in sympathetic nerves to blood vessels is normally regulated quite efficiently by inhibitory feedback from mechanoreceptors sensitive to pressure changes in the circulatory system. The question arises, how is it that in the potentially hypertensive animal an increase in sympathetic activity could occur without invoking inhibitory feedback from the receptors sufficient to restore it to its original level? One possibility is that suggested by Foklows and others. These authors believe that sympathetic activity is only periodically or spasmodically increased and that during these episodes there is some abolition of the baroreceptor reflex. If this occurs often enough it may lead to morphological changes in the arterial walls which themselves sustain the hypertension. There is some evidence that weak, repeated stimulation of the hypothalamic defense region in rats leads to hypertension and that hypertensive rats are more responsive than normal rats to acute mental stress, and this might support such a hypothesis.

Another possibility is that the ability of the barorecep-
tors to prevent sympathetic activity rising is inefficient and therefore a sustained excitatory input to sympathetic neurones can overcome the increase in baroreceptor inhibition. On the other hand, it may be because the sensitivity of the baroreceptors or their central connections is changed. However, recent work by Nosaka and Wang strongly suggests that the latter is unlikely.

In the present experiments we determined the degree to which sympathetic activity is inhibited by rises in arterial blood pressure (BP) in normotensive rats and in hypertensive rats. The effect of such pressure changes on heart rate also was examined.

**Methods**

The experiments were carried out in nine spontaneously hypertensive male rats (SHR) from original Okamoto strain F1, generation, 15-21 weeks old, weighing 290-350 g, and nine normal Wistar male rats (normotensive control rats, NTR) (Nihon Rat Co.), 11-18 weeks old, weighing 400-530 g. They were anesthetized with sodium pentobarbital, 45-50 mg/kg given intraperitoneally, or in one case with chloralose, 25 mg/kg given intravenously after initial ether induction. In each experiment BP was recorded from one carotid artery via a polyethylene cannula filled with heparinized 0.9% NaCl and connected to a pressure transducer, the output of which was displayed on one channel of pen recorder (Nihon Khoden). At intervals throughout a period of 2 weeks before each experiment, systolic BP was measured on the caudal artery of unanesthetized rats. The method used was that of detection of the BP pulse by means of a photoelectric cell, the output of which was displayed on a pen recorder. The pressure in a cuff placed proximally around the tail was gradually increased until the BP pulse was just obliterated. This point was determined in three or more trials and the mean was taken as systolic BP. In six NTR and six SHR, the renal nerve on the left side was exposed retroperitoneally. Recordings of action potentials from the central cut end of the nerve were made using bipolar silver wire electrodes. Exposed tissues were covered in warm (37°C) paraffin oil.

Exposed tissues were covered in warm (37°C) paraffin oil. In six rats (three NTR, three SHR), the effects of the nerve were made using bipolar silver wire electrodes. Exposed tissues were covered in warm (37°C) paraffin oil. The cathode follower output of the oscilloscope was fed into a pulse counter and window discriminator (Hewlett-Packard) and the integrated output was displayed on one channel of the pen recorder. The counting period of the pulse counter was set to 1 second or 1.2 seconds.

In six rats (three NTR, three SHR), the effects of the pressure responses on heart rate were compared. Heart rate was recorded with an integrator (Nihon Khoden RT-5) triggered by the QRS complex of the electrocardiogram (ECG) the output being displayed on the pen recorder.

Temperature of the rats was maintained at or close to 37°C by means of a DC heating pad.

The cardiovascular mechanoreceptors were stimulated by producing a rise in systemic arterial pressure, using the α-agonist phenylephrine, given intravenously. Drugs were given via a cannula of known capacity (0.1 ml) placed in a jugular vein. Phenylephrine was prepared in a series of concentrations enabling different amounts of the drug to be given in the same volume of liquid, this being equal to the capacity of the cannula. This was done to ensure that all the drug was given at one time during the washing-in with 0.4 ml of 0.9% NaCl.

Recordings were made of sympathetic nerve activity in multiferber preparations of sympathetic nerves. To test the effectiveness of the cardiovascular mechanoreceptors in influencing this nerve activity we chose to measure the duration of silence, i.e., complete inhibition of all sympathetic nerve activity, elicited by a rise in systemic arterial BP of 5-10 mm Hg. This can be measured accurately and it is likely to be proportional to the intensity of pressure receptor input.

**Results**

**SYMPATHETIC NERVE ACTIVITY**

In the following experiments the duration of inhibition of sympathetic nerve activity resulting from different rises in BP was examined and compared in two groups of rats, one normotensive and the other, hypertensive.

**Normotensive rats**

The systolic BP of six unanesthetized NTR was 121 ± 7.0 (mean ± se) mm Hg. In these six rats following anesthesia, phenylephrine given intravenously in the dose range 0.25-10 μg elicited rises in mean BP varying from 5 to 80 mm Hg. Each dose was repeated three times. The effect of the increased BP on the duration of inhibition of activity in the sympathetic nerve is illustrated for each of the NTR (Fig. 1). The pressure necessary to produce

![Graphs showing the duration of complete inhibition of activity in the renal nerve (ordinate) in six anesthetized normotensive control rats following increases in mean blood pressure (BP) (abscissa) above the resting levels. The increases in mean BP were produced by intravenous phenylephrine (0.25-10 μg).](https://example.com/graph.png)
reflexly an observable silence in sympathetic nerve activity lay between 120 and 140 mm Hg (threshold pressure). For the smaller pressure changes between 130 and 160 mm Hg the duration of inhibition showed a gradual increase. Above this there was a marked lengthening of the inhibitory period, reaching a maximum of 60-100 seconds at 165-185 mm Hg, that is, for changes in pressure of 35-60 mm Hg. In these six NTR the maximum pressure change above threshold ranged from 35 to 60 mm Hg (mean, 45 mm Hg) and the related inhibition of sympathetic activity had a duration between 40 and 100 seconds (mean, 59 seconds).

After section of both vagus nerves and occlusion of the remaining carotid artery, rises in BP elicited by phenylephrine no longer produced any changes in sympathetic discharge. Thus the inhibition was most likely due to pressure-sensitive afferents in carotid sinus and vagal nerves.

Spontaneously Hypertensive Rats

The mean systolic blood pressure of six unanesthetized SHR measured at intervals over a period of 2 weeks was 179 ±9 mm Hg, that is, considerably higher than that of the NTR. As in the NTR, after anesthesia and surgery the systolic BP was changed, usually being much lower after these procedures. In this group of rats the curves relating the duration of inhibition of sympathetic activity to the pressor response was very different from those obtained for NTR (Fig. 2). First, the pressure necessary to produce reflexly an observable silence in sympathetic nerve activity (threshold pressure) was higher, varying from 140 to 220 mm Hg. Second, pressor responses up to 260 mm Hg elicited inhibition in the sympathetic nerve of only short duration, even though the changes in pressure were sometimes as much as 120 mm Hg. The maximum duration of the inhibition in any one experiment varied between 10 and 40 seconds (mean, 25 seconds). This was for the maximum pressure changes of 40-80 mm Hg (mean, 58 mm Hg) above threshold pressure. Thus the long periods of inhibition observed for smaller changes in mean BP in the NTR were never seen in the SHR. The duration of the

"silent period" in sympathetic activity was proportional not only to the intensity of the pressure input to the baroreceptors but also to the duration for which the pressure stimulus lasted above the minimal level producing silence. The data from all experiments have been plotted to show this relationship (Fig. 3). The slope of the calculated regression line was much steeper in NTR than in SHR for a similar range of durations of the adequate pressure stimulus. Again it is clear that stimuli of similar pattern and magnitude affecting the cardiovascular me-
chanoreceptors were unable to elicit a period of complete sympathetic inhibition of as long duration in the SHR as in the NTR (Fig. 4). The top left panel shows the longer duration of the silent period in renal nerve activity in NTR (6 seconds) following a small, short-lasting rise in the BP of 30 mm Hg (from 130 mm Hg to 160 mm Hg). The silence produced in nerve discharge in SHR for a similar rise in mean BP from 170 mm Hg to 200 mm Hg lasted only 3 seconds even though the rise in BP was maintained for a longer period than that in NTR. The records in the right panel of Figure 4 further illustrate this difference but here the effects of more maintained rises in BP in NTR and SHR are compared.

As in the NTR, section of vagus nerves and carotid occlusion in the SHR abolished the effect of the pressor responses on sympathetic activity. The result of this procedure on the level of sympathetic activity was also compared in the two groups of rats. In the NTR sympathetic activity increased almost 4-fold (Fig. 5), whereas in the SHR, although sympathetic activity increased this was usually small, being some 26% in the example in Figure 5.

The resting level of sympathetic activity illustrated for NTR and SHR in this graph is that recorded in a multifiber or few-fiber preparation of a renal nerve and therefore is not indicative of the overall discharge rate in any one fiber. In three SHR vagus nerve section resulted in a small increase in renal nerve activity, from 31 ± 7.0 impulses/sec to 39 ± 10 impulses/sec (26%) in one rat (Fig. 5); from 56 ± 21 to 60 ± 9 impulses/sec (23%) in the second rat; and from 14 ± 4.0 to 24 ± 4.0 impulses/sec (65%) in the third. In contrast, in three NTR after section of the vagus nerve, renal nerve activity increased from 11 ± 6 to 40 ± 12 impulses/sec (380%) in one; from 20 ± 12 to 79 ± 15 impulses/sec (390%) in another (Fig. 5); and from 18 ± 10 to 66 ± impulses/sec (366%) in a third.

Activity in sympathetic nerves, innervating pressure-sensitive regions, could modify the response to pressure changes. Activity in sympathetic activity following a rise in mean BP was compared before and after section of both cervical sympathetic nerves. No differences were observed. The inhibition in NTR was also not affected by this procedure.

HEART RATE CHANGES

The “resting” heart rate in anesthetized rats had similar values in both SHR and NTR. The mean value for NTR was 392 ± 50 beats/min and for SHR somewhat lower, 372 ± 52 beats/min, the difference not being significant.

The effect on heart rate of raising the mean BP with phenylephrine is illustrated in Figure 6, which shows the results obtained in three NTR and three SHR. Although there is some scatter and variability of heart rate responses in each experiment it is evident, in both groups of rats, that larger increases in mean BP are associated with larger decreases in heart rate. In the SHR, heart rate responses were examined for changes in mean BP above a resting level of 165, 180, or 200 mm Hg, whereas in the NTR the changes in mean BP were produced at a resting level of 150 mm Hg. Consequently the curves relating BP change and heart rate decrease are shifted to the right in the SHR.
Atropine given intravenously in doses of 0.5 mg/kg abolished the slowing of heart rate produced by the rises in mean BP in both SHR and NTR. This indicates that the bradycardia was due to a reflex increase in vagal activity to the heart and was neither partially due to a reflex decrease in cardiac sympathetic activity nor, possibly, to a direct effect of phenylephrine on the heart.

**Discussion**

The present results show that the threshold pressure needed to produce reflexly an observable silence in the multiunit recording of sympathetic activity in renal nerves is raised in the SHR (Figs. 1 and 2). This aspect of our work confirms the results of Nosaka and Wang. These authors measured the falls in BP elicited by graded changes in the perfusion pressure in both isolated carotid sinus regions of NTR and SHR. Construction of stimulus-response curves showed that the relationship between the pressure change and the reflex BP decrease was similar in both groups of rats, although the curves were shifted to the right in the SHR. Thus the initial perfusion pressure in the carotid sinus on which the subsequent changes were superimposed needed to be 160 mm Hg in the SHR compared to 100 mm Hg in the NTR. The stimulus-response characteristics of the receptors from carotid sinus and aortic regions are similar in NTR and SHR, but in the latter the curves for this relationship again are shifted to the right; therefore Nosaka and Wang suggested that in the SHR the baroreceptor reflex wasreset at a higher pressure level. The resetting was thought to be a consequence of changes in the arteriolar walls in the pressure-sensitive regions, occurring secondary to the hypertension. Other aspects of the work reported here support this finding; that is, the cardiovascular mechanoreceptor reflex influence on heart rate is little impaired apart from the shift to the right in stimulus-response curves of the SHR.

It would seem then that the method used in our experiments to test the effectiveness of the cardiovascular mechanoreceptors was adequate to give a picture of their functional ability similar to that shown in the experiments of Nosaka and Wang. However, our study also revealed that the inhibition of all recorded renal nerve activity by the baroreceptors in SHR was of much shorter duration than that produced in NTR. Thus if we examine the duration of complete silence in the renal nerve record (Figs. 1–4) it is observed that the cardiovascular pressure-sensitive receptors are not able to inhibit sympathetic activity as effectively in the SHR as in the NTR when stimulated by rises in systemic arterial BP of equal magnitude and duration in the two groups of rats or even larger magnitude in the SHR. This can best be seen by calculating the mean of the maximum pressure change above the threshold pressure for each group of rats and the mean duration of inhibition of sympathetic activity which this produced. Thus in the six NTR a change of 45 mm Hg above threshold produced an inhibition 59 seconds in duration, whereas in the six SHR a change of 58 mm Hg above threshold produced an inhibition only 25 seconds in duration. This probably means that the excitatory drive onto sympathetic neurons can better overcome baroreceptor inhibition in SHR than in NTR, since a reduced sympathetic drive in SHR would not be compatible with this ability to recover from inhibition. It is likely that this more powerful excitatory drive results in an increased sympathetic activity, as indicated by the experiments of Okamoto et al., Iriuchijima, and Numao and Iriuchijima. This might explain the observation that removal of the influence of all the buffer nerves led to a rather small increase in renal sympathetic activity in the SHR compared to that in the NTR. Since the sensitivity of the baroreceptors is little changed as judged by their influences on heart rate and as shown by Nosaka and Wang, it might be argued that renal sympathetic activity did not increase by much in the SHR because it was already high. If we assume there is a raised level of sympathetic activity, this could be the reason for the seeming contradiction between some of our results and those of the latter authors, who measured BP. Changes in this reflect not only changes in sympathetic activity but also changes in the response of the effector organ. In the SHR the latter will undoubtedly be different from that in NTR because of the changes in wall to lumen ratio. Thus, although the pressure receptors may reflexly reduce sympathetic activity enough in the SHR to produce an absolute change in BP similar to that in the NTR, this reduction could well be superimposed on an increased sympathetic discharge. Measurements of changes in BP would not expose this. We consider the present results are therefore complementary to those of Nosaka and Wang.

Indeed, all the evidence supports the observations of the latter authors that the baroreceptors are reset at a higher pressure level. However, it is not sufficiently accurate to state that they now act to maintain the new pressure. In fact the inefficiency of the reflex revealed by its inability to produce sustained suppression of all renal sympathetic activity will, if this is so in other vasomotor nerves, result in a continued tendency for BP levels to increase due to an increase in sympathetic activity even though this may be gradual.

A recent study by Lais et al. is in conflict with this conclusion, but there are many features of this latter investigation which are puzzling. The hindlimb vessels of SHR were shown to be more responsive than those of NTR to infused norepinephrine, yet electrical stimulation of the lumbar sympathetic chain over a range of frequencies elicited a similar change in resistance for both groups of rats. Also, using the doubtful method of recording multifiber nerve activity to compare nerve discharge rates, they considered these to be similar in the lumbar sympathetic nerves of SHR and NTR. However, they were careful to base the main body of their conclusions on comparison of changes in lumbar sympathetic nerve activity which were induced reflexly. In addition they demonstrated that, paradoxically, section of lumbar sympathetic nerves led to less change in vascular resistance in SHR. Such findings are difficult to reconcile although the authors do attempt to do this. They point out that if there is an increase in sympathetic activity in SHR this should be associated with an increased turnover of catecholamines, whereas there is much evidence that suggests that in SHR...
catecholamine turnover in peripheral tissues is reduced.26-30 However, there is an alternative explanation. It may be that once the hypertensive state is established there are fewer vasomotor nerve terminals in the walls of the peripheral blood vessels as a consequence of the increased lateral pressure.31 If this is the case then one could expect to see a decrease in turnover of catecholamine in peripheral tissues even though the few remaining nerves are firing more frequently. One might also expect to observe a hyperresponsiveness of vessels to vasoconstrictor agents,31 as has been reported in SHR by Lais et al.34 Nevertheless, these considerations are hypothetical. To establish whether there is an increase in sympathetic activity in SHR requires a statistical analysis of identified single-fiber recordings from sympathetic nerves. A similar criticism may be made of the recent work by Judy et al.,32 whose results are in contrast to those of Lais et al.34 From multifiber recordings in anesthetized and conscious rats these workers obtained evidence indicating that there is an increase in sympathetic nerve activity in SHR. The results of both groups are equivocal, and until evidence from single-fiber studies is available these arguments will continue.

The present experiments go a little way toward clarifying the confusion but it is difficult to explain our findings other than to suggest that there is an increase in activity in the renal nerves in SHR. This conclusion finds support in the work on splanchnic nerves by Okamoto et al.,3, 5-6 Numao and Iriuchijima,23 and Judy et al.32 It may be that the difference lies in the different sympathetic nerves used by different workers, as suggested by Judy et al.32 All studies suggesting an increase in sympathetic nerve activity in SHR. The results of both groups are equivocal, and until evidence from single-fiber studies is available these arguments will continue.

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Myocardial Function and Coronary Blood Flow Response to Acute Ischemia in Chronic Canine Diabetes

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SUMMARY To examine the influence of preexistent diabetes mellitus on left ventricular performance and coronary blood flow responses to acute ischemia, mild normoglycemic diabetes was induced in nine mongrel dogs after three doses of alloxan, (20 mg/kg, iv), at monthly intervals. Hemodynamic measurements and coronary blood flow (Hg clearance) were obtained before and after the onset of ischemia. This was produced by occlusion of the proximal left anterior descending coronary artery via a balloon-type catheter in nine anesthetized diabetic dogs and 10 nondiabetic dogs. During the 1st hour of ischemia in the diabetic group, the end-diastolic pressure rose from 7 ± 1.1 (mean ± SE) mm Hg to 23.8 ± 2.3 without a significant increase of end-diastolic volume. In controls end-diastolic pressure rose from 8.6 ± 1.1 mm Hg to 15.3 ± 1.4, and end-diastolic volume was significantly increased, so that the ratio of end-diastolic pressure and volume was significantly higher in the diabetic group (P < 0.005). Although indices of contractility did not differ, stroke volume and work reductions were significantly greater in diabetes, despite the fact that coronary blood flow was reduced to a similar extent. Size of the ischemic areas appeared comparable as judged by distribution of dye injected distal to the occlusion. Since potassium loss and sodium gain in the inner and outer layers of ischemic tissue did not differ between the two groups, the intensity of ischemia seemed similar. Glycogenolysis was unimpaired in the diabetic ischemic muscle but triglyceride levels remained elevated. Morphologically the diabetic myocardium was characterized by a diffuse accumulation of periodic acid-Schiff-positive glycoprotein in the interstitium, which was thought to contribute to the substantial reduction of ventricular performance.

ALTHOUGH the influence of acute regional ischemia on left ventricular function has been well defined in the previously normal animal, the response of the ventricle affected by a chronic metabolic or structural abnormality has not been described. Acute myocardial infarction has been reportedly associated with a greater incidence of pump failure and higher mortality in diabetes mellitus. Although the increased mortality from cardiac disease complicating diabetes mellitus has been traditionally attributed to accelerated atherosclerosis of the coronary arteries, this is a disputed issue since recent evidence in studies using more quantitative methods and age-matched controls has shown that the complicated lesions of atherosclerosis may occur to only a slightly greater extent in diabetics.

In a previous study from this laboratory, we observed altered myocardial function in chronic diabetes mellitus in dogs, associated with accumulation of periodic acid-Schiff (PAS)-positive glycoprotein in the myocardial interstitium without coronary obstructive lesions; this morphological abnormality also has been observed in man. To examine the response of the diabetic myocardium during acute regional ischemia as compared to normal controls the following study was undertaken.

Methods

Two groups of healthy male mongrel dogs 2-4 years old and weighing 21-28 kg were studied. The dogs had no clinical evidence of disease for 6-8 weeks before admission to the study groups. Hematocrit and serum albumin were initially normal and both groups received the same diet consisting of 8% fat, 22% protein, 58% carbohydrate, 9% ash and 3% crude fiber. One group (n = 10) served as controls with normal glucose tolerance by intravenous testing. The other group (n = 9) was made diabetic with low doses of alloxan at monthly intervals. To produce mild normoglycemic diabetes, alloxan monohy-
Reflex regulation of sympathetic activity in the spontaneously hypertensive rat.
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doi: 10.1161/01.RES.40.6.571

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
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