Modified Characteristics of the Aortic Baroreceptor Activities in the Spontaneously Hypertensive Rat

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The characteristics of the aortic baroreceptor reflex activities in the Spontaneously Hypertensive Rat (SHR) were studied by electrophysiological approaches. After systemic blood pressure was reduced by hyperventilation to a sufficiently low level (50 to 60 mmHg), mixture solution of noradrenaline and angiotensin was infused intravenously so that a variety of systemic blood pressure levels which caused a full range of the aortic baroreceptor firing could be obtained. The aortic baroreceptor activities were recorded simultaneously with systemic blood pressure, and the firing rates of the former were plotted against the various levels of the latter. These experimental procedures were carried out both on SHR and control rats, and the results were compared between the two groups.

The results could be summarized as follows.
1. The mean blood pressure-firing rate curve in SHR showed a shift to the higher blood pressure level.
2. The range of blood pressure within which the aortic baroreceptors showed a response was significantly expanded in SHR in comparison with controls.
3. Natural blood pressure in SHR was above the value which was postulated to represent the greatest reflex producing point of the mean blood pressure-firing rate curve, while in controls their normal blood pressure was below or around the point.

These findings indicate that the aortic baroreceptor function in SHR was reset to the higher blood pressure. Besides, it is suggested that SHR will not be favoured with so effective a buffer action of baroreceptors as in controls against an abnormal blood pressure-raising disturbance such as stress.

Concerning the Spontaneously Hypertensive Rat (SHR), considerable amount of work has been published on the course of the development, the cardiovascular diseases, the endocrine changes, the pharmacological characteristics, the catecholamine metabolism, the histo- and the histometrical findings in the autonomic nervous system and so on. These were summarized in OKAMOTO's recent review on SHR. Several reports, most of which were reviewed in it gave the evidences showing that the autonomic nervous system in SHR is modified in a way and that the neural factor plays a significant role in the hypertension. As to the baroreceptors, the feed back system in the neural cardiovascular regulation, THANT et al. reported the effect of acute sinoaortic denervation in SHR and stated that young SHR showed a greater pressor response to this procedure than the age-matched controls, while the response of adult SHR was almost the same as that of the controls. However, functional characteristics of baroreceptors in SHR have not been touched so much by this experiment, which revealed one extremity of baroreceptor characteristic curve and whose results might also have included central and peri-

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pheral sympathetic responsiveness to the negative feed back circuit elimination.

The resetting mechanism of baroreceptor function in chronic renal hypertension seems to have been established since McCUBBIN et al's report. Nevertheless, the baroreceptor function in the spontaneous hypertension, which resembles the human essential hypertension in the aspect of the development, has not fully been revealed, as mentioned above. Any other type of experimental hypertension in which normal animals are changed hypertensive by artificial manipulations within a more or less short time must be different from the human essential hypertension in its development. Since a different way of development of hypertension may produce a different pattern of vascular changes around baroreceptor areas, it seems to be indispensable to know more in detail how the baroreceptors are changed in spontaneous hypertension before the idea of reset baroreceptors is applied to the essential hypertension. It was for these reasons that the present investigation was designed to evaluate the characteristics of the aortic baroreceptors in SHR, which is a colony of spontaneous hypertensive animals.

**Materials and Methods**

**Materials** Seven male SHR (F14 to F19), 8 to 10 months old, were used in this experiment. Eight male normotensive rats of Wistar strain supplied by the Animal Center Laboratory of Kyoto University, from the ancestor of which SHR had been separated, were used as the controls. The body weight of these animals was within the range of 340 to 400 gm.

**Operative Procedures** The animal was anesthetized with an intravenous injection of α-Chloralose (60 mg/kg) and fixed in a supine position. Atropin sulfate was given intraperitoneally (5 mg/kg) in order to reduce the excessive secretion of respiratory tract during the cervical operation. The left femoral artery and vein were cannulated by thin polyethylene tubings. Subsequently, the skin covering the ventral cervical portion was removed. Platysma, left sternocleidomastoideus and infrahyoid muscles were cut off. Then, the left common carotid artery together with the left vago-sympathetic trunk was carefully exposed. The exposed area was filled with warm liquid paraffin. Another thin polyethylene tubing was introduced through the left external

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**Fig. 1. Outline of Recording Devices.**

Abbreviations: CRO: cathode ray oscilloscope; IWO: ink writing oscillograph.

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carotid artery into the left common carotid artery for the purpose of monitoring the blood pressure which approximated to the one at the baroreceptor area in the aortic arch. After these procedures, the aortic depressor nerve was searched for under a dissecting microscope with great caution. The best method to find the aortic depressor nerve was as follows. First, the left superior laryngeal nerve was identified just ventrally to the carotid bifurcation. In half of the cases the aortic depressor nerve could be identified as a thin separate filament which diverged rectangularly from the superior laryngeal nerve and ran towards the heart along the lateral to posterior wall of the common carotid artery. In other cases the aortic depressor nerve ran only a short course to join the sympathetic or vagus nerve. These candidates of the aortic depressor nerve were submitted to the identification by the following electrophysiological recording. The proximal portion of the prepared nerve filament was tied with a thin silk thread and cut just cranially to it.

**Recording of Blood Pressure** The blood pressure was picked up by a strain gauge transducer (Nihon Kohden, Model MP-4T) and recorded on a channel (A) of an ink writing oscillograph. However, owing to the limited number of channels of our recording apparatus only the mean blood pressure was recorded except for temporary checking of systolic and diastolic pressures. This selection was based on BRONK and STELLA's report\(^{13}\) that baroreceptor firing had a good correlation to mean blood pressure.

Just before the cervical operation the blood pressure was checked through the tubing in the left femoral artery. Afterwards, continuous recording of blood pressure was made through the tubing in the left common carotid artery simultaneously with that of the aortic baroreceptor firing. The former recording was used as the blood pressure of the animal in normal condition, i.e., the condition in which baroreceptors were not touched, and the latter as a variable to give changes in baroreceptor firing.

**Recording of Aortic Depressor Nerve Activities** (Fig. 1) Following the intramuscular injection of gallamine (20 mg/kg), the animal was ventilated artificially with air. Then, the animal was placed on a copper plate through which the body was grounded.

The aortic depressor nerve was placed on paired stainless steel electrodes and dipped into a liquid paraffin pool together with the electrodes. The preparation of the electrodes and the method to fix the nerve to them were the same as described in the previous report.

The nerve activities were fed into a differential preamplifier (Nihon Kohden, Model AVB-1) and displayed on a channel \(A'\) of a dual beam oscilloscope (Nihon Kohden, Model VC-6). At the same time, the activities were picked out from the \(Y\)-axis of the same channel and capacity-coupled to a slicer circuit, where the signals below a given voltage were cut off. Then, the signals were led to a pulse generator (Nihon Kohden, Model MSE-3) and converted into the standardized pulses. These standardized pulses were fed into an RC-integrator which transformed them into the standardized pulse-integrated activities ("firing rate"). The firing rate was amplified by a DC amplifier and recorded on a channel \(B\) of the ink writing oscillograph. The blood pressure was recorded on the other channel \(A\) synchronously with this firing rate. Incidentally, the standardized pulses were displayed on the other channel \(B'\) of the dual beam oscilloscope.

**Artificial Alteration of Blood Pressure** The polyethylene tubing which was cannulated into the left femoral vein was connected to a 5 cc injection syringe filled with physiological saline solution containing noradrenaline (30 \(\mu\)g/cc) and angiotensin (Hypertensin CIBA, 15 \(\mu\)g/cc). Then, the animal was hyperventilated by increasing the tidal volume and frequency of artificial respiration. This procedure was designed for the purpose of reducing the blood pressure level at the onset of noradrenaline-angiotensin infusion as well as for the purpose of inhibiting the chemoreceptor firing. When the blood pressure was lowered far from the original level, the infusion was started by raising the syringe up to 20 cm above the body level, while the aortic depressor nerve activities and mean blood pressure were recorded simultaneously. By this method the infusion was made at the speed of 1 cc/5 min., and a gradual rise in blood pressure was induced (about 30 sec. from start of pressure rise to its peak). In a minute after the peak was reached, the infusion was stopped and the blood pressure was allowed to return to the original level. The amount of the solution used in each infusion was less than 1 cc.

In two normotensive animals, the upper portion of the sternum, the left clavicle and the left first and second ribs were removed to expose the aortic arch region. Then, a cotton ball soaked with the same solution as was used for the infusion was applied on this region in order to
observe a direct action of the drugs on the baroreceptor.

Data Analyses (Fig. 2) In the two channel tracings of the ink recorder the firing rate of the aortic baroreceptors were read and plotted against various given levels of the mean blood pressure. Thus, a mean blood pressure-firing rate curve was drawn in each animal. The minimum pressure that elicited a response of the baroreceptor (Pmin) was estimated in the curve. The pressure level at which the firing showed the maximum value (Pmax) was also determined. Then, the equation that approximated to the linear portion of the curve was calculated by the method of least squares. After the values of the two points at which the above equation intersected F = Fmin and F = Fmax (F: firing rate; Fmin and Fmax: minimum and maximum firing rates, respectively) were calculated, the middle point of these two was determined (Pe). Pe was considered to be the value that represented the linear portion, at which the baroreceptors showed the most effective response to the pressure. Provided the equation of the linear portion was

### Table I

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Fmax</th>
<th>Fmin</th>
<th>Pmax - Pmin</th>
<th>Pe</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>176 ± 20*</td>
<td>59 ± 29</td>
<td>28.3 ± 19*</td>
<td>128 ± 24*</td>
</tr>
</tbody>
</table>

**Abbreviations:***
- **Fmax:** maximum firing rate
- **Fmin:** minimum firing rate
- **F:** blood pressure level to give Fmax
- **Pmin:** blood pressure level to elicit a baroreceptor response (threshold pressure)
- **Pe:** middle value of the two points at which the line approximating to the linear portion intersects F = Fmax and F = Fmin.

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expressed as $F = aP - b$, $P_e$ was given by the following equation:

$$P_e = (F_{\min} + F_{\max})/2a + b \quad (a, b: \text{constants})$$

All the data in this experiment were statistically analysed by Student's small sample 't' test.

**RESULTS**

Blood Pressure Change by Hyperventilation and Noradrenaline-Angiotensin (Table I) The blood pressure level was easily reduced when the animal was hyperventilated by increasing the tidal volume and frequency of the artificial respiration. The blood pressure level at the onset of noradrenaline-angiotensin infusion was around 60 mmHg ($59 \pm 29$ mmHg, Mean ± S.D., in SHR; $58 \pm 9$ mmHg in controls). When the infusion started, blood pressure rose gradually to reach the peak in about 30 seconds. The peak blood pressure level was higher in SHR than in controls ($263 \pm 19$ mmHg, Mean ± S.D., in SHR; $194 \pm 17$ mmHg in controls). However, correlation between tidal volume, frequency of respiration, $pO_2$ or $pCO_2$ in blood and blood pressure level or correlation between total dosage of noradrenaline-angiotensin and blood pressure level was not evaluated simply because they were not the primary purpose of our present study.

Relationship between Aortic Depressor Nerve Activities and Blood Pressure At a blood pressure level which was lowered by hyperventilation, the aortic depressor nerve showed only a few, fasciculated discharges which appeared synchro-

ously with systoles (Fig. 3a). As the blood pressure was raised by the noradrenaline-angiotensin infusion and reached a certain level, the firing began to increase in frequency and voltage (Fig. 3b). When the blood pressure was elevated up to another higher level, the potential voltage and frequency of the activities showed the maximum. At this level the burst-like firing with silent phase was replaced by a continuous one (Fig. 3c). At a still higher level above this point, however, the activities showed a slight reduction in voltage and frequency to regain the burst-like appearance (Fig. 3d).

Fig. 4 presented simultaneous recording of the mean blood pressure and the firing rate of the aortic depressor nerve on an ink writing oscillograph. The mean blood pressure-firing rate curves, which were obtained by plotting the firing rate against the mean blood pressure in such simultaneous recordings as shown in Fig. 4, were shown in Fig. 5.

As seen in these figures, there was no difference in the maximum discharge frequency or in the maximum potential voltage of the aortic depressor nerve activities between SHR and controls. In SHR, however, the nerve firing occurred more laggingly at a low blood pressure and the maximum firing was attained at the higher blood pressure level than in controls. In other words, in SHR the mean blood pressure-firing rate curve showed a remarkable shift to the right, that is, to the higher level of blood pressure in compari-
Fig. 4. Response of Aortic Baroreceptors to Rise in Mean Blood Pressure.
Right: SHR; Left: control. Upper trace: time interval (1 sec.).
Middle trace: mean blood pressure. Lower trace: firing rate of aortic baroreceptors.
Abbreviations Preop. P: blood pressure level before operations (before artificial respiration);
p: pulse pressure.

Fig. 5. Firing Rate Plotted Against Mean Blood Pressure
Abscissa: mean blood pressure; ordinate: firing rate of the aortic baroreceptors in impulses/sec.
Open circles indicate curves of controls, while closed circles are those of SHR.

$P_{\text{min}}$, the threshold pressure to fire the baroreceptor, was not statistically different between these groups. $P_e$, the middle value which was postulated to represent the linear portion of the mean blood pressure-firing rate curve, was also greater in SHR. When the initial blood pressure level at preoperative stage was compared with $P_e$, the former was located always above the latter in SHR, while the former was below or around the latter in controls.

In addition, the difference of $P_{\text{max}}$ and $P_{\text{min}}$, the range within which the baroreceptor was able to respond to pressure changes, was also greater in SHR.

Noradrenaline-Angiotensin Application on Aortic Baroreceptor Area While recording the aortic depressor nerve activities and mean blood pressure, a small cotton ball soaked with noradrenaline-angiotensin solution was applied on the aortic wall between diverging points of the left subclavian artery and the left common artery. No detectable change, however, was observed in the baroreceptor firing or in the mean blood pressure within 10 minutes after the application.

**Discussion**

The baroreceptors in carotid sinus and aortic arch have been energetically investigated in many animals with physiopharmacological[13-17] and

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morpological\textsuperscript{18,19} approaches. Recently, dynamic quantitative analyses of the baroreceptor reflex mechanism have been arousing the interest of researchers of this field\textsuperscript{20}.

As to the baroreceptors in the rat, however, available reports do not exceed a few in number. Moreover, these few reports\textsuperscript{11,21–23} have no more information than descriptions of electrophysiological activities or of anatomy of the aortic depressor nerve. So far as the authors know, the baroreceptor in the rat has not been studied in any analytical way. However, the rat is one of the most ordinary animals used in hypertension research. Therefore, the scarcity of the amount of work in this field seems to be unreasonable.

Difficulty of studying the baroreceptor function in the rat exists partly in its complicated pathways\textsuperscript{11,21–23} which was also observed in the present experiment. Furthermore, even if the aortic depressor nerve may be identified and submitted to recording, we may encounter another difficulty in connecting this baroreceptor area to an adequate pump system which is necessary for a dynamic analysis, and even a surgical procedure to cause mechanical obstruction of the aorta, such as reported by Ninomiya and Irisawa in the cat\textsuperscript{24} seems to be impossible. In addition, attempts to record the carotid sinus nerve activities in the rat have not been succeeded in the world. Owing to these limitations, our present study did not cover an analysis of dynamic characteristics, but revealed only a steady state relationship between the firing rate and the mean blood pressure.

In this experiment systemic infusion of pressor substances was employed to give a variety of pressures on the aortic baroreceptors. For this purpose we used a mixture of noradrenaline and angiotensin because with this mixture solution higher diastolic pressure and smaller pulse could be obtained than in the case of simple noradrenaline infusion and because tachyphylaxis shown in simple angiotensin infusion could be eliminated.

According to our results the mean blood pressure-firing rate curve in SHR showed a shift to the right in comparison with controls. In other words, the portion of the curve at which the baroreceptor showed the most effective reflex activities deviated to the higher level of blood pressure. This finding may correspond to the resetting mechanism that has been established by many researchers in renal hypertension of dogs and rabbits\textsuperscript{12,25–27} However, unlike their reports, \(P_{\text{min}}\), threshold pressure, in SHR was statistically equal to that of controls. Instead, \(P_{\text{max}}\) (pressure level which gave the maximum firing) and, therefore, the difference between \(P_{\text{max}}\) and \(P_{\text{min}}\) were remarkably greater in SHR. This does not only mean that SHR showed a simple shift in the curve of baroreceptor characteristics, but also means that SHR showed widening of the range of pressure within which the baroreceptor can respond to a rise in pressure. The causative mechanism of the latter phenomenon may be explained by the following postulation. According to Burton\textsuperscript{8} the collagenous tissue in the vessel wall has a protective role as a 'jacket' against stretch. It has also been reported that when arterial sclerotic lesions occurred in both common carotid and peripheral arterial walls of SHR, sinoaortic denervation gave only a weak or no response according to the severity of the lesions\textsuperscript{29} This was probably because the distensibility of arterial wall of baroreceptor sites was reduced due to arteriosclerosis. Provided that such a lesion including fibrotic process had occurred in baroreceptor sites in SHR used in our present experiment, the baroreceptors, protected from stretch by less distensible aortic wall, could not fire so actively at weak stimuli as in controls, but it could keep firing over a wider range of stretch stimuli. On the other hand, some investigators\textsuperscript{30,31} reported that the baroreceptor endings were degenerated in hypertension. However, the baroreceptor of SHR showed a firing of which maximum potential voltage or frequency was not reduced as compared with controls. Therefore, modified pattern in the mean blood pressure-firing rate curves in SHR does not seem to be reasonably explained by such a degeneration of baroreceptors themselves.

It was an unexpected finding that the baroreceptor showed a reduction in firing at the higher pressure level above a certain one where the maximum firing was observed. According to Warner\textsuperscript{32} the relationship between the frequency of buffer nerve impulses (\(F\)) and pressure (\(P\)) is given by

\[
F = k_1(P - P_0) + k_2(dP/dt)
\]

where \(P_0\) is the threshold pressure and \(k_1\) and \(k_2\) are constants. Accordingly, the reduction in firing rate may be related to the fact that \(dP/dt\) became smaller as the rate of rise in blood pressure by infusion diminished gradually. Sometimes, however, the firing began to reduce even

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when the blood pressure was still rising without diminution in the rate. Therefore, the alternative explanation that the reduced firing of the baroreceptor might be a result of acute degeneration or exhaustion due to an abnormal pressure loading is more plausible.

In order to guess how the aortic baroreceptors act at a blood pressure level in the natural condition, $P_e$, a middle point of the effective portion of the mean blood pressure curve, and the initial blood pressure level were compared. In SHR, the natural blood pressure level was always above $P_e$, while the relation was reversed in controls. This implies that SHR will not be favoured with so effective a buffer action as in controls if an abnormal pressure raising disturbance such as stress is loaded.

We must admit that our present experiment included a few weak points to be improved in future. First, the variable to cause the baroreceptor firing was brought about by systemic blood pressure change which was induced pharmacologically because mechanical pressure regulation was not available at that time. Secondly, for the same reason, the speed in changing pressure level might not be regulated constantly. As to the first point it has been reported that pressor substances fire the baroreceptor even when applied directly on the baroreceptor area. Although we could not observe such an effect in our experiment, we do not intend to go so far as to exclude the direct action of these drugs. In regard to the second point, although the pressor substance infusion was made as gradually as possible in order to minimize time factor, it was still doubtful whether this factor was successfully eliminated. However, according to our calculation based on the actual tracings the time factor ($k_2 dP/dt$ in Warner's equation), that is, the difference between the actual firing rate ($F$) and the firing rate to steady pressure [$k_1 (P - P_o)$] was within 5% of the actual firing rate. This means that our approximation had an error of less than 5%. For all that, however, these problems remain to be overcome in future investigation.

REFERENCES

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