Central Resetting of Baroreflex in the Spontaneously Hypertensive Rat

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SUMMARY The role of central nervous system in the resetting of baroreflex was investigated in 5-month-old spontaneously hypertensive rats (SHR) of Okamoto strain. Age-matched Wistar-Kyoto (WKY) rats were used as normotensive controls. The aortic nerves, which in the rat, contain few or no chemoreceptor fibers, were stimulated electrically using a wide range of stimulus frequencies. The depressor responses (expressed as percent decrease in blood pressure as compared to its blood pressure value prior to aortic nerve stimulation) produced by these stimulations were significantly smaller in SHR than those in WKY. In another series of experiments, changes in the efferent limb of the baroreflex arc (i.e., greater splanchnic nerve activity) in response to stimulation of the baroreceptor afferents in the aortic nerve were recorded. Inhibition of the greater splanchnic nerve activity due to aortic nerve stimulation was found to be significantly smaller in SHR than in the WKY. Control sympathetic nerve activity was greater in SHR than in WKY. These results suggest that the central bulbospinal nervous system may be another site for resetting of baroreflex in hypertension. (Hypertension 5:346-352, 1983)

Key Words • aortic nerve • baroreceptors • blood pressure • splanchnic nerve

ARTERIAL baroceptors constitute an important regulatory mechanism of cardiovascular function.1 Resetting of baroreflex has been demonstrated in hypertensive patients2 as well as in animals with experimentally induced hypertension.3-6 Resetting of baroreceptor function was demonstrated in the spontaneously hypertensive rat (SHR),3,5,7 when it became available as a model of human essential hypertension.8 The mechanism of baroreceptor resetting of baroreflex is not clear at present. On the afferent limb of this reflex, changes in the receptor elements,12-14 as well as vascular wall in which they are embedded,3,9,15 have been implicated as causes of baroreflex resetting. However, there is a possibility that resetting may occur at other components of the baroreflex arc. Central resetting of baroreflex has been suggested from time to time,3-5,10 but to the best of our knowledge, never clearly demonstrated. The results presented in this communication suggest that central resetting of baroreflex may indeed occur in spontaneously hypertensive rats.

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Methods

Forty male, 5-month old, SHR of Okamoto strain and 35 age-matched Wistar-Kyoto (WKY) rats were obtained from Taconic Farms (New York) and maintained under standard laboratory conditions for 1 week prior to experimentation. During this time, blood pressures were monitored periodically by tail-cuff method20 to verify the normotensive or hypertensive status of the animals.

Blood Pressure Responses in Decerebrate Rats

These experiments were carried out in 15 SHR and 10 WKY. The details of decerebration procedure have been described elsewhere.20 Briefly, external and internal carotid arteries were ligated under halothane anesthesia. The animal was then fixed in a stereotaxic instrument, and bilateral parietal craniotomy was performed. The cortex was aspirated to visualize the colliculi, and a midcollicular transection was performed. The cranial cavity was then carefully packed with oxidized cellulose. Anesthesia was discontinued, and the animal was fixed in a supine position. Body temperature was monitored and maintained at 37°C by a temperature controller (Bailey Instruments, BAT78). These preparations were stable for at least 10 to 12 hours, and their cardiovascular and respiratory parameters were comparable to those of conscious restrained animals.30 Throughout the duration of the experiment, the animals urinated spontaneously periodically and no distension of the bladder was observed.
The carotid sinus and aortic nerves were identified under an operation microscope (Carl Zeiss, OPMI-1H). The carotid sinus nerves were sectioned bilaterally. The aortic nerves were also sectioned low in the neck. In 90% of the animals used, the aortic nerves of both sides were separate, traveling rostrally parallel to the vagus up to the carotid bifurcation where it joined the superior laryngeal nerve, which in turn joined the vagus nerve just below the nodose ganglion. The left or right aortic nerve was placed on a bipolar platinum iridium stimulation electrode and immersed in a pool of warm (37°C) paraffin oil. The activity of the caudal end of the greater splanchnic nerve was amplified using a bandpass amplifier (Ortec, 4660). The low bandpass filter was set at 100 Hz in order to avoid 60 cycle interference and low frequency mechanical and electrical noise while the high bandpass was set at 10 kHz. The amplified activity was displayed on an oscilloscope (Tektronix, 7623A) and recorded on an FM tape recorder (Hewlett-Packard, 3968A). The stored data on the tape recorder was later played back into a visicorder (Honeywell, 1858) for making permanent records.

Analysis of the Nerve Activity

Spine activity of the whole preceliac nerve segment was analyzed on a signal averaging (Nicolet, 1070) system. The recorded nerve activity was then fed into a signal shaper (Nicolet, SH72). The threshold was set to exclude background noise, and count rate analysis was performed using a frequency/time histogram analyzer (SH71). The system is capable of counting spikes at intervals as low as 20 μsec.

Statistical Analysis

Significance of difference between means was ascertained using unpaired student’s t test. Changes in blood pressure and SNA compared to control were expressed as percent changes. Differences were considered significant at p < 0.05.

Results

Blood Pressure Responses in Decerebrate Rats

Mean blood pressure of decerebrate WKY normotensive rats (98 ± 6 mm Hg) was significantly lower (p < 0.01) than the mean blood pressure (166 ± 4 mm Hg) of SHR. Periodic determination of blood gases in SHR and WKY showed that these values (pCO2 35 ± 5 mm Hg; pO2 95 ± 8 mm Hg; pH 7.42 ± 0.04) were comparable to the same values reported elsewhere.

Left aortic nerve stimulation at variable frequencies for 1 minute produced a fall in blood pressure in all rats. Baroreceptor responses consisted of an initial dynamic phase which was followed by a steady-state phase. In the SHR, the two phases of blood pressure fall in response to the aortic nerve stimulation were distinct while the differences between these two phases were subtle in WKY (fig. 1).

A comparison of decrease in mean blood pressure in response to the aortic nerve stimulation during dynamic (initial 15 seconds following aortic nerve stimulation) and steady-state (15 seconds just before the termi-
FIGURE 1. Depressor responses in decerebrate 5-month-old SHR (left panel) and age-matched WKY (right panel). The depressor response was produced by electrical stimulation (2 V, 3 msec, 50 PPS) of the left aortic nerve. Identical responses were produced by the stimulation of the right aortic nerve. Control mean blood pressure (166 ± 4 mm Hg) in SHR (n = 15) was significantly higher (p < 0.01) than that of WKY (98 ± 6 mm Hg; n = 10). Aortic nerve stimulation produced a smaller depressor response in SHR when compared to the WKY; note also that during the steady state the blood pressure tended to recover to control levels despite maintained stimulation of the aortic nerve. In the WKY rats, on the other hand, the blood pressure remained at depressed levels as long as aortic nerve stimulation was maintained.

nation of the aortic nerve stimulation) phases is shown in figure 2. In the WKY, the depressor responses (expressed as percent decrease in blood pressure as compared to its blood pressure value prior to aortic nerve stimulation) to the aortic nerve stimulation at different frequencies were not statistically different during steady-state (hatched bar) and the dynamic phase (blank bar). In the SHR, on the other hand, the depressor responses to the aortic nerve stimulation at the frequencies of 25, 50, and 75 were significantly smaller (p < 0.05) during steady-state when compared to that during the dynamic phase. A comparison of depressor responses to the aortic nerve stimulation in SHR and WKY is shown in figure 3. The stimulus frequency was varied between 2 to 150 pulses/sec (the 2 V-intensity and 3 msec duration of the stimulus were kept constant). At each frequency, the dynamic as well as steady-state depressor responses were statistically smaller (p < 0.05 to 0.005) in SHR than in WKY.

Blood Pressure in Anesthetized Rats

Mean blood pressures in urethane-anesthetized WKY and SHR were 91 ± 5 mm Hg and 180 ± 5 mm Hg, respectively. These values were comparable to the corresponding values in decerebrate preparations. The blood pressure responses to the aortic nerve stimulation were slightly smaller, but not statistically different from those obtained in decerebrate preparations. The dynamic as well as steady-state blood pressure responses to the aortic nerve stimulation were smaller in WKY when compared to the corresponding values in SHR. Figure 4 shows the dynamic depressor responses to the aortic nerve stimulation in SHR and WKY.

FIGURE 2. Differences in depressor responses to the aortic nerve stimulation during dynamic and steady-state phases. Top panel: WKY (n = 10). Bottom panel: SHR (n = 15). The left aortic nerve was stimulated at constant intensity (2 V) and duration of stimulus (3 msec) while the frequency of stimulus was varied between 2 and 75 pulses per sec (PPS). The depressor responses plateaued at 25 PPS in WKY, as well as SHR. At the frequencies of 25, 50, and 75 PPS, the difference in the depressor responses during dynamic (blank bars) and steady-state (hatched bars) phases were statistically significant (p < 0.05) in SHR than the corresponding values in WKY.

Sympathetic Nerve Activity

Sympathetic nerve activity in the segment of greater splanchnic nerve between cardiac ganglion and celiac ganglion is predominantly postganglionic. It is characterized by a typical burst pattern synchronous with the cardiac cycle with occasional respiratory modulation. Control sympathetic nerve activity in SHR (i.e., prior to the aortic nerve stimulation) was 263 ± 25 spikes/sec. This activity was significantly greater (p < 0.05) than the corresponding value (196 ± 18 spikes/sec)
sec) of control sympathetic nerve activity in WKY. Aortic nerve stimulation at different frequencies varied between 2 and 150 pulses/sec (at constant intensity and duration, 2 V and 3 msec, respectively) produced an inhibition of sympathetic nerve activity associated with fall in blood pressure in WKY as well as SHR. A typical response is shown in figure 5. The WKY inhibition of sympathetic nerve activity persisted as long as the aortic nerve stimulation was maintained; as soon as the stimulation was terminated, the splanchnic nerve activity returned to control levels. On the other hand, inhibition of sympathetic nerve activity in response to identical aortic nerve stimulation was smaller in SHR. During the steady-state, the splanchnic nerve activity tended to recover to control levels despite maintained stimulation of the aortic nerve. Figure 6 shows that at all frequencies of the aortic nerve stimulation, the inhibition of sympathetic nerve activity during dynamic as well as the steady-state was significantly smaller ($p < 0.05$ to $0.01$) in SHR than in WKY.

**Discussion**

Under normal physiological conditions, the baroreceptor reflex regulates blood pressure so that it is maintained at or around a setpoint. Various components of baroreflex arc are: 1) baroceptors; 2) the vessel walls in which the receptors are embedded; 3) baroreceptor

**Figure 3.** Comparison of depressor responses to the aortic nerve stimulation in decerebrate WKY and SHR. Solid line = WKY ($n = 10$); dashed line = SHR ($n = 15$). Depressor responses to the stimulation of the left and right aortic nerves were identical; these data were therefore pooled. Top: dynamic responses. Bottom: steady-state responses. During dynamic as well as steady-state, the depressor response (expressed as percent decrease in mean blood pressure) were significantly smaller ($p < 0.05$-$0.005$) in SHR when compared to those in WKY.

**Figure 4.** Comparison of dynamic depressor responses to the aortic nerve stimulation in urethane anesthetized WKY and SHR. Mean blood pressures of anesthetized SHR ($n = 10$) and WKY ($n = 10$) were $180 \pm 5$ mm Hg and $91 \pm 5$ mm Hg, respectively. Note that the depressor responses were significantly smaller ($p < 0.05$-$0.01$) in SHR (dashed line) when compared to those in WKY (solid line). These dynamic responses were similar to those in decerebrate rats, as shown in figure 3.
FIGURE 5. Sympathetic nerve activity (SNA) in SHR and WKY. This figure shows a typical tracing of SNA in WKY (left tracings) and SHR (right tracings). In each panel: top trace represents left greater splanchnic nerve activity. Middle trace represents systolic-diastolic blood pressure. Bottom trace represents spike activity of the pre-celiac segment of the splanchnic nerve. The sympathetic activity prior to the aortic nerve stimulation was greater in SHR when compared to the corresponding value in WKY. Note that the aortic nerve stimulation for 1 minute inhibited SNA more in WKY than in SHR.

afferent fibers; 4) bulbar, suprabulbar, and spinal neurons involved in baroreflex; 5) efferent pathways. In hypertensive humans as well as animals, baroreflex is reset to function at higher blood pressure levels. The mechanism of resetting has been ascribed to changes in baroreceptors themselves as well as to changes in the vessel walls in which they are embedded. In the spontaneously hypertensive rats, the hypertrophy of the aortic and carotid sinus media induced by hypertension contributes to baroceptor resetting. These results are in agreement with those reported in other experimental models of hypertension. However, resetting of baroceptor can occur in the absence of structural vascular changes, suggesting that changes (such as higher pressure threshold and reduced sensitivity to pressure changes) in receptor elements may also contribute to baroceptor resetting. Most of these studies have been carried out in isolated carotid sinus or aortic arch preparations.

These experiments do not exclude the possibility that other components of the reflex arc may be involved in the resetting of baroreflex. Studies in intact preparations in which baroreflex function was tested by drug-induced blood pressure changes could not elucidate if, in addition to changes in baroreceptors and vessel walls, there are other sites of baroceptor resetting. In the present study, these two components were bypassed, and the baroreflex was studied by activating baroceptor afferents in the aortic nerve and recording directly changes in efferent limb of the arc. The rats were artificially ventilated to avoid secondary effects via respiratory changes. Heart rate changes were not analyzed in this study because gallamine was used to immobilize the animals. This neuromuscular blocking agent is known to have vagolytic action. Since the aortic nerve of the rat contains little or no chemoreceptor activity, electrical stimulation of this nerve is expected to activate only baroceptor afferents.

Depressor responses to the aortic nerve stimulation were attenuated in decerebrate spontaneously hypertensive rats when compared to age-matched decerebrate WKY rats. Similar results were obtained in intact urethane anesthetized rats. Seeber et al. in 1972 reported similar results with electrical stimulation of the aortic nerve in dogs made chronically hypertensive by wrapping one kidney with cellophane followed 1 week later by removing the contralateral kidney. Attenuation of responses could be due to changes in: 1) afferent fibers; 2) central cardiovascular neuronal pools; 3) efferent baroceptor pathways; or 4) end organ, i.e., arterioles. It may be argued that SHR may have a lesser
number of fibers in the aortic nerve when compared to the same nerve in the WKY rats. This possibility has been excluded by the electron microscopic studies carried out by other authors. Measurement of the aortic nerve under a microscope (× 40) showed no significant difference in the diameter of the aortic nerve of WKY (100 ± 5 μ; n = 7) and SHR (95 ± 7 μ; n = 10). The role of the last component, i.e., incomplete relaxation of arterioles (due to structural adaptations) in response to reduction in sympathetic tone, was excluded in the present study by recording directly from the efferent limb of the baroreflex arc. Smaller reduction in splanchnic nerve activity in response to aortic nerve stimulation in SHR, when compared to WKY, suggested that resetting may occur in the central cardiovascular neuronal pools. The exact site of the resetting in central nervous system is not clear at present. Since the depressor responses to the aortic nerve stimulation were smaller in decerebrate SHR when compared to the decerebrate WKY, it is possible that the site of resetting is in bulbo spinal region because hypothalamus and other higher centers were absent in these rats. Sympathetic nerve activity is known to be increased in SHR. Our results are in agreement with these reports. The mechanism by which the activity of the sympathetic nervous system is increased in hypertension is unknown. It is conceivable that stimulation of baroceptors is unable to inhibit central bulbo spinal baroreflex neurons in hypertension because of their augmented activity. It may be pointed out that direct punctate stimulation of the medullary pressor areas has been reported to produce more pronounced pressor responses in SHR than in normotensive controls.

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