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Attenuation of Cardiac but not Vascular Component in Baroreflex of Spontaneously Hypertensive Rats

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Abstract

The cardiac and vascular components of the baroreceptor reflex in spontaneously hypertensive rat (SHR) and stroke-prone spontaneously hypertensive rat (SHRSP) were compared against their counterparts in normotensive Wistar Kyoto rat (WKY). SHR, SHRSP and WKY of 12-16 weeks old were chronically instrumented for intra-arterial recording of blood pressure. Intravenous injections of phenylephrine and nitroprusside were used to challenge their baroreflex. The products of blood pressure change and the half time required for the pressure to return to the control value were used as the quantitative estimation of the blood pressure stabilizing capability. The cardiac component of the baroreflex was obtained from the change in the blood pressure stabilizing capability after blockade of β and muscarinic receptors by atenolol and atropine, respectively. The vascular component was obtained by subtracting the cardiac component from the total stabilizing capability which was the difference after blockade with a ganglionic transmission blocker, hexamethonium. We found the cardiac component of the baroreflex of the hypertensive rats was significantly less sensitive than that of the WKY. In contrast, the vascular component of the baroreflex of the three strains did not differ significantly. Therefore, we concluded that the 12-16 week old SHRs were able to maintain a stable blood pressure due to the intact vascular component of the baroreflex.

Key Words: blood pressure control, cardiac sympathetic tone, cardiac vagal tone, heart rate control, SHR, SHRSP

Introduction

It has been well established that baroreceptor control of heart rate (HR) is diminished in spontaneously hypertensive rats, including the spontaneously hypertensive rat (SHR) (4,14,16) and the stroke-prone spontaneously hypertensive rat (SHRSP) (9). The threshold and range of the baroreflex are higher in the hypertensive rats, and the gain of the reflex, as measured with HR change per unit of blood pressure (BP) change, diminishes in the hypertensive rats.

The major function of the baroreceptor reflex is to regulate BP. Previous evidence suggested that baroreceptor control of the BP in hypertension is largely preserved. For examples, similar BP responses are produced with the same amount of suction applied to the neck of normotensive and hypertensive human patients (10). In anesthetized rabbits, Angell-James and George (1) demonstrated that hypertensive rabbits have similar blood pressure and vascular responses to alternations in carotid sinus pressure. In addition, muscular sympathetic responses of hypertensive humans (17) and renal sympathetic responses of SHR (7, 11, 14) were found to be similar to those of normotensive subjects. Luft et al. (9) also demonstrated that the splanchnic sympathetic responses of the SHRSP are similar to those of the WKY.

Several of the studies cited above were

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performed with SHR or SHRSP; some of these investigations (9, 14) had recordings of conscious animals. To our knowledge, no direct study of BP regulation of the baroreflex has yet been performed in conscious, spontaneously hypertensive rats. Good correlation exists between sympathetic activities and BP. The sympathetic activity, nevertheless, is an indirect index of the vasculature tonus and BP. Therefore, in this study the BP stabilizing capability of the baroreflex was used as an index to make a direct comparison of the baroreflex control of BP in chronically instrumented hypertensive (SHR, SHRSP) and normotensive (WKY) rats. The basic principle of this index is that an animal with more sensitive baroreflex has the ability to return BP to its prestimulation level faster than one with less sensitive baroreflex upon challenging BP fluctuation. Thus, an animal with more sensitive baroreflex should have a smaller and shorter change in BP. The same principle can be applied to the same animal under different conditions. The difference between the waveform of BP change produced by vasoactive agents and that of the same animal treated with combined beta-1 and muscarinic blockers was used as a quantitative measurement of the cardiac component of the BP stabilizing capability of the baroreflex. Moreover, the vascular component of the BP stabilizing capability of the baroreflex was assessed with an additional ganglionic blocker, hexamethonium.

Materials and Methods

General Preparation

Male WKY, SHR and SHRSP, seven each, aged 12-16 weeks, were used. They were raised under an identical condition of temperature (20-25°C), light-dark cycle (12 hours light: 12 hours dark) and relative humidity (60-70%). Fu-show Rat Chow and water were available ad libitum. Five to six rats were housed in one cage. The systolic arterial pressure fulfilled the following criteria: SHRSP above 220 mmHg, SHR between 180 and 200 mmHg, and WKY below 150 mmHg. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Arterial and venous catheters were implanted into the right femoral artery and vein, respectively. The venous catheter had a tube in a tube design by drilling a small hole in a PE-160 tube and inserting a piece of PE-10 tubule into it. The PE-160 tube was connected serially to a piece of PE-50 tubule approximately 3 cm in length for cannulation. Next, the PE-10 tubule was threaded into the PE-50 tubule all the way to the tip which was cut flush so that outlets of both tubules were drained directly into the venous blood. Connection points were sealed airtight with silicone glue. The outer tube was used for continuous infusion of saline or blockers; the small inner tubule was used for bolus injection of vasoconstricting or vasodilating drugs. The catheters were tunneled subcutaneously and exteriorized from the nape. After surgery, the animals were allowed to recover in individual cages for at least 48 hours, and the catheters were flushed daily with diluted heparin (100 U/ml in saline).

On the day of testing, the arterial catheter was connected to the pressure transducer (Statham P23D, Gould) for measurement of systemic arterial pressure (SAP), mean systemic arterial pressure (MSAP) and HR. The infusion tube of the venous catheter was connected to an infusion pump. After 10 to 15 min of stabilizing time, the basal BP and HR data were recorded (the control state). This recording and the following tests were performed with the rat conscious and unrestrained in its home cage.

Protocol for Drug Administration

In the control state, saline was infused at the rate of 1 ml/(kg*hr). Bolus administration of phenylephrine (PE) 0.5, 1.5, 5.0 μg/kg and nitroprusside (NP) 1.0, 2.0, 5.0 μg/kg was given in a random sequence pre-arranged. The drugs were made fresh before the individual experiments. Dilutions were made according to the dosage and body weight of the rat so that a 0.1 ml drug solution was given first followed by a 0.2 ml saline flush (the dead space of the inner tubule was less than 0.2 ml). After control state, atropine (3 mg/kg) bolus administration was given. Ten min later, atenolol (3 mg/kg) bolus administration was given. Another 10 min later, saline infusion was switched to infusion of a mixture of atropine, 3 mg/(kg*hr), and atenolol, 3 mg/(kg*hr), in saline (1 ml/(kg*hr)). This was called cardiac blocked state. The six dosages of PE and NP were repeated. Next, a bolus of hexamethonium (HX, 15 mg/kg) was administered, followed by infusion of a mixture of HX, 15 mg/(kg*hr), and atropine and atenolol in saline at the same rate as in cardiac blocked

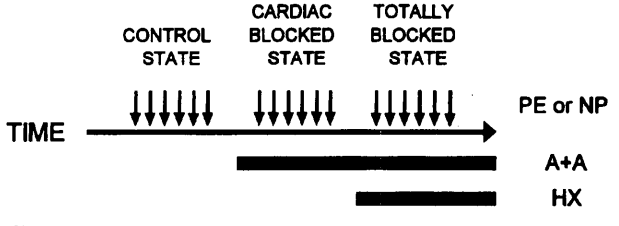

Fig. 1. Protocol of drug administration. The large horizontal arrow denotes the progress of time. Each small vertical arrow indicates a bolus injection of either phenylephrine (PE) or nitroprusside (NP). A+A: atenolol and atropine infusion. HX: hexamethonium infusion.

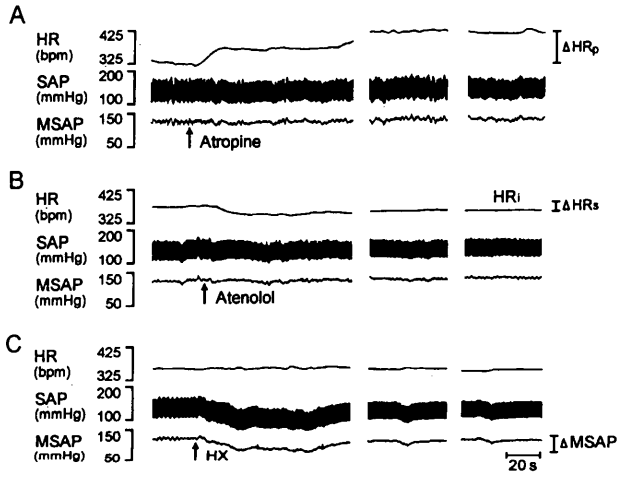

Fig. 2. Methods used in the calculation of cardiac vagal tone ($\Delta HR_p/HR_i$, A), cardiac sympathetic tone ($\Delta HR_s/HR_i$, B), and vascular tone (ΔMSAP/MSAP before HX, C).

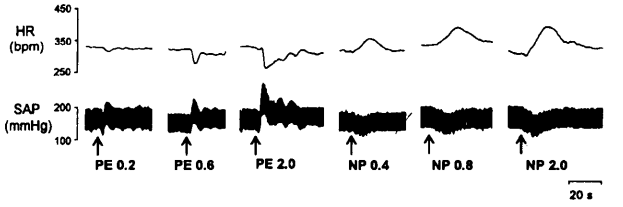

Fig. 3. An illustrative example of BP and HR effects produced by various dosages (in μg) of phenylephrine (PE) and nitroprusside (NP) in a SHR.

state. This was called the totally blocked state. PE and NP boluses were repeated. The entire protocol is illustrated diagramatically in Figure 1. The intervals between each PE or NP dose in the protocol was determined by the complete return of the BP and HR to prestimulation level (at least 10 times the 0.5 ΔT, see below).

Chemicals

Atropine sulfate, atenolol, heparin, hexamethonium bromide, L-phenylephrine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium nitroprusside was obtained from Merck (Darmstadt, Germany).

Data Analysis

Values given in the text and figures are means±S.E. One way analysis of variances was performed to compare data obtained in the three strains of rats. Two way analysis of variance was undertaken to separate the strain factor from interaction with the drug factor. P = 0.05 was used as significance threshold. The following measurements were taken: the difference between peak and initial HR (ΔHR), the difference between peak and initial MSAP (ΔMSAP), and the half time (0.5ΔT) for the returning of the systolic arterial pressure from its peak to initial value. The measurements of ΔMSAP and 0.5ΔT are illustrated in Figure 5B. Here, the products of ΔMSAP and 0.5ΔT were used as a quantitative measurement of BP fluctuation. Three sets of ΔMSAP*0.5ΔT values were obtained for the PE and NP effects in the control state ($\Delta MSAP_C$ * $0.5\Delta T_C$), the cardiac blocked state ($\Delta MSAP_A$ * $0.5\Delta T_A$) and the totally blocked state ($\Delta MSAP_H$ * $0.5\Delta T_H$), respectively. For each dose of either PE or NP, calculations were made for a set of cardiac components ($\Delta MSAP_A * 0.5\Delta T_A - \Delta MSAP_C * 0.5\Delta T_C$) and vascular components ($\Delta MSAP_H * 0.5\Delta T_H - \Delta MSAP_A * 0.5\Delta T_A$) of BP stabilizing capabilities of baroreflex. The baroreflex gain for the control of the HR of each rat was derived from the slope of the

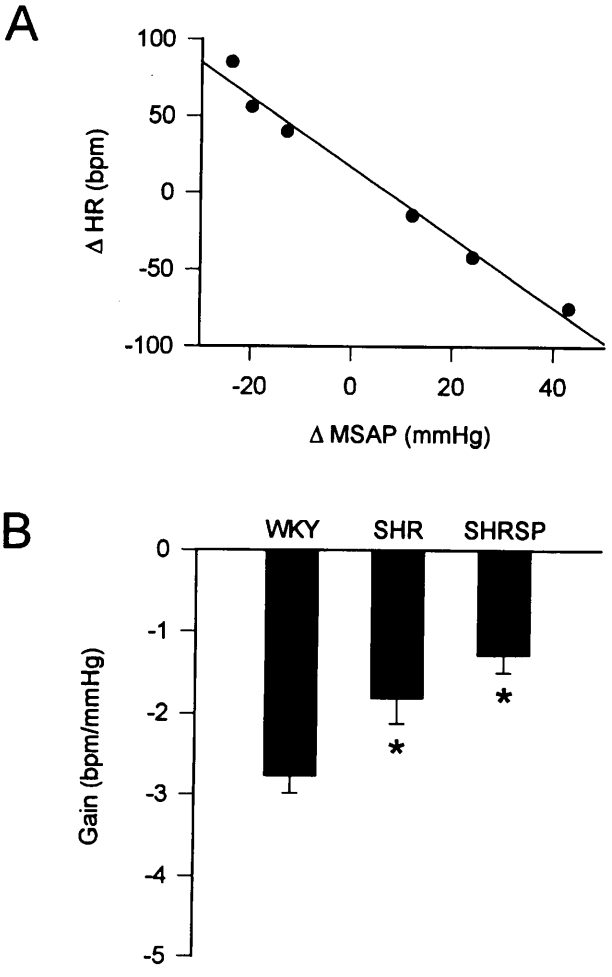

Fig. 4. (A) Estimation of the gain of the baroreceptor reflex in the control of HR by linear regression method. Data as in figure 3. (B) Comparison of the gain of the HR control of the baroreflex in the three strains of rats (n = 7, respectively). *: P < 0.05

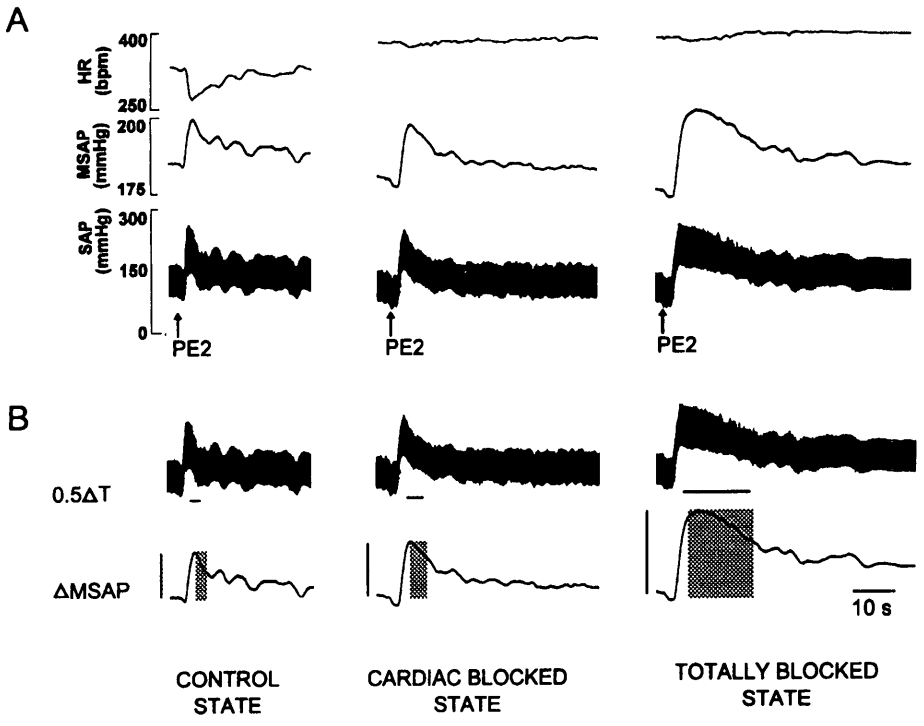

Fig. 5. (A) An illustrative example of BP and HR effects produced by the same amount (2 μg) of phenylephrine (PE) injected intravenously in the control state, cardiac blocked state and totally blocked state in a SHR. (B) Method used in calculating of ΔMSAP and Δ0.5T. Note that the products of ΔMSAP and Δ0.5T (shaded areas) were the largest in the totally blocked state, and the smallest in the control state.

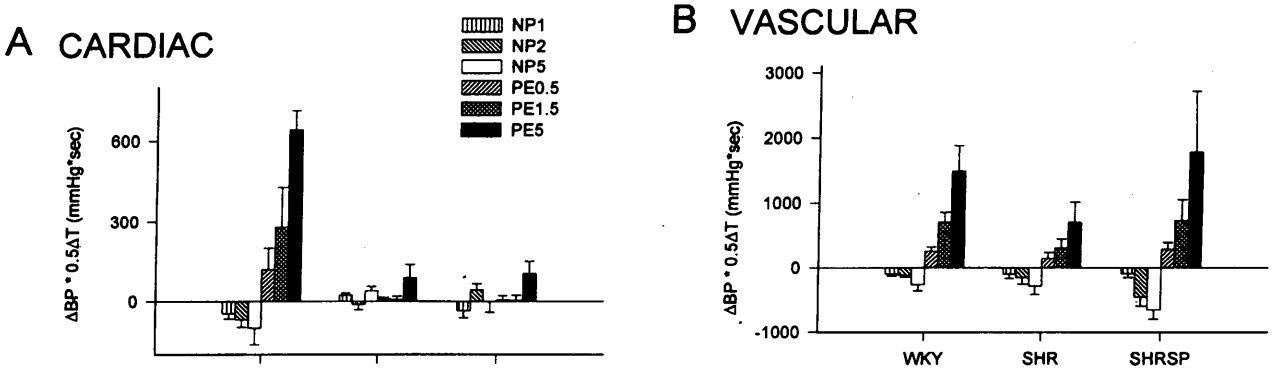

Fig. 6. Comparison of cardiac component (A) and vascular component (B) of the BP stabilizing capability of the baroreflex (as measured with ΔMSAP*0.5ΔT, in mmHg*sec) of the WKY, SHR and SHRSP (n = 7, respectively) with different dosages of phenylephrine (PE) or nitroprusside (NP). Note significantly stain difference in the cardiac component but not in the vascular component.

linear regression of the seven ΔHR/ΔMSAP values in control state (Figure 4A). The cardiac sympathetic and vagal tone were represented by ΔHR/HRi (Figure 2), obtained after atenolol or atropine boluses injections, respectively. HR_i was the intrinsic HR after beta-1 and muscarinic blockade. The vascular tone was calculated as ΔMSAP/MSAP, where MSAP was the MSAP value before HX bolus injection and ΔMSAP was the maximal drop of MSAP by the HX bolus.

Results

At the beginning of the control state before any drug was administered, the resting MSAP of the hypertensive rats (206±7 and 159±9 mmHg for SHRSP and SHR respectively) were significantly higher than those of the normotensive WKY (113±6 mmHg, $P < 0.0001$). The HR values of the three strains did not significantly differ (352±15, 326±15 and 343±13 for SHRSP, SHR and WKY respectively, $P = 0.46$).

Typical responses elicited by PE and NP in a conscious SHR in its control state are shown in Figure 3. PE administration induced an increase in BP, followed by a decrease in HR; NP induced a decrease in BP, followed by an increase in HR, respectively. The magnitudes of responses were proportional to the dosages. The gain of the HR control of the baroreceptor reflex thus derived is shown in Figure 4A. This gain was smaller in the SHR and it was the smallest in the SHRSP as compared to the WKY (Fig. 4B). The differences were statistically significant ($P = 0.0018$).

The baroreceptor reflex control of the HR diminished markedly after combined atenolol, atropine and/or HX treatments. An illustrative example is shown in Figure 5A. In this SHR, the same amount of PE (2 μg) produced progressively stronger BP changes in the control, cardiac blocked and totally blocked states. Scarcely any HR change occurred in the cardiac blocked or totally blocked states. HRi values of the three groups of rats were not significantly different (339±9, 359±7 and 339±15 bpm for WKY, SHR and SHRSP respectively, $P = 0.35$). The differences in the BP changes in different states were used to calculate the cardiac and the vascular components of the BP stabilizing capabilities of the baroreceptor reflex (Fig. 5B). These results are summarized in Figure 6. WKY had a significantly stronger cardiac component than the hypertensive rats ($P = 0.0005$, Figure 6A). In contrast, the strengths of the vascular component of the three strains were not different ($P = 0.27$, Fig. 6B).

The differences in cardiac sympathetic tone, cardiac vagal tone and vascular tone in the three strains of rats as estimated by percentage change were not statistically significant. When expressed as absolute change, Hx produced significantly larger decrease in BP in the SHRSP (-45±6 mmHg) than either SHR (-24±5 mmHg) or WKY (-22±5 mmHg).

Discussion

The cardiac and vascular components of the baroreflex of spontaneously hypertensive rats were assessed in this study by direct measurements of BP stabilizing capabilities of the animals under differential blocked conditions. That the cardiac blockers had effectively and specifically blocked the cardiac autonomic innervations could be ascertained by observing the large shifts in the heart rate caused by the drugs. Thereafter the heart rate remained essentially a flat line even with large fluctuations of blood pressure (Figure 5), and remained flat upon intravenous injection of another blocker, hexamethonium (Figure 2). Whether hexamethonium used effectively removed all the ganglionic transmission was not ascertained in this study. The amount used was the same as that in our previous publication (20) and higher than those used by other published study for the same purpose (6, 8, 12). Whether hexamethonium would have had effects on the central nervous system will not affect our conclusion because after a total blockade of ganglionic transmission, the central nervous system cannot have much effect on the cardiovascular functions anymore. Therefore, the results obtained in this study indicated that the baroreflex of hypertensive rats had a defective cardiac component such that total blockade of the sympathetic and parasympathetic innervation of the heart had only a minute effect in the BP stabilizing capability of the hypertensive rats (Fig. 6A). This observation correlates well with the finding that heart rate control of the baroreflex in the spontaneously hypertensive rats is significantly diminished (4,9,16,18, and Fig. 4B in this study).

More importantly, the vascular component of the baroreflex of the hypertensive rats was found to be as effective as the normotensive WKY (Fig. 6B). Because the vascular component was very much stronger than the cardiac component (as revealed in a comparison of Fig. 6A with 6B), the BP stabilizing capabilities of the baroreflex of the hypertensive rats were as good as those of the WKY. Outwardly, both hypertensive and normotensive rats were capable of maintaining a stable blood pressure level. This conclusion, although implied by the experimental results of sympathetic nerve recording (7, 9, 11, 14), could only be demonstrated by directly measuring of the BP fluctuation, done so in this study.

Hypertensive rats such as SHR and SHRSP are known to have less sensitive baroreceptors (2, 16). The baroreceptors of these animals have higher threshold; they operate within a higher blood pressure range; and their responsiveness, measured as number of spikes per unit change in blood pressure, is significantly decreased. All of these properties fit very well with the finding that cardiac component of the baroreflex is defective in SHR and SHRSP (4, 14, 16 and the present paper). What is not apparent is why the vascular component of the baroreflex should be preserved in these animals. A possible reason is that neurons in the vasomotor areas, such as the rostral ventrolateral medulla and dorsomedial medulla, are hyperreactive in the SHR and the SHRSP (15, 19). In contrast, responsiveness of cardiovagal preganglionic neurons in the nucleus ambiguus in the SHR and the SHRSP are not significantly altered (3). Therefore, a diminished input from the baroreceptor of the hypertensive rats may produce relatively normal amount of responsiveness of the vasomotor sympathetic fibers but a diminished cardiovagal response.

Our second goal was to compare the tonic activities of the cardiac sympathetic, cardiac vagal and vascular nerve activities of the hypertensive versus the normotensive rats by differential blockade with atenolol, atropine and hexamethonium, respectively. This has been achieved many times previously by other investigators (5, 6, 8, 12, 13) and by us (20). Our findings that the sympathetic tone of the hypertensive rats, when normalized to the baseline BP or HR level, were not significantly different from that of the WKY correlate with these previous studies. Thus, we conclude that, after establishment of a high blood pressure level, sympathetic activity may not be an influential factor in maintaining hypertension. The cardiac component of the baroreflex is impaired already in the 12 to 16 weeks old hypertensive rats. However, the baroreflex control of the blood pressure in the same age hypertensive rats is still functioning properly due to the intact vascular component.

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