Cardiac Parasympathetic Output of Nucleus Ambiguus is not Altered in Spontaneously Hypertensive Rats

R.-J. Chiou¹, R.-F. Chen² and C.-T. Yen²

¹ Dept. of Anatomy, Taipei Medical College, Taipei, Taiwan, R.O.C.
² Dept. of Zoology, Taiwan University, Taipei, Taiwan, R.O.C.

ABSTRACT

The distribution and reactivity of cardiac parasympathetic neurons in the ventral medulla of hypertensive rats (spontaneously hypertensive rats [SHR] and stroke-prone spontaneously hypertensive rats [SHRSP]) were compared with age matched normotensive rats (Wistar and Wistar-Kyoto [WKY]). Rats were anesthetized with α-chloralose and urethane. L-glutamate (Glu, 10 mM, 11 nl) was microinjected into the medulla to map the distribution of the cardioinhibitory neurons in and around the nucleus ambiguus (NA). The distribution pattern of the bradycardiac sites was similar in the four strains of rats. Differences in the maximal bradycardic responses were not statistically significant either. In comparison, hypertensive rats had significantly larger depressor responses to Glu microinjected into the caudal ventrolateral medulla. Taken together, the results suggest that the ventral medulla of hypertensive rats has enhanced sympathetic reactivity, whereas the parasympathetic cardioinhibitory mechanisms of the NA of hypertensive rats are not significantly altered. Therefore, the diminished cardiovagal response in the baroreflex of hypertensive rats may be due to an alteration on the input side.

Key words: Caudal ventrolateral medulla, Heart rate, Blood pressure, Hypertension, Glutamate, SHR, SHRSP

INTRODUCTION

The neural mechanism is one of the major factors contributing to the development and maintenance of essential hypertension. Numerous experimental and clinical studies have shown that sympathetic activity and reactivity are increased in experimental models of hypertension and in some essential hypertensive patients (Judy et al., 1976; Tuck, 1986; Julius, 1991; Yang et al., 1993, 1995; Head, 1994). The parasympathetic system, which is equally important in the control of cardiovascular functions, has been largely ignored. Few publications have reported on possible changes of parasympathetic activity in hypertension (Van Zwieten, 1992; Amerena and Julius, 1995). Julius et al. (1971) indicated that both an increase in sympathetic activity and a decrease in parasympathetic tone underlie the increased heart rate and elevated cardiac output found in borderline hypertensive patients. A similar conclusion was reached by Korner et al. (1973) who reported a significantly smaller vagal effect on heart rate (HR) in established hypertension. A few recent studies also showed that there is reduced parasympathetic HR control in
experimental animals and hypertensive patients (Ricksten and Thoren, 1981; Friberg et al., 1988; Whitescarver et al., 1990; Grossman et al., 1992; Langewitz et al., 1994; Truett et al., 1996; Minami et al., 1997; Chandler and DiCarlo, 1998), although others report different results (Daffonchio et al., 1995; Ferrari et al., 1996; Han et al., 1998). Therefore, the potential importance of parasympathetic cardiac control in hypertension should be further explored.

Another important issue has been raised by a recent report from our laboratory (Han et al., 1998). By analyzing the pressure wave and heart rate changes following intravenously administered pressor or depressor agents, we were able to differentiate the cardiac and vascular components of the baroreflex. Interestingly, whereas the cardiac component of the baroreflex of spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SHRSP) was severely impaired, the vascular component of the baroreflex of hypertensive rats did not significantly differ from that of their normotensive relatives. What could the mechanism be underlying this differential change in the control of the heart and vasculature in hypertension? The present study sought to answer this question by comparing the distribution and reactivity of the central vagal component of the baroreflex, i.e., the nucleus ambiguus (NA) of the medullary oblongata of hypertensive and normotensive rats. Comparisons of the distribution and reactivity of the sympathetic component of hypertensive rats have been previously published (Yang et al., 1995).

MATERIALS AND METHODS

Experiments were performed on adult male SHRSP, SHR, Wistar-Kyoto (WKY), and Wistar rats between 12 and 16 weeks of age. The SHPSP, SHR, and WKY were obtained from the Animal Center of the Institute of Biomedical Sciences, Academia Sinica, Taipei. The Wistar rats were purchased from the Animal Center, National Taiwan University Hospital, Taipei. The systolic arterial blood pressure of the animals was examined, and only those with pressure > 230 mmHg for SHPSP, pressure between 200 and 230 mmHg for SHR, and pressure < 170 mmHg for WKY and Wistar rats were used. Surgical and recording procedures complied with NIH (USA)- recommendation for animal use and care.

The rats were anesthetized intraperitoneally with 6 ml/kg initially of a mixture of urethane (450 mg/kg) and α-chloralose (60 mg/kg) in saline, and supplemental doses (2 ml/kg i.v.) were administered when needed. The trachea was intubated for artificial ventilation with the rate and tidal volume adjusted to an expiratory end-tidal CO₂ concentration of 4.0% - 4.5% as monitored with a capnograph (Normocap, Datex). The rectal temperature was maintained at 37.0 ± 0.1 °C with a homeothermic blanket control unit (Harvard). The femoral artery and vein were cannulated for measurement of systemic arterial blood pressure (BP) and for administration of drugs, respectively. HR was monitored through a biotachometer triggered by the pressure pulse. A bipolar stainless steel electrode with fire-polished ball tip was attached to the diaphragm through an opening in the abdomen to record the electromyogram (EMG). All parameters were continuously displayed on a polygraph (TA-11, Gould) and stored on a tape recorder (Neuro Data DR-886) for analysis with a computer. All signals were digitized at 512 samples/s by using an MP100 A/D converter (BIOPAC System, Goleta, CA). The EMG signal was
integrated by a digital integrator with a 20-ms resetting time.

The head of the rat was fixed in a Kopf stereotaxic apparatus in the prone position with the bite bar 10 mm below the interaural line. The dorsal surface of the brain stem was exposed. The medial-lateral and anterior-posterior coordinates of the obex were identified and used as a reference point for brain stimulation.

Microinjections were administered through a two-barrel recording-injection micropipette designed by Tsai et al., (1997). The injection barrel (0.75 mm O.D., tip diameter 20 μm) was filled with 10 mM L-glutamate (Glu) solution in artificial cerebrospinal fluid (ACSF: 124 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 1.25 mM KH₂PO₄, 26 mM NaHCO₃, and 11 mM glucose, pH 7.4) and 0.01% horseradish peroxidase (HRP), which served as a marker for identification of the injection site. Preliminary trials (Fig. 1) showed that with this concentration and in doses of 100 to 300 pmole, repeated injections could be given to the same site at an interval of less than 5 min without affecting the magnitude of the response, indicating that no significant depolarization blockade or tissue damage occurred. Eleven nanoliters of the Glu solution was injected with a pneumatic pressure pump (Medical System, PPS-2). The injection volume was monitored by measuring the movement of the fluid meniscus within the pipette barrel under the operating microscope equipped with a reticle in the eyepiece. A dose of 11 nl was chosen for mapping of the chronotropic cardioinhibitory sites in the caudal ventrolateral region of the medulla (around the NA) and for determining the maximal bradycardiac response, because it is the volume injected with the smallest division of the micrometer, and because this dose produced a near-maximal bradycardiac effect (Fig. 2). The same volume of ACSF was injected as a vehicle and volume control. The micropipette was positioned at a 20° angle. Injections sites were separated by 300 μm in the dorsoventral, mediolateral, and rostrocaudal directions. The main mapping plane was around the obex along

![Figure 1](image_url)

**Figure 1.** Control experiment showing the reproducibility of the microinjection method. From A to F, 110 pmole of Glu was injected into a medullary bradycardiac locus at intervals of approximately 3 min. Note similar depressor and bradycardiac responses with different numbers of air pulses (from one [E] to four [A and F] pulses).
Figure 2. Control experiment showing the dose-response relationship of the microinjection method. Amounts of Glu injected were gradually increased from A to H by varying the duration of the air pulse while holding the injection pressure constant. Durations of the air pulses were 100 ms (A), 150 ms (B), 200 ms (C), 300 ms (D), 400 ms (E), 500 ms (F), 600 ms (G) and 700 ms (H). The interval between injections was 3 min. The volumes of the injections were below the detection limit (A and B), below 11 nl (C), 11 nl (D), 16 nl (E), 12 nl (F and G), and 27 nl (H). Note that at the injection volume of 11 nl, the bradycardiac response was on the rising slope of the dose-response curve.

Figure 3. Three types of cardiovascular responses observed with microinjection of 110 pmole of Glu. (A) Decrease of BP (depressor); (B) decrease of HR (bradycardia); and (C) decrease of BP accompanying the decrease of HR (depressor and bradycardia).

The rostrocaudal direction. Every plane mapped had at least three tracks in each half of the medulla.

At the end of each experiment, the rat was sacrificed by intracardiac perfusion with saline followed by a 10% formalin saline solution. The brainstem was removed and blocked for serial frozen sectioning (50 μm) at the coronal plane. The sections were then processed with the cobalt- and nickel-
Parasympathetic Control in Hypertensive Rats

intensified diaminobenzidine (DAB) method (Adams, 1981) for histochemical demonstration of HRP deposits and counterstained with acetylcholinesterase (Woolf and Butcher, 1981). Chemical stimulation sites were reconstructed from sections containing the electrode tracks and marks of HRP with reference to the stereotaxic atlas of Paxinos and Watson (1998).

**Data Analysis**

Changes in HR resulting from Glu stimulation of the medulla were calculated by subtracting control values from the minimum values after stimulation and compared by Student's non-paired t-test (two-tailed). One-way analysis of variance and Tukey's test were used to compare the maximal bradycardiac responses at different medullary levels. The data are presented as the mean ± S.E. P < 0.05 was considered statistically significant.

**RESULTS**

Three types of cardiovascular responses were encountered when 11 nl of 10 mM Glu solution (110 pmoles) was microinjected into the caudal portion of the ventrolateral region of the medulla. These response types are illustrated in Fig. 3. The focus of the present study is the bradycardiac response (Fig. 3B). Fig. 4 illustrates an example of our method for the localization of the bradycardiac sites. With the aid of acetylcholinesterase staining, the NA could be accurately localized, as illustrated in Fig. 5. In this Wistar rat, three complete planes of bilateral medullary mapping were performed. The relationship of the bradycardiac sites with the NA can be clearly observed. Figs. 6 and 7 show the locations of all the bradycardiac and depressor points, respectively, plotted in a standard series of cross-sections of the rat medulla (Paxino and Watson, 1998). The general patterns of distribution of the bradycardiac sites were similar in hypertensive and normotensive rats (Fig. 6). In comparison, the depressor sites of hypertensive rats (SHR and SHRSP) produced significantly stronger depressor effects than did normotensive rats in absolute magnitude (than WKY and Wistar), or in percentage response (than Wistar). The magnitude of the BP decrease was 58 ± 9, 57 ± 4, 45 ± 2 and 30 ± 4 mmHg for SHRSP, SHR, WKY, and Wistar respectively. Percentage-wise, these values were 39 ± 5 %, 41 ± 2 %, 39 ± 1 %, and 26 ± 3 %, respectively.

The maximal bradycardiac response was determined in each animal. The maximal bradycardiac responses were −144 ± 43 bpm (n = 4) in SHRSP, −205 ± 42 (n = 5) in SHR, −129 ± 25 (n = 10) in WKY, and −274 ± 27 (n = 6) in Wistar rats. After adjustment with the baseline control value, the percentage difference was not statistically significant. The patterns of distribution of the maximal bradycardiac responses to Glu injection at each level of the medulla along the anteroposterior axis did not significantly differ among the four strains either (Fig. 8).

**DISCUSSION**

In the present study, we found that the distribution and reactivity of cardiac parasympathetic neurons in spontaneously hypertensive rats and their normotensive relatives did not significantly differ, as indicated by the similar distribution patterns of bradycardiac sites in the ventrolateral medulla and the similar maximal bradycardiac responses after Glu microinjection.
Figure 4. An illustrative example of complete mapping of the strongest bradycardiac locus and maximal bradycardiac value in a rat. Twenty-one injection tracts were made bilaterally in three anteroposterior planes separated by 300 μm. Changes of HR are labeled at their respective injection sites.
Parasympathetic Control in Hypertensive Rats

Figure 5. A representative example of localization of injection sites with HRP-DAB reaction and acetylcholinesterase counterstain. Cardiovascular responses obtained with Glu injection are shown on the top panel with their matching injection sites in the lower panel. A, B, D, and F: 11 nl; C: 16 nl; E: 22 nl. Note the dark injection tract. Enlarged view of the injection sites is shown in the lower right photograph. Note HRP-labeled neurons in the nucleus ambiguus (NA, arrowheads)
Figure 6. Distribution of bradycardic sites after microinjection of 110 pmol of L-glutamate into the ventrolateral region of the medulla in Wistar (n = 5), Wistar Kyo (WKY, n = 10), spontaneously hypertensive (SHR, n = 5), and stroke-prone spontaneously hypertensive (SP, n = 4) rats from levels 0.5 mm rostral (A 0.5) to 0.5 mm caudal (P 0.5) to the obex. Smallest solid circles: non-responsive injection sites; small circles: sites that elicited bradycardic response < 30%; medium circles: bradycardic response > 30%; large circles: bradycardic response > 10%.
Figure 7. Distribution of depressor sites after microinjection of 110 pmole Glu solution into the ventrolateral region of the medulla. Smallest circles: non-responsive sites; small solid circles: sites that decreased BP below 30% of control value; large solid circles: sites that decreased BP more than 30%; small solid triangles: sites that decreased both BP and HR below 30%; large solid triangles: sites that decreased BP and HR more than 30%.
R.-J. Chiou, R.-F. Chen and C.-T. Yen

Figure 8. Comparison of the maximal bradycardiac response to L-glutamate injection at each level of the medulla along the anteroposterior axis in the four strains of rats. No significant difference was found.

In this series of experiments, we used non-paralyzed, artificially ventilated rats with an intact neural axis and an intact baroreceptor reflex. The interpretation of results under these conditions could be complicated by the interaction of respiration and baroreflex. Only the sites that showed obvious bradycardiac responses without depressor or with minimal depressor responses were included in the data analysis to avoid the contamination of potent sympathetic sites. Many different types of respiratory responses were also obtained after Glu injection into the caudal ventrolateral regions of the medulla. We used a small dose (11 nl, 10 mM) of Glu for injection to reduce the effective spread of the injectate and to minimize respiratory and baroreflex interactions. We attempted to demonstrate Glu spread by adding 0.01% HRP in the Glu solution to observe HRP deposition in the extracellular spaces of the brain tissue. The HRP reaction worked very well in identifying electrode tracks and injection loci. In the present study, electrode tip displacements of 200-300 μm usually eliminated the bradycardiac effect. This suggests a limited spread of Glu relative to the geometry of the underlying neuronal circuits. The cardioinhibitory effect of Glu injection did not result from nonspecific effects of volume or pressure of the injectate since control injection of an equal volume of the vehicle (artificial
Parasympathetic Control in Hypertensive Rats

cerebrospinal fluid) within the same length of time produced no discernible effect.

The NA is the most important cardiovagal region of the brain. It has been estimated that in the cat, more than 90% of the preganglionic cardiovagal neurons reside in the NA (Hsieh et al., 1998). Similar estimations of cardiovagal neurons of the rat (Chen, 1994) put the value at 78%. Direct excitation of neurons in the rat NA produced profound bradycardia. This was completely blocked by either an ipsilateral vagal transection or an intravenous dose of the muscarinic blocker, atropine (Chen, 1994). Thus, results of the present study likely reflect the predominant central cardiovagal control mechanism.

Recent studies suggest that there is reduced parasympathetic cardiac control in experimental animals (Truett et al., 1996; Pelat et al., 1999; Verwaerde et al., 1999) and hypertensive patients (Alicandri et al., 1982; Drummond et al., 1990; Chakko et al., 1993; Langewitz et al., 1994). On the surface, the finding that the responsiveness of the parasympathetic medullary center is not altered in hypertensive rats in the present study might seem at odds with the prevailing wisdom. Closer examination of the literature reveals that the major evidence has been derived from human patient studies (Korner et al., 1973; Alicandri et al., 1985; Drummond et al., 1990; Chakko et al., 1993; Langewitz et al., 1994). The most convincing data from an animal model is that from the hypertensive dog (Truett et al., 1996; Pelat et al., 1999; Verwaerde et al., 1999). When SHR was examined, Daifonchio et al., (1995) found no specific HR or BP spectral power change. Ferrari et al., (1996) examined chronically instrumented SHR for HR and BP variability change and concluded that chronic hypertension altered none of the observed cardiovascular regulatory mechanisms. Our recent work (Han et al., 1998) found no difference in cardiac vagal tone of consciously behaving SHR and SHRSP either. Thus in spontaneously hypertensive rat models, it is not clear whether parasympathetic control does indeed change. In fact, the heart of the SHR is more sensitive to direct stimulation of the vagal nerve than is that of the WKY (Ferrari et al., 1992; Head, 1994).

The evidence for marked impairment of the baroreflex control of the heart of the SHR and SHRSP, on the other hand, is solid (Struyer-Boudier et al., 1982; Head and Adams, 1988; Whitescarver et al., 1990; Widdop et al., 1990; Minami and Head, 1993; Han et al., 1998). Since bradycardia mainly depends on vagal activation (Head, 1994), diminished cardiac parasympathetic control may be involved. Abnormalities may occur in the baroreceptor afferent input, the central nervous system or the heart. The baroreceptors of SHR have been shown to be less sensitive, to have higher threshold, to operate within a higher blood pressure range, and to show significantly decreased responsiveness (Brown et al., 1976). Our results suggest that the responsiveness of cardiovagal preganglionic neurons in the nucleus ambiguus of the medulla is not significantly altered. As described in the preceding paragraph, the responsiveness of the heart of the SHR has been found to be more sensitive (Ferrari et al., 1992; Head, 1994). Therefore, the diminished cardiovagal response of the SHR after peripheral baroreceptor stimulation may be due to alterations on the input side.

Arterial baroreceptor stimulation affects both heart rate (mainly parasympathetic) and blood pressure (mainly sympathetic). By analyzing heart rate changes and blood pressure change separately, we recently demonstrated that the cardiac and vascular components of the baroreflex are differentially modified in SHR and SHRSP. How could the same depressed input cause
depressed heart rate changes without affecting blood pressure responsiveness. Our answer to this question lies in the differential change of the sympathetic center versus the parasympathetic center in the SHR and SHRSP. Whereas the pressor rostral ventrolateral medulla and dorsal medulla, and the depressor caudal ventrolateral medulla of the SHR and the SHRSP become significantly more responsive (Yang et al., 1995), the responsiveness of NA, the major parasympathetic preganglionic nucleus, is not altered (the present study). Therefore, a diminished baroreceptor input plus a relatively normal parasympathetic center produces a diminished heart rate response, while a diminished input plus a more sensitive sympathetic center produces a relatively normal blood pressure response (albeit abnormally higher basal pressure and sympathetic levels) in the hypertensive rats.

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Parasympathetic Control in Hypertensive Rats

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Struyver-Boudier, H. A., R. T. Evenwel, J. F. 123
K.-J. Chiou, R.-F. Chen and C.-T. Yen


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先天性高血壓大鼠延腦疑核區控制心跳的
迷走機制正常

邱瑞珍1、陳瑞芬2、嚴震東2

1台北醫學院解剖學科
2台灣大學動物學系

摘要
本研究探討先天性高血壓大鼠(SHR)及中風型先天性高血壓鼠(SHRSP)
延腦後段腹側區控制心臟功能的副交感性神經細胞的分佈與反應能力是
否與其正常血壓的控制組 WKY 及 Wistar 有所不同。大鼠以氯醛鈉及尿酯
作腹腔麻醉。以微注射方式用 L-甘胺酸(Glu, 10mM, 11nl)興奮延脳疑核
附近的區域來探測降心跳點的分佈與最強能力。我們發現高血壓大鼠疑核
降心跳區域的位置、大小及心跳作用能力均與正常血壓控制組沒有顯著的
差異。同時控制延脳腹外側後段的降血壓反應，則發現高血壓大鼠降血壓
能力大於正常血壓控制組大鼠，顯示交感神經控制區有明顯差異。同時考
量此二結果可以解釋為何高血壓鼠在感壓反應偵測器減弱的狀況下有交
感神經反應能力增加，心跳反應減弱的區別改變。

關鍵詞：心跳、血壓、高血壓、延脳腹外側區後段、甘胺酸

125