行政院國家科學委員會專題研究計畫　成果報告

正常血壓及高血壓大鼠延腦心臟副交感神經元活性及中樞連結之比較

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中文摘要

本計畫利用自發性高血壓大鼠(Spontaneously hypertensive rat；SHR)(9-12 週齡)為高血壓模式動物，並以其同齡近親正常血壓之WKY(Wistar-Kyoto rat)及一般的Wistar 鼠為對照組，來探討高血壓動物控制心臟的副交感神經系統是否產生異常。本實驗採用可以同時紀錄神經元活性和注射藥物的玻璃電極，在延腦閂門附近的疑核(Nucleus Ambiguus; NA)區域，以麩氨酸(L-glutamate 10 mM, Glu)刺激，進行降心跳反應的劑量－反應實驗，以最大反應、閾值反應和劑量－反應曲線的斜率，來比較三種大鼠延腦副交感神經機構之反應性；同時亦記錄明顯降心跳反應位置的神經元活性，分析其神經元的放電型態。實驗結果發現，雖然三種大鼠的最大降心跳反應與閾值劑量沒有統計上的差異，但是有趨勢顯示SHR所需的閾值劑量與飽和劑量均較大。而且造成最大降心跳反應的飽和劑量在SHR中明顯大於正常血壓鼠；劑量－反應曲線的斜率則以Wistar鼠為最大。由實驗結果推測，SHR的疑核可能對麩胺酸刺激的反應性變低，需要較大的刺激才會引起最大的降心跳反應。在三種大鼠的疑核中，大多數神經元具有呼吸節律的放電活性，具有心跳節律的神經元活性極少。

關鍵詞：疑核；自發性高血壓大鼠；心搏徐緩；麩氨酸；心臟副交感神經元活性

Abstract

In the present study, the neuronal activities and HR responses to glutamate solution (Glu, 10 mM) stimulation of the cardiac vagal preganglionic neurons (CVPNs) in nucleus ambiguus (NA) of the 12-16 week old SHR were compared with aged matched normotensive WKY (Wistar-Kyoto) rats and Wistar rats. Microinjection of different doses of 10 mM Glu into the NA produced dose-dependent responses of heart rate (HR) in normotensive and hypertensive rats. The maximal HR changes and threshold dose of Glu were not significantly different in three strains, but there was a tendency that the threshold dose of SHR was largest, then that of WKY rats and Wistar rats. The saturation dose of Glu to elicit the maximal HR change was also significantly larger in SHR than that in Wistar rats. The maximal slope of dose-response curve in Wistar rats is steepest in three strains. The results suggested that the reactivity of CVPNs in NA to Glu stimulation might be lower in SHR than that in Wistar rats. Most neurons in NA had respiratory firing patterns, few pulse-related neurons were observed in three strains of rats.

Keywords: nucleus ambiguous, SHR, bradycardia, glutamate, cardiac vagal preganglionic neuron

Introduction

Neurogenic mechanisms play a key role in both rapid and long-term regulations of blood pressure (BP). The abnormality of autonomic nervous control of the heart may play an important role in genesis of the hyperkinetic state during development of hypertension (Lucini et al., 2002). It is believed that enhanced sympathetic activity and reactivity are major factors contributing to the essential hypertension (Wyss et al., 2004). But the parasympathetic activity in SHR is not well known. Previous evidences suggested that cardiac but not vascular component of the baroreflex of the spontaneously hypertensive rat (SHR) was significantly less sensitive than that of normotensive rat (Han et al., 1998). The activity of cardiac vagal preganglionic neurons (CVPNs) is a major determinant of HR. Resting HR is normally dominated by the tonic activity of CVPNs and is influenced to a lesser extent by sympathetic cardiac activity in humans, dogs, cats and rats (Levy and Warner, 1994; Andresen et al., 2004). It is of interest to determine whether the CVPNs, which counteracts with sympathetic system, exhibit higher or lower reactivity.

In the present study, the neuronal activity and HR response to Glu stimulation of the CVPNs in NA of the 12-16 week old SHR were compared with aged matched normotensive WKY and Wistar rats.

Materials and Methods

General preparation

Experiments were performed on 12-16 weeks old adult male SHR (n=5) and age-matched WKY (n=4) and Wistar rats (n=7). All animals were anesthetized intraperitoneally with urethane (1.3 g/6 mL/Kg , initially) and supplemental doses (0.2 g/kg, i.v.) were administered when required. The rectal temperature was maintained at 37.0 ± 0.5 °C with a homeothermic blanket control unit (Harvard). The femoral artery and vein were cannulated for measurement of BP and for administration of drugs, respectively. HR was monitored through a biotachometer (Grass 7P4G) triggered by the pressure pulse. In addition, an electrocardiogram (ECG) was also recorded (Grass P511 amplifier, 2000X, 10-1000Hz) and used to measure HR. A bipolar stainless electrode with fire-polished ball tip was attached to the diaphragm through an opening in the abdomen to record the diaphragm electromyogram (EMG) via a Grass amplifier (P511, 5000X, 30-1000Hz).

Unit activity recording and medullary glutamate stimulation

The head of the rat was fixed in a Kopf stereotaxic instrument in the prone position. The dorsal medulla was exposed and the obex were identified and used as a reference point for brain stimulation.The recording-injection microelectrode was constructed before experiment (Shaw et al., 2001). The injection barrel of the recording-injection microelectrode were filled with 10 mM Glu solution in 0.9% NaCl with 0.01% HRP that served as a marker for identification of the Glu injection site. The Glu solution was ejected by a pneumatic-pressure pump (Medical System, PPM-2). The main mapping plane was around the obex along the rostrocaudal direction and 1.6-2.2 mm lateral to the middle line.

On approaching the NA and/or when a single-unit activity appeared, the neuronal activity was recorded several minutes for analysis of its firing pattern. The neural activity was amplified (10000X) by Grass P511 amplifier and filtered (300-3000Hz). Then, different doses of Glu were ejected with different pulse durations in a random order to stimulate the neurons and to evoke bradycardiac effects. The dose-dependent relationship between the bradycardiac effects and the doses of Glu was evaluated.

All signals, including BP, unit activity, ECG, diaphragm EMG and Glu injection, were stored on a tape recorder (Neuro Data DR-886) for offline analysis with computer. Unit activities and their relationship with cardiac or respiratory cycles were analyzed by the software OfflineSorter and NeuroExplorer (Plexon). Other signals were analyzed by the software Chart 4 (ADInstrument, Version 4.2).

At the end of each experiment, the rat was perfused. Serial coronal frozen sections (50 μm) through the medulla oblongata were cut. The sites of Glu stimulation were reconstructed from sections containing the electrode tracks and marks of HRP with reference to the stereotaxic atlas of Paxinos and Watson (1998).

Dose-response curve

To establish the dose-response relationship, changes in HR in individual injection sites with different doses of Glu were fitted to a sigmoidal curve with a logistic equation (Sigmaplot Version 8.02, SPSS Inc.)

$$y = d + \frac{a - d}{1 + \exp[b(x - c)]}$$

where x is the volume of Glu injection, y is HR response, a is the maximum y, b is a slope parameter, c is the x at the midrange of the curve. The threshold dose, maximum dose and maximum gain of Glu stimulation was calculated according to the method of Chen and Chang (1991).

Threshold dose = c+2/b
Saturation dose = c-2/b
Maximum gain = Gmax = $|b(a - d)/4|$

Statistics

For group comparisons, data were analyzed by one-way analysis of variance. Data were presented as mean ± SE. $p<0.05$ was considered statistical significance.

Results and Discussion

Bradycardiac response to Glu stimulation of NA

Microinjection of different doses of Glu into the NA produced dose-dependent responses in HR in normotensive and hypertensive rats. Figure 1 shows a representative example of HR responses to various amounts of Glu stimulation in Wistar rats. When sub-threshold dose of Glu solution was applied, there is no change of HR could be observed

(Figure 1A). With the increasing dose, the larger bradycardiac effect could be elicited (Figure 1B and 1C). The maximal HR changes to Glu stimulation were -142 ± 20 bpm in Wistar rats, -204 ± 30 bpm in WKY and -171 ± 30 bpm in SHR. They were not significantly different between three strains of rats.

The threshold dose of Wistar rats, WKY rats and SHR were 2.3 ± 0.5 nL, 9.2 ± 9.6 nL and 13.6 ± 7.3 nL, respectively. The saturation dose of Wistar rats, WKY rats and SHR were 4.4 ± 0.9 nL, 37.6 ± 12.2 nL and 61.7 ± 20.5 nL, respectively. There was a tendency that the threshold dose of SHR is largest, then that of WKY rats and Wistar rats, but there is no significant difference between them. However, the saturation dose of SHR is significantly higher than that of Wistar rats.

The averaged dose-response curves were shown in Figure 2. Compared with that of Wistar rats, the curve of WKY rat shifted rightward and that of SHR shifted more. The slope of Wistar rats is steeper than WKY rats and SHR. However, the slope of WKY rats and SHR were similar.

The firing patterns of unit activities around bradycardiac sites of NA

The unit firing activities were classified into five patterns. They were pulse-related rhythm, expiratory rhythm, inspiratory rhythm, regular firing rhythm and others. The percentage of different firing types in three strains was shown in Figure 3. Units with inspiratory rhythm were more than others. The firing patterns of most neurons around NA were respiratory-related. Units with pulse-related rhythm were rare.

In the present study, the maximal HR responses to Glu stimulation of NA were not different between Wistar rats, WKY rats and SHR, which confirmed the previous investigation (Chiou et al., 2000). But our results also demonstrated that there was a tendency that the threshold dose of Glu stimulation for evoking HR change is largest in SHR and the saturation dose of Glu for evoking maximal percentage of HR change was larger in SHR. It may suggest that the function of vagal nuclei in medulla might be abnormal in SHR.

In the future, we will compare the number and distribution of the medullary CVPNs after wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) injected into the heart. Next, we will record the activity of CVPNs and observe the effect of Glu stimulation of nucleus tractus solitarius (NTS) in vivo or in vitro. We will also examine the correlation between activities of medullary CVPNs and that of NTS with multi-site, single- or multi-channel recording during baroreceptor heart-rate reflex. We will assess whether the impairment of baroreflex exists in neuronal transmission between NTS and NA in SHR. In the tract-tracing experiments, biotin-conjugated dextran (BD) and WGA-HRP will be injected to NTS and the heart, respectively. We will examined whether the number of NA projecting neurons in NTS or their fiber terminals around the CVPN s are reduced in SHR. These data should be helpful for understanding the difference of functional organization of medullary neural circuit controlling baroreflex bradycardia between the hypertensive and normotensive rats.

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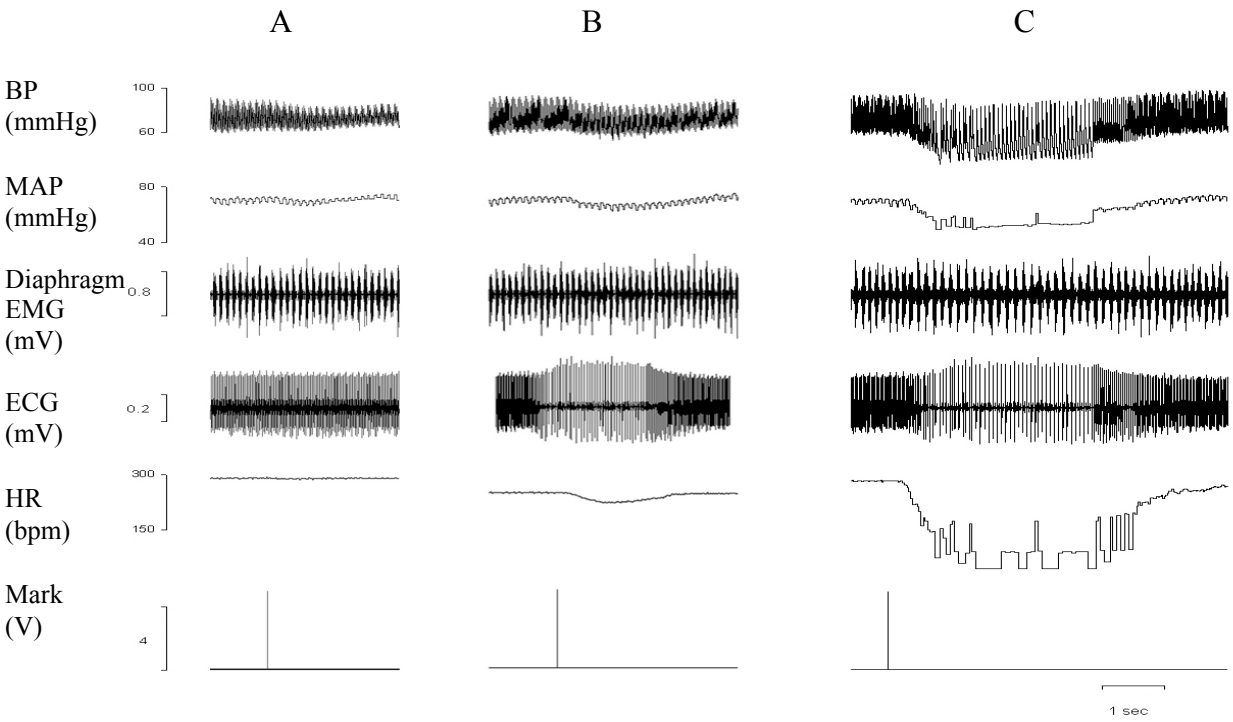

Figure 1 A representative example (Wistar rat 5) of HR responses to various amounts of L-glutamate (Glu, 10 mM) stimulation in Wistar rats. This figure shows the dose-dependent response to Glu stimulation. The traces from top to bottom : BP, arterial blood pressure; MAP, mean arterial blood pressure; Diaphragm EMG, diaphragm electromyogram; ECG, electrocardiogram; HR, heart rate; Mark: ejected volume of Glu. A. 0.7 nL, B. 3 nL, C. 5.6 nL.

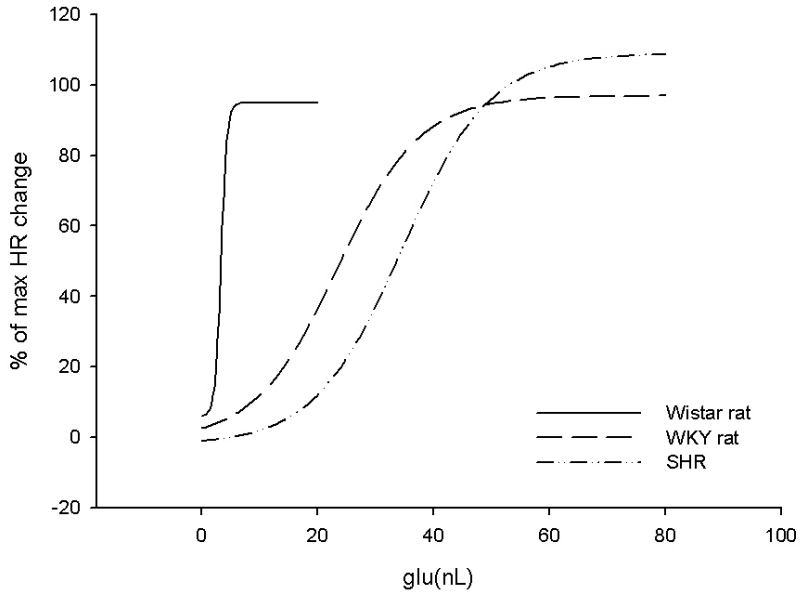

Figure 2 Averaged dose-response curves showing relationship between evoked bradycardiac responses and doses of Glu microinjected in NA in Wistar rats, WKY rats and SHR.

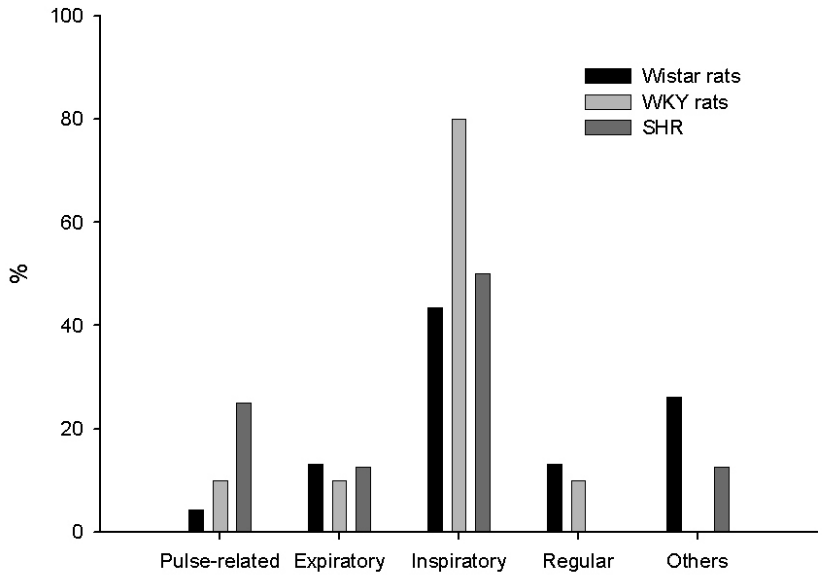

Figure 3 The proportions of different firing patterns of NA neurons in three strains. Note that units with inspiratory rhythm were most abundant.