Depressor effect of closed-loop chip system in spontaneously hypertensive rats

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Abstract

We previously reported that a closed-loop chip system was designed to decrease arterial pressure in normal rabbits and rats. In the present study, the depressor effects of the chip system were investigated in spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY). The arterial pressure was recorded, sampled, operated and processed in the chip system. The chip system instantaneously controlled arterial pressure by stimulating the left aortic depressor nerve according to the feedback signals of arterial pressure. The closed-loop chip system effectively decreased mean arterial pressure (MAP) and heart rate (HR) in both SHR and WKY rats. It decreased the duration and the maximal MAP level of the pressor response evoked by either intravenous injection of phenylephrine or cutaneous nociceptive stimulation in SHR, but had no significant effect on the magnitude of the increase in MAP. Furthermore, the chip system significantly increased the baroreflex gain in SHR, but not in normal WKY rats. These results suggest that the closed-loop chip system effectively decreases the arterial pressure and increases baroreflex gain in SHR. The chip system does not abolish the arterial pressure responses to accidental pressor events, but decreases the duration and the maximal MAP level of the pressor responses.

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1. Introduction

It is well known that baroreflex is a powerful buffering mechanism which counteracts short-term fluctuations in arterial pressure. Although the abnormal baroreflex function has been found in several animal models of hypertension (Andresen et al., 1980; Coleridge et al., 1984; Lantelme et al., 1998) and patients with hypertension (Grassi et al., 2000; Mussalo et al., 2002), the role of baroreflex in long-term control of arterial pressure is still uncertain. Recent studies have indicated that continuous activation of the bilateral carotid sinuses produces sustained hypotension in normal dogs during a one-week period (Lohmeier et al., 2004), and that the baroreflex plays an important role in the long-term control of the arterial pressure (Lohmeier, 2001; Malpas, 2004; Lohmeier et al., 2004, 2005b). These findings suggest a possibility to decrease arterial pressure by activating the baroreflex in hypertension.

As early as 1960s, an implantable device called baropacer was designed to treat serious and drug-resistant hypertension by stimulating the carotid sinus nerves (Bilgutay et al., 1965; Neistadt and Schwartz, 1967; Schwartz et al., 1967). An improved device was used for the treatment of hypertension in 1970s (Brest et al., 1972). It was found that the device did chronically decrease arterial pressure in those patients. However, the baropacer was not easily accepted by most patients because of the technical limitations such as its big size, poor battery, failure in external control of the stimulating current, and discomfort caused by current leakage. Furthermore, the device was designed as an open-loop system and could not receive the feedback information of the arterial pressure, which was difficult to control the arterial pressure as exactly as desired. The approach for treatment of hypertension
with the baropacer was eventually abandoned owing to its technological limitations. Nowadays, it is probable to break through these limits by developing a new device to control arterial pressure accurately because of the rapidly developed modern technology. It is more exciting that a persistent reduction in arterial pressure could be caused by one-week activating the bilateral carotid sinuses in normal dogs with a developed method (Lohmeier et al., 2004, 2005a,b). Thus, it is possible to treat hypertension with the long-term baroreflex activation.

We have designed a special closed-loop chip system which is used to control arterial pressure by activating the baroreflex. The chip system mimics the negative feedback model of physiological closed-loop regulatory system, and instantaneously controls arterial pressure according to the feedback signals of real-time arterial pressure. In the past few years, the chip system has been evaluated in vitro and vivo, and its depressor effect has been confirmed in normal rabbits and rats in acute experiment (Gao et al., 2004, 2005, 2006). In the present study, the depressor effect of the chip system was investigated in spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY). The effects of baroreflex activation with the chip system on the accidental pressor events mimicked by either intravenous administration of phenylephrine or cutaneous nociceptive stimulation were evaluated. Furthermore, the change of baroreceptor reflex sensitivity (BRS) caused by the chip system was determined with repeated bolus intravenous injections of graded doses of phenylephrine.

2. Methods

Experiments were carried out in 28 male SHR and 22 normal WKY rats weighing between 250 and 300 g. All animals were obtained from Shanghai Laboratory Animal Center. The procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

2.1. Chip system

The chip system has been reported in our previous studies (Gao et al., 2004, 2005, 2006). Briefly, it was primarily composed of a microprocessor, an A/D convertor, a built-in bridge amplifier, a connecting interface of transducer and stimulating electrodes. The arterial pressure was recorded, sampled, operated and processed in the chip system. According to the input information of the arterial pressure, the output signals in different frequencies produced by the chip system were used to stimulate the left aortic depressor nerve (ADN). Then, the stimulation of the ADN caused the decrease in mean arterial pressure (MAP). Although the duration and the amplitude of the stimulus were kept at 0.2 ms and 6.0 V respectively, the frequency of the stimulus varied between 0 and 100 Hz according to the difference between the built-in artificial set point in the chip and the real-time MAP. That is, an artificial arterial pressure resetting mechanism was established by the chip system to modify the original set point of arterial pressure in the body. The data of the MAP were saved in the memory of the chip, and were transferred to a personal computer by way of on-line or off-line.

2.2. Program of chip system

When the range of MAP was between 80 and 160 mmHg, the frequency of stimulus increased following the elevation of the MAP. Thus, the increased stimulation of the ADN resulted in an enhanced depressor reflex followed by a decline of MAP. When the MAP exceeded 160 mmHg, the frequency of stimulation reached its maximal value (100 Hz). However, when MAP was less than 80 mmHg, the stimulation stopped automatically. It was reported that the relationship between the actual values of the pressure (input) and the frequency of stimulation (output) was almost completely accorded with the design of the chip system (Gao et al., 2006).

2.3. Surgical procedure of acute experiment

Each rat was anesthetized with intraperitoneal injection of urethane (1.2 g/kg). Tracheotomy was performed and an intubating cannula was connected to a rodent ventilator. The right femoral artery and femoral vein were cannulated for recording of MAP and administration of drugs, respectively. Arterial pressure was recorded with a special designed solid-state pressure transducer embedded at the tip of the catheter which was connected to a built-in bridge amplifier and a data acquisition system in the chip. Heart rate (HR) was determined by the arterial pressure waves. The arterial pressure and HR were simultaneously recorded on a Powerlab data acquisition system (ADInstruments, Australia). The left aortic depressor nerve (ADN) was isolated and identified. In order to keep an effective response of the ADN to long-term stimulation, it was critical to assure that all small vessels irrigated the ADN as well as possible during the ADN isolation. A pair of thin silver electrodes was hooked around the nerve preparing for the electrical stimulations of the chip system. The ADN and electrodes were immersed in silicon elastomer (WPI Inc., FL) to fix the ADN on the electrodes, isolate surrounding tissues and nerves, and avoid water loss of the ADN.

2.4. Evaluation of the BRS

The baroreceptor reflex was induced by repeated bolus intravenous injections of graded doses of phenylephrine (1, 5, 10, 20, and 40 μg/kg) (Smyth et al., 1969; Machado, 2001; Piccirillo et al., 2003; Watson et al., 2004). The effects of phenylephrine completely recovered to the control level within 2 min. Phenylephrine injections were separated with an interval of at least 3 min for a complete recovery. The BRS was
evaluated in each rat by fitting a least-squares regression line for the relationship between the changes in HR and the changes in MAP for each data point obtained with graded injections of phenylephrine (Oliveira et al., 1996). The slope of the line expressing the relationship (beats·min$^{-1}$·mmHg$^{-1}$) was used as an index of the BRS. The BRS was respectively evaluated before, during and after the ADN stimulation.

2.5. Evaluation of the effect of chip system on accidental pressor response

To determine whether the chip system partially or completely blocked the pressor response caused by acute accidental pressor events, the effect of the ADN stimulation with the chip system on the acute pressor responses was investigated before, during and after the stimulation. Two different methods were used to mimic the accidental pressor events in the present study. One was the bolus intravenous injection of phenylephrine (20 μg/kg), and the other was cutaneous nociceptive stimulation which was carried out by pinching an area of approximately 1 cm$^2$ of the skin of the abdominal area for 30 s (pinching force was 3 kg) (Uchida et al., 2005). The pressor response was evaluated by both the duration and the maximal level of the increased arterial pressure. The duration of the pressor response was calculated at the level which was 10% of the maximal level of the pressor response.

2.6. Experiment protocol

All rats were subjected to electrical stimulation of the ADN with the chip system lasting for 60 min. At first, the depressor effects of the chip system were determined in 8 SHR and 8 WKY rats. Secondly, the effects of the chip system on the pressor responses induced by bolus intravenous injections of two doses of phenylephrine (5 μg/kg and 20 μg/kg) were investigated in 6 SHR and 6 WKY rats. Thirdly, the effects of the chip system on the pressor response induced by cutaneous nociceptive stimulation were evaluated in 6 SHR. At last, the effects of the chip system on the BRS were determined in 8 SHR and 8 WKY rats.

2.7. Drug

Phenylephrine was obtained from Sigma Chemical Co.

2.8. Statistical analysis

Comparisons between two observations (before and after administration) in the same animal were assessed by paired $t$ test. One-way ANOVA, followed by the Student’s $t$ test was used when multiple comparisons were made. The criterion for statistical significance is set at $P<0.05$. All data are presented as mean±SE.
3. Results

3.1. Depressor effects of chip system

Stimulation of ADN with the chip system significantly decreased the MAP and the HR in SHR (−35.4±6.8 mmHg; −20.4±9.0 bpm) and WKY rats (−17.1±6.9 mmHg; −50.8±10.5 bpm) compared with the baseline (Fig. 1). It caused a greater fall in MAP but a smaller fall in HR in SHR than in WKY rats. Although the chip system did not normalize the MAP completely in severe hypertensive rats whose systolic pressure exceeded 200 mmHg, it did show a great depressor effect (−55.4±10.6 mmHg, n=3). Fig. 2 shows representative recordings of arterial pressure in SHR and WKY rats. During the period of stimulation, the arterial pressure was well controlled at a lower level with little fluctuation. When the chip system ceased stimulating, both the MAP and the HR returned to 90%–95% of the baseline immediately, and recovered in 1 min without significant rebound.

3.2. Effects of chip system on pressor response evoked by phenylephrine

Intravenous injection of phenylephrine resulted in a rapid and transient pressor response. Stimulation of the ADN with the chip system significantly decreased the duration of the pressor response evoked by intravenous administration of phenylephrine compared with control and recover in both SHR and WKY rats (Fig. 3). Although the magnitude of pressor responses was not changed, the maximal level of the MAP after administration of phenylephrine was much lower during the stimulation of ADN than control or recover (Fig. 4).

3.3. Effects of chip system on pressor response evoked by nociceptive stimulation

Cutaneous nociceptive stimulation caused a pressor response accompanied with increased HR. Stimulation of ADN with the chip system significantly decreased the duration of the pressor response evoked by cutaneous nociceptive stimulation in SHR. Although the magnitude of pressor responses was not decreased, the maximal level of the MAP during the period of the pressor response was much lower during the ADN stimulation than baseline (Fig. 5).

3.4. Effects of chip system on the BRS

The BRS was determined by repeated bolus intravenous injections of graded doses of phenylephrine. The BRS in SHR was much lower than in WKY rats. The stimulation of the ADN significantly increased the BRS in SHR, but not in WKY rats (Fig. 6).

4. Discussion

We previously reported that a closed-loop chip system was designed to control arterial pressure instantaneously according to the feedback signals of real-time arterial pressure. The

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Fig. 2. Representative recordings of arterial pressure in SHR and normal WKY rats. The arterial pressure was well controlled at a lower level with little fluctuation by the stimulation caused by the chip system.
Chip system successfully and steadily kept the arterial pressure at a lower level in either normal rabbits or normal rats (Gao et al., 2004, 2005). Pretreatment with either methyl atropine or bilateral vagotomy abolished the bradycardia effect of the chip system but had no significant effect on hypotension (Gao et al., 2006). In the present study, we found that the chip system steadily decreased the arterial pressure to a lower level which was close to the normal arterial pressure in SHR. There was no significant adaptation during the stimulation with the chip system and no significant rebound after the cessation of the stimulation in all rats. In 3 severe hypertensive rats whose systolic pressure exceeded 200 mmHg, the chip system achieved significant depressor effect by about −50 mmHg although it didn’t lower the blood pressure to the normal level. These results indicate that the chip system effectively decreases the arterial pressure in hypertensive animal models.

The chip system caused a greater fall in MAP but a smaller fall in HR in SHR than in normal rats, suggesting that the depressor effect induced by the chip system was less dependent on its bradycardia effect in hypertensive rats than in normal rats. The results are consistent with our previous findings that pretreatment with either methyl atropine or vagotomy completely blocked the bradycardia but had no significant effect on depressor response induced by the stimulation with the chip system (Gao et al., 2006). These results suggest that the fall in arterial pressure induced by the chip system is mainly due to the sympathoinhibitory component of the baroreflex. It might be a potentially encouraging advantage of the chip system that the chip system decreased arterial pressure without much interfering with the heart rate.

Wondering whether the chip system fixed the arterial pressure at an unchangeable level, we investigated the effects of the chip system on the mimicked daily accidental pressor events. It was found that the chip system did not reduce the magnitude of pressor response evoked by the alpha receptor agonist phenylephrine or nociceptive stimulation. These results indicate that the arterial pressure can still respond to the pressor events evoked by either vasoconstrictive drug or nociceptive stimulation during the ADN stimulation with the chip system, which was beneficial to the animal in adapting the various rapid changes in the environment when using the chip system to control arterial pressure. However, the ADN
stimulation with the chip system significantly decreased the duration and the maximal MAP level of the pressor response caused by phenylephrine in either SHR or WKY rats. The chip system had a similar effect on the pressor response evoked by cutaneous nociceptive stimulation in SHR. The shortened duration of the pressor response might be explained as the enhanced baroreflex activation increasing the body’s ability on buffering blood pressure. These findings are important because the decreased duration and maximal MAP level of the pressor response might reduce or eliminate the risk of the cardiovascular accidents in the hypertensive state.

It is well known that the BRS is impaired in either hypertensive patients or experimental hypertensive models, as exemplified by the blunted baroreceptors and the decreased baroreflex gain (McCubbin et al., 1956; Brown et al., 1976; Andresen et al., 1978, 1980). We found that the chip system significantly improved the BRS in hypertensive rats. It was possible that the improved BRS was caused by establishing an artificial closed-loop control system. When the chip worked, the signals of arterial pressure were recorded and operated by the chip system and were sent back to the body which bypassed the blunted baroreceptor. Therefore, the closed-loop chip system might raise the baroreflex gain. There was no significant difference in the BRS between the baseline and the recover, suggesting that the continuous activation of baroreflex lasting an hour did not impair the baroreflex function in SHR.

Implanted chips are new-developed medical devices which have been used to treat some diseases such as diabetes and Parkinson’s disease (Nasser et al., 2002a,b; Hernando et al., 2004; Hovorka, 2006). However, the chip system used to control arterial pressure in this study shows its particular features. Firstly or most importantly, the working principle of the chip system is a closed-loop system instead of an open-loop system. In other words, the chip system instantaneously controls blood pressure by changing the frequency of the ADN stimulation according to the feedback signals of the arterial pressure. Secondly, it effectively and stably decreases the arterial pressure in acute experiment of hypertensive animal models and normal animals. Thirdly, the data in the chip can be easily saved or communicated with personal computers by way of on-line or off-line. Fourthly, the program operated by the chip system can be easily changed or updated according to the different experiment design or depressor requirement. Lastly, the size of the chip is small, and electric power consumption is very low. Thus, the chip is suitable for implantation in the body. Therefore, the chip system could be suitable for complex control of the arterial pressure as an implantable device, which is strengthened by...
the findings that the hypotensive response was sustained throughout the entire 7 days of baroreflex activation (Lohmeier et al., 2004, 2005a). The insufficiency of the present study is that the depressor effect of the chip system is only investigated in acute experiments without the results of long-term study. However, the present study did indicate that the chip system had an effective acute antihypertensive effect and could be used to decrease arterial pressure. Our findings urge us to determine the long-term effects of the chip system on arterial pressure in conscious normal animals and hypertensive models.

In conclusion, the closed-loop chip system has a significant depressor effect in hypertensive animal models and normal animals during acute experiments. The chip system increases the baroreflex gain, and decreases the duration and the maximal MAP level of the pressor responses to accidental pressor events in SHR.

Acknowledgments

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References


