

Original Article

The effect of angiotensin II in nucleus tractussolitarius in chronic neuropathic pain induced hypertension

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Abstract: Chronic neuropathic pain (CNP) is a global disease with a high incidence rate. Hypertension is one of its terrible complications. This experiment was designed to study the mechanism of this terrible event with detecting if Ang II in NTS through BRS participates in this higher blood press. Twenty rats randomize to 2 groups: Sham group and CNP group. Rats in CNP group received chronic constriction injury of the infraorbital nerve, while Sham group was exposed but was not ligated. Compared with Sham group, the pain threshold in CNP group was significantly declined since 10 days after surgery and lasted to 40 days ($P<0.05$). Forty days after operation, CNP rats' MAP were significantly increased ($P<0.05$), while BRS decreased obviously ($P<0.05$), which were improved by microinjection prevented by microinjection of AT₁R blockade (losartan) ($P<0.05$). Consistently, central infusion of Ang II produces the increased BRS. It is suggested that increased Ang II system in the NTS may play an important role in mediating the cardiovascular dysfunction after CNP.

Keywords: Ang II, chronic neuropathic pain, nucleus tractussolitarius, hypertension

Introduction

According to the 2014-2015 Global Year against Neuropathic Pain Campaign, there are 7% to 8% of the general population suffers from neuropathic pain [1]. Increasing evidence shows that, except the painful experience, chronic neuropathic pain (CNP) affects the regulation of other physiological processes in the body, such as anxiety, depression and sleep disorders [1, 2]. Hypertension is one of the changes which have been numerously reported among CNP patients. Many articles have reported the significant positive relationship between resting blood press and pain sensitivity in chronic pain patients [3-5]. Impaired baroreflex sensitivity may play an important role in this relationship.

Nucleus tractussolitarius (NTS) which is the location of the first synapse in the baroreceptor reflex pathway [6] may be involved in the processing of hypertension after neuropathic pain. NTS also plays an important role in pain regula-

tion for stimulating it inducing antinociception [7]. However, the exactly mechanism of NTS for hypertension after chronic neuropathic pain has not been explicated. It is well known that the increased angiotensin II (Ang II) in the NTS contributes to hypertension. Microinjection of Ang II into the NTS inhibits baroreflex function, whereas blockade of Ang II facilitates the BRS [8]. It is not clear whether Ang II is involved in this processing of blood pressure change after CNP. Therefore, this study was designed by microinjected Ang II and AT₁R blocker losartan into the NTS to clarify if Ang II and NTS were associating with the changes of BRS after chronic neuropathic pain which was developed from chronic constriction injury to infraorbital nerve.

Methods

Experimental animals and groups

A total of 50 male Sprague-Dawley rats (190-220 g) were obtained from a single vendor

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(Sino-British SIPPR/BK Laboratory Animal Ltd, Shanghai, China). They were housed temperature-controlled cage and 12-h light-dark cycle. Food and water were available ad libitum. All experiments were approved by the Institutional Care and Use Committee of the Second Military Medical University, and conformed to the US National Institute of Health Guide for the Care and Use of Laboratory Animals. Three days before the experiments, the rats were placed singly in special brown and non-transparent cages and calmed for 20 min before measurement. Von-Frey filaments were used to stimulate the rats' whisker pads for five times on each side to allow the rats to adapt to the stimulation of Von-Frey filaments. The basal mechanical pain threshold values were recorded, and rats that were very sensitive to the Von-Frey filaments were excluded. After the training was completed, rats that were adapted to the training were randomly divided into the model group (CNP group, 10 animals) and the sham group (10 animals). The methods for general surgery, chronic neuropathic pain, tail arterial pressure measuring and baroreflex measurement were described in previous studies by our laboratory.

Chronic neuropathic pain model [9]

The model, which was according to our formal work, was established using the chronic constriction injury of the infraorbital nerve, branch of the trigeminal nerves in rats. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.35 mL/100 g). A 1-cm incision was made at approximately 0.5 cm below the zygomatic bone in the rats' cheeks. The subcutaneous tissues, muscle, and surrounding fascia were blunt separated to expose the infraorbital foramen. The infraorbital nerve traveled from the infraorbital foramen was observed in a fan-shape distribution. A glass dissecting needle was used to free the infraorbital nerve from the proximal end for approximately 4 mm. The infraorbital nerve was ligated using two pieces of absorbable thread (4-0 chromic catgut suture) under a microscope; the spacing was approximately 2 mm with proper strength to mainly form a constriction ring. The incision was sutured. The animals were normally fed. The infraorbital nerve of the sham group was exposed using the same method but was not ligated.

Measurement of the mechanic pain threshold

Changes in the behavioral reaction of the animals were observed on preoperative day 3 and postoperative per 10 days, and the testing was performed using the Von-Frey filaments. The stimulation strengths from low to high were 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 26.0 g. Each stimulation strength was tested five times on the bilateral whisker pads of the rats. The mechanical pain threshold value was the corresponding stimulation strength of one or more items presented by the rats, as follows: (1) Dodge actions such as backward movement, turning around, or shaking the head: To avoid the stimuli, the rats would curl their body, move closer to the cage walls, or hide their face and head under their body. (2) Scratching their face: The presentation was scratching the stimulated region on the face more than three times. (3) Aggressive behaviors: The rats grasped and bit the stimulating device and exhibited attack actions.

Measurement of blood pressure (BP) and heart rate (HR)

The rat tail arterial pressure measuring instrument was turned on, and the temperature was set at 37°C to pre-heat for 10 min. The calibration was performed in real-time according the pressure signals. The fixed box was adjusted according to the rats' body size. The rats were gently placed in the box, and flipping the rats in the box was avoided if possible. A pressurizing sleeve was placed on the tail root of the rats, and the temperature probe was inserted into the fixed box to observe the temperature changes at any time, to ensure the stability of the rat body temperature. The BP was measured when the rat pulses were stabilized. During the measurement, pressure was applied using the pressurizing sleeve to a value above the systolic BP to block the blood flow in the tail artery, and the pulse gradually disappeared. When the externally applied pressure decreased to the systolic pressure, the pulse reappeared, thereby signifying the systolic pressure. With a continuous decrease in the externally applied pressure, the wave amplitude of the pulse continuously increased. When the applied pressure decreased to the diastolic pressure, the pressure on the tail artery applied by the pressurizing sleeve disappeared, and the wave amplitude of the pulse reached

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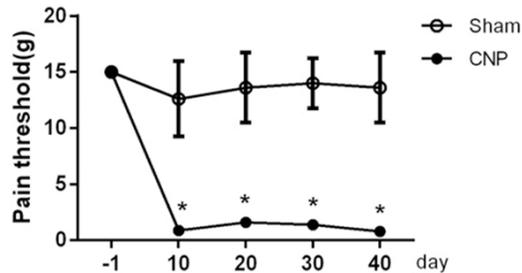


Figure 1. Changes in the mechanic pain threshold in rats before and after CNP surgery. * $P < 0.05$, compared with Sham group in same time.

the maximum value, which was the diastolic BP. Based on the BP curve, the values including the systolic pressure, diastolic pressure, and HR were fitted using the pressure measurement software. Each rat was measured 10 times with an interval of 2 min. The average value was used as the final BP value.

General surgery at 40 days after CNP

Forty days after CNP surgery, rats with CNP and sham operation were anesthetized with intraperitoneal α -chloralose (40 mg/kg) and urethane (800 mg/kg). The trachea was cannulated and artificial ventilation with mixed 100% oxygen and room air was used for assistance. The left femoral artery was cannulated for recording BP and heart rate (HR) by PowerLab/8PS (ADInstruments, Australia). Both the left and right femoral vein were cannulated for fluid infusion and drug injections. Rats were placed in a stereotaxic frame (Narishige, Japan) and the dorsal surface of the medulla oblongata was exposed by removing part of the occipital bone and dura from incising of the atlanto-occipital membrane. Temperature of the rats were maintained at about 37°C with an temperature controller (World Precision Instruments, USA).

NTS microinjections [10]

NTS microinjections were according to our former experiment. The CNP rats were used to explore the effect of microinjection of the AT_1R blocker losartan into the NTS on changes in BP and BRS. NTS Microinjections were made by three-barrel micropipettes (tip: 20-30 μ m) using a pneumatic pressure injector (World Precision Instruments, USA). The microinjection volume was 50 nl. The position of NTS was 0.4-

0.5 mm rostral, 0.5-0.6 mm lateral, and 0.4-0.5 mm deep to calamus scriptorius, and functionally identified by a rapid depressor response (>25 mmHg) to 1 nmol L-glutamate injection. The interval between bilateral injections was within 60 seconds. L-glutamate and losartan were dissolved in artificial cerebrospinal fluid (aCSF). At the end of each experiment, the injection sites were identified by microinjection of 50 nl 2% pontamine sky blue for analysis of injection area.

Measurement of baroreflex sensitivity

The most common method for quantitatively assessing BRS is based on the Oxford and Modified Oxford techniques by altering BP pharmacologically [11]. In our study, we used nitroprusside sodium (100 μ g/kg) to decrease BP (40-50 mmHg), and then increased it (140-150 mmHg) with phenylephrine (80 μ g/kg) [10]. The sensitivity of baroreflex was calculated as the changes in HR (beat/min) per unit changes in MAP (mmHg) ($\Delta HR/\Delta MAP$).

Intra-cerebroventricular infusion [10]

This experiment was performed to verify the effect of central angiotensin II on MAP elevation. The controlled, intact sham rats were anesthetized with α -chloralose (40 mg/kg) and urethane (800 mg/kg), ip. The atlantooccipital membrane was exposed and punctured into the fourth ventricle by a stainless-steel cannula, which was verified by seeing the cerebrospinal fluid running out through the cannula. The cannula was connected to a 0.5 ml syringe via 30 cm of flexible tubing. Ang II was infused at the rate of 300 μ l/h for one hour and the concentration was 150 pmol/100 μ l. The MAP and HR were observed 30 and 60 min after central infusion of Ang II.

Statistical analysis

All data were presented as Mean \pm SE. Paired *t*-tests was used to compare MAP, HR and BRS changes between CNP and sham groups. Difference was defined as significant at $P < 0.05$.

Results

Changes in the mechanic pain threshold in rats before and after CNP surgery

Two groups' mechanic pain thresholds were tested before and after surgery in 40 days. It

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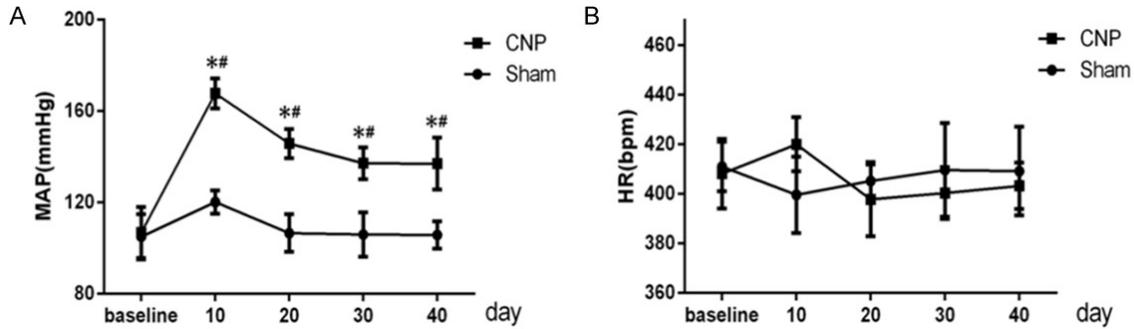


Figure 2. Changes in MAP and HR in rats before and after CNP surgery. * $P < 0.05$, compared with Sham group in same time. # $P < 0.05$, compared with baseline.

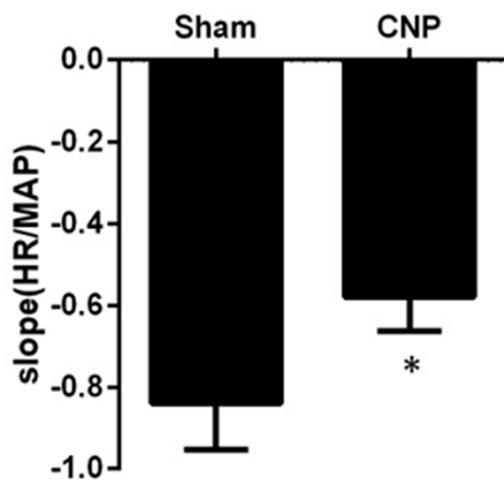


Figure 3. BRS of two groups after 40 days of operation. * $P < 0.05$, compared with Sham group.

slightly decreased in Sham group with no difference before and after surgery. While, the threshold significantly reduced in CNP group 10 days after surgery (0.88 ± 0.67 g vs. 12.60 ± 3.36 g, $P < 0.05$) and lasted to 40 days (**Figure 1**).

Changes in MAP and HR in rats before and after CNP surgery

Before and after the surgery, MAP and HR of the rats were continuously measured using the non-invasive tail artery pressure measuring method every 10 days. The MAP before and after surgery in Sham group did not exhibit significant changes ($P > 0.05$). The mean arterial pressure in CNP group began to increase on postoperative day 10, and the value was significantly higher than that before surgery and the sham group and lasted to 40 days ($P < 0.05$)

(**Figure 2A**). However, the changes in HR at all points between these two groups were not significantly different ($P > 0.05$) (**Figure 2B**).

Changes of BRS in CNP

The two group rats' BRS was measured in 40 days after operation. It was found that the slope of BRS in CNP group was significantly reduced compared with Sham group (-0.58 ± 0.08 vs. -0.84 ± 0.11 bpm/mmHg, $P < 0.05$) (**Figure 3**).

Effects of Ang II in the NTS on BRS

The changes of BRS in CNP rats were measured 30 min after bilateral microinjection of losartan into the NTS ($n = 5$). The decreased BRS was significantly improved after NTS injection of losartan (**Figure 4A**).

Control rats were received central infusion of Ang II for determining the effect of AT_1R activation on BRS. After Ang II treatment (30 and 60 min), BRS changes was monitored. The BRS decreased significantly ($P < 0.05$) (**Figure 4B**).

Discussion

Major findings obtained from the present study are: 1) rats with CNP show a significant amplification in MAP and BRS, which is prevented by microinjection of AT_1R blockade (losartan); 2) central infusion of Ang II produces the increased BRS. Based on the present results, it is suggested that increased Ang II system in the NTS plays an important role in mediating the cardiovascular dysfunction after CNP.

CNP is one of the most common diseases with 7% to 8% of the general population. CNP influences patients' daily life quality by itself, but

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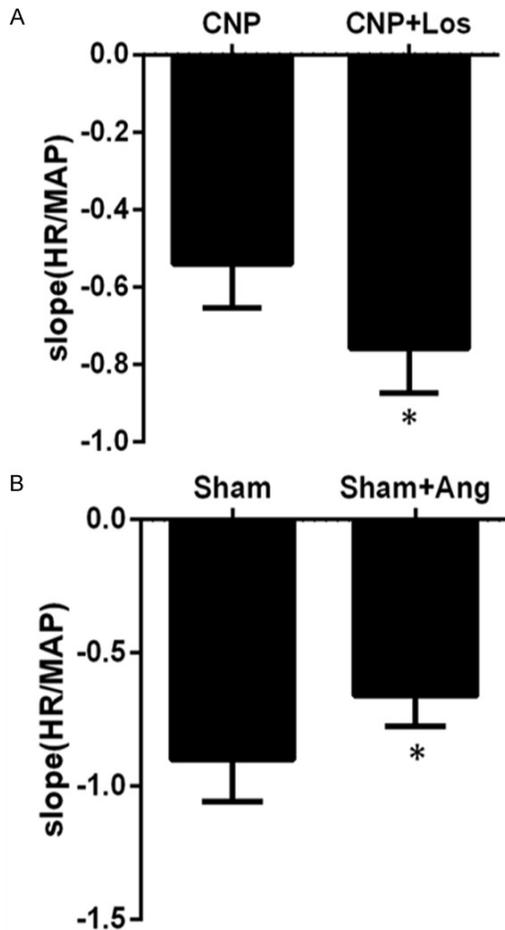


Figure 4. Effects of Ang II in the NTS on BRS. A. The changes of BRS in CNP rats were measured 30 min after bilateral microinjection of losartan into the NTS. * $P < 0.05$, compared with CNP group in same time. B. The changes of BRS in Sham rats were measured 30 min after bilateral microinjection of Ang II into the NTS. * $P < 0.05$, compared with Sham group in same time.

also its complications, including anxiety, depression, agrypnia, and so on. Hypertension is one of these complications. Many articles have reported the significant positive relationship between blood press and pain sensitivity in CNP patients and animal experiments. Here, compared with sham group, we also detected the increased blood press in CNP rats, while no obvious change was obtained in HR.

Persistent excessive sympathetic activation greatly contributes to the pathogenesis of hypertension. Many reports have described that NTS was an important brainstem nucleus, and it played pivotal role through BRS in autonomic cardiovascular regulation [12]. Ang II is

one of the neurotransmitters involved in the processing of the cardiovascular reflexes within the brainstem. But no study has detected if Ang II participate in this CNP induced hypertension. Therefore, this work was aimed to test the hypothesis that the NTS including Ang II system is involved in mediating processing of this increased blood press in CNP.

Our study found that BRS in CNP rats was significantly decreased. And, this declined BRS can be improved by microinjection of the AT_1R antagonist losartan into the NTS. Furthermore, we have demonstrated that AT_1R activation by central infusion of Ang II in sham operative rats reduced BRS changes as same as evoked by CNP. The function of baroreflex is important to stabilize the resting BP. The current results verified our hypothesis that the activation of AT_1R in the NTS contributes to the MAP elevation in CNP rats by the mechanism of BRS reduction, and blockade of AT_1R by losartan significantly blunts this function.

The AT_1R is reported to be expressed in the NTS and involved in central control of BP and baroreflex transmission, and increased Ang II is an important mechanism responsible to cardiovascular dysfunction in hypertension and heart failure [13, 14]. However, the exact mechanism by which change in Ang II system in the NTS is produced by CNP is not clear. Ang II system is activated by increase of generation, decrease of angiotensin II resolution, up-regulation or super-sensitivity of AT_1R , it could lead to permanent and progressive hypertension. It is possible that CNP probably activate the renin angiotensin system and affect the maintaining of resting MAP.

In conclusion, CNP can increase the level of MAP, and decrease BRS, which is prevented by microinjection of AT_1R blockade (losartan). However, central infusion of Ang II produces the increased BRS. It is suggested that increased Ang II system in the NTS may play an important role in mediating the cardiovascular dysfunction after CNP.

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Disclosure of conflict of interest

None.

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