Capsaicin facilitates carotid sinus baroreceptor activity in anesthetized rats

Hao ZHANG, Yi-xian LIU, Yu-ming WU, Ze-min WANG, Rui-rong HE[1]

Department of Physiology,Institute of Basic Medicine,Hebei Medical University,Shijiazhuang 050017,China

KEY WORDS capsaicin; carotid sinus; baroreceptor; ruthenium red; glibenclamide

ABSTRACT

AIM: To study the effect of capsaicin on carotid sinus baroreceptor activity (CBA). **METHODS:** The functional curve of carotid baroreceptor (FCCB) was constructed and the functional parameters of carotid sinus baroreceptor were measured by recording sinus nerve afferent discharge in anesthetized rats with perfused isolated carotid sinus. **RESULTS:** Low-concentration of capsaicin (0.2 μmol/L) had no significant effect on CBA, while perfusion of the isolated carotid sinus with middle-concentration of capsaicin (1 μmol/L) could shift FCCB to the left and upward, with peak slope (PS) increased from (2.47 %±0.14 %)/mmHg to (2.88 %±0.10 %)/mmHg (P<0.05) and peak integral value of carotid sinus nerve discharge (PIV) enhanced from 211 %±5 % to 238 %±6 % (P<0.01). The threshold pressure (TP) and saturation pressure (SP) were significantly decreased from 68.0±1.1 to 62.7±1.0 mmHg (P<0.01) and from 171.0±1.6 to 165.0±0.6 mmHg (P<0.01). By perfusing with high-concentration of capsaicin (5 μmol/L), FCCB was shifted to the left and upward further and the changes of the functional parameters such as PS, TP, and SP were concentration-dependent. Pretreatment with ruthenium red (100 μmol/L), an antagonist of vanilloid receptor subtype 1 (VR_1), blocked the effect of capsaicin on CBA. Preperfusion with glibenclamide (20 μmol/L), a K_{ATP} channel blocker, could eliminate the effect of capsaicin on CBA. **CONCLUSION:** Capsaicin exerts a facilitatory role on the isolated carotid baroreceptor in a concentration-dependent manner. The facilitatory action of capsaicin may be attributed to the opening of K_{ATP} channels mediated by VR_1.

INTRODUCTION

Capsaicin, a pungent ingredient of hot pepper, is a specific activiator of nociceptive sensory neurons with C and A_δ fibers[1] and its effects are mediated through vanilloid receptor subtype 1 (VR_1)[2]. The cardiovascular effects of capsaicin were studied extensively[3,4]. Smith *et al*[5] observed that capsaicin caused a rapid dose-dependent reflex fall in blood pressure when injected intra-arterially into the hindlimb vasculature of anesthetized rats. Capsaicin can cause vasodilation in the coronary arteries and has positive inotropic and chronotropic effects on the heart[6]. Xue and He demonstrated that microinjection of capsaicin into area postrema induced the increases in mean arterial pressure, heart rate, and renal sympathetic nerve activity[7], and intracarotid injection of capsaicin could activate neurons of the brainstem nuclei involved in cardiovascular regulation such as nucleus paragigantocellularis lateralis, locus coeruleus, and nucleus tractus solitarius[8,9]. Recently, our lab has shown that capsaicin facilitated carotid baroreflex[to be published]. Whether capsaicin affects carotid baroreceptor activity (CBA) remains to be clarified as yet. The major goal of the present study

[1] Correspondence to Prof Rui-rong HE. Phn 86-311-606-2490. Fax 86-311-606-2490. E-mail syho@hebmu.edu.cn
Received 2003-11-17 Accepted 2004-04-01

was to observe the effects of capsaicin on CBA in anesthetized male rats with perfused isolated carotid sinus through direct recording of carotid sinus nerve activity (CSNA), and to elucidate the mechanism involved.

MATERIALS AND METHODS

Sprague-Dawley rats (♂, 340 g±40 g, grade II, Certificate No 04036), obtained from the Experimental Animal Center of Hebei Province, were anesthetized with ip urethane1.0 g/kg. The trachea was cannulated for ventilation.

Perfusion of left carotid sinus The method of isolating the carotid sinus has been described previously[10]. Briefly, the left carotid sinus area was fully exposed after turning rostrally the trachea and esophagus. Sternohyoideus muscles and superior laryngeal nerves were sectioned. The bilateral aortic nerves and recurrent laryngeal nerves were all sectioned. The common, external, and internal carotid arteries and smaller arteries originating from these vessels were exposed and ligated, while carefully leaving the left carotid sinus nerve undisturbed. Ligation of the occipital artery at its origin from the external carotid artery excluded chemoreceptors from the isolated carotid sinus, thereby preventing chemoreceptor activation secondary to decreased carotid sinus pressure. Plastic catheter introduced into the left common carotid artery in the anterograde way (served as inlet tube) was attached to a peristaltic pump (1210, Harvard) which controlled the intrasinus pressure (ISP). The intrasinus pressure was monitored by a pressure transducer (MPU-0.5A, Nihon Kohden, Japan) connected with inlet tube. A plastic catheter inserted into the external carotid artery served as an outlet tube. The carotid sinus was then perfused with warm oxygenated modified Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 5.6, pH 7.35-7.45).

Recording of sinus nerve afferent discharge The carotid sinus nerve was cut near the glossopharyngeal nerve and desheathed carefully. The isolated sinus nerve and surrounding structures were immersed in warm (37 ºC) liquid paraffin to avoid drying of the tissues. The sinus nerve was placed on a bipolar platinum electrode connected to a bioelectrical amplifier (AB-621G, Nihon Kohden, Japan). Bioelectrical signal was fed to a polygraph (RM-6240, Chengdu Instrument Factory), with an integral time setting 5 s. ISP and discharge of sinus nerve were recorded synchronously, and at the end of the experiment, the integral of sinus nerve activity (ISNA) was obtained and measured.

Protocols According to the computer controlled program, ISP was altered in a stepwise manner by perfusing left carotid sinus with K-H solution. After ISP was lowered from 100 mmHg to 0 mmHg, it began to increase slowly to 240 mmHg in a staircase manner, and then decreased to 0 mmHg in the same manner, and again stabilized at 100 mmHg. Each step of the staircase changed the ISP by 30 mmHg and lasted for 15 s. The functional curve for ISP-ISNA relation was constructed, and the functional parameters of carotid baroreceptor such as peak slope (PS), peak integral value (PIV), threshold pressure (TP), saturation pressure (SP), and operation range (OR) were determined. TP was the ISP at which ISNA began to increase by 15 % in response to increase in ISP. SP was the ISP at which ISNA just showed no further increase with an increase in ISP. OR was calculated as the difference between SP and TP.

On perfusing carotid sinus with K-H solution, the functional curve of carotid baroreceptor (FCCB) was drawn, obtaining the control parameters of TP, SP, OR, PS, and PIV. ISP was then fixed at 100 mmHg for 20 min, and K-H solution containing capsaicin 0.2, 1, and 5 μmol/L was then perfused to examine the changes in ISNA, followed by measurement of the parameters again. Finally the carotid sinus was perfused with K-H solution as a postcontrol.

The effect of ruthenium red (RR) on the response to capsaicin was examined. After the control parameters of CBA were obtained, the isolated carotid sinus was perfused with K-H solution containing RR 100 μmol/L for 15 min, and the above parameters were measured. Then capsaicin 1 μmol/L was added to perfuse the carotid sinus area. The parameters were measured within 20 min, and then the drugs were washed out with K-H solution. To determine whether the effect of capsaicin 1 μmol/L was caused by activation of ATP-sensitive potassium channel (K_{ATP} channel), one group in the experiment was pretreated with glibenclamide for 15 min before capsaicin was added.

Drugs Capsaicin (Sigma) was dissolved in normal saline containing 10 % ethanol and 1 % Tween-80 and then diluted to final concentration with saline. RR (Sigma) was dissolved in normal saline. Glibenclamide (Tianjin Institute of Medical and Pharmaceutical Industry) was dissolved in dimethylsulfoxide (100 μmol/L). The

same amount of diluted ethanol and Tween-80, dimethylsulfoxide was also added to normal K-H solution as control. No change was observed during perfusion with these solvents.

Statistics All data were presented as mean±SD. The significance of group differences was determined by one way ANOVA and *q*-test. $P<0.05$ is considered as statistically significant.

RESULTS

Effect of capsaicin on carotid baroreceptor activity By perfusing carotid sinus with K-H solution and elevating ISP from 0 to 240 mmHg in a stepwise manner, ISNA was increased. There was no difference in CBA parameters among controls. As compared with control groups, capsaicin concentration-dependently increased PIV and PS, and decreased TP and SP, shifting FCCB to the left and upward (Tab 1, Fig 1). The above effects occurred within 10 min after perfusing carotid sinus with capsaicin, and reached the peak during 15-30 min.

Fig 2 is an original tracing showing the effects of capsaicin on ISNA.

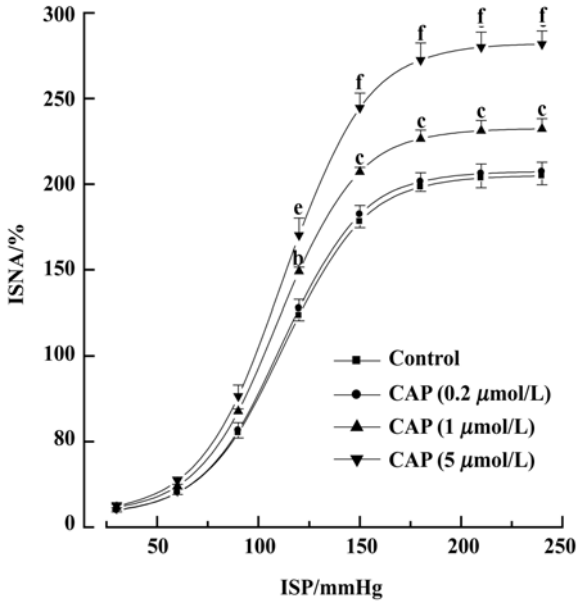

Fig 1. Effects of different concentrations of capsaicin on functional curves of carotid baroreceptor in anesthetized rats. *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* 0.2 μmol/L. $^{e}P<0.05$, $^{f}P<0.01$ *vs* capsaicin 1 μmol/L. ISP: intrasinus pressure; ISNA: integral of sinus nerve activity.

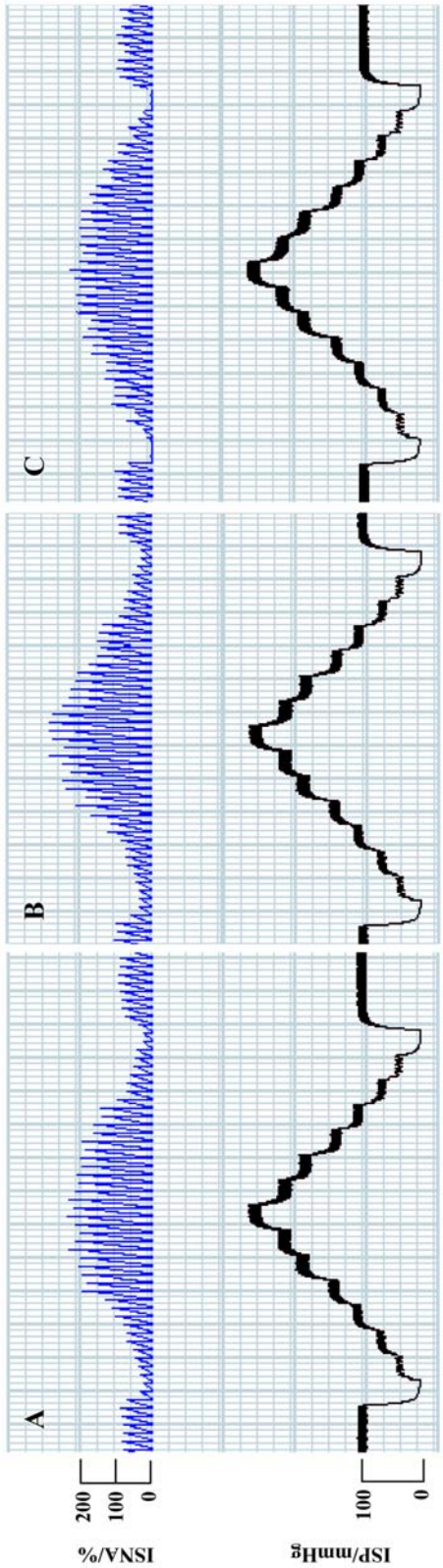

Fig 2. Original recording showing the responses of ISNA to intrasinus perfusion with capsaicin. ISP: intrasinus pressure; ISNA: integral of sinus nerve activity. A: Control; B: Perfusion with capsaicin 1 μmol/L; C: washing out.

Tab 1. Effect of capsaicin on functional parameters of carotid baroreceptor in anesthetized rats. *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{e}P<0.05$, $^{f}P<0.01$ *vs* capsaicin 0.2 μmol/L. $^{h}P<0.05$, $^{i}P<0.01$ *vs* capsaicin 1 μmol/L.

Drugs	TP/mmHg	SP/mmHg	OR/mmHg	PS/mmHg^{-1}	PIV/%
Control	68.0±1.1	171.0±1.6	103.0± 0.6	2.47 %±0.14 %	211±5
Capsaicin 0.2 μmol/L	68.0±1.6	170.0±2.1	103.0±1.0	2.56 %±0.16 %	213±6
Capsaicin 1 μmol/L	62.7±1.0^{cf}	165.0±0.6^{ce}	102.0± 0.6	2.88 %±0.10 %^{be}	238±6^{cf}
Capsaicin 5 μmol/L	59.7±1.5^{ch}	161.0±1.5^{ch}	101.0±1.5	3.38 %±0.14 %^{ci}	293±8^{ci}

TP: threshold pressure; SP: saturation pressure; OR: operation range; PS: peak slope; PIV: peak integral value of carotid sinus nerve discharge.

Tab 2. Effects of ruthenium red (RR) 100 μmol/L and glibenclamide (Gli) 20 μmol/L on the responses of carotid baroreceptor to capsaicin 1 μmol/L. *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{e}P<0.05$, $^{f}P<0.01$ *vs* capsaicin.

Drugs	TP/mmHg	SP/mmHg	OR/mmHg	PS/mmHg^{-1}	PIV/%
Control	68.8±1.2	173.0±2.4	105.0±2.1	2.41 %±0.15 %	210±5
Capsaicin	62.9±1.3^{c}	166.0±1.7^{c}	103.0± 2.0	2.86 %±0.12 %^{b}	236±6^{c}
RR	69.9±2.1	175±3	107.0±1.6	2.44 %±0.14 %	209±6
RR+capsaicin	69.1±1.5^{c}	174±3^{f}	105.0± 2.2	2.52 %±0.15 %^{f}	211±5^{f}
Control	70.1±1.12	175.0±2.4	105.0±2.1	2.41±0.11	209±6
Capsaicin	63.6±2.3^{c}	167.0±3.5^{c}	103.0±2.0	2.88±0.14^{b}	236±7^{c}
Gli	71.1±2.1	173.0±3.3	105.0±1.6	2.42±0.12	210±5
Gli + capsaicin	69.8±1.5^{e}	175.0±2.4^{f}	104.0±3.1	2.51±0.15^{f}	212±6^{f}

Effect of RR on capsaicin responses By perfusing the isolated carotid sinus with capsaicin 1 μmol/L, the FCCB was shifted to the left and upward, with enhancement of PS and PIV, while TP and SP were significantly decreased. Pretreatment with RR 100 μmol/L *per se* did not induce any change in functional parameters of carotid baroreceptor, but blocked the effect of capsaicin 1 μmol/L (Tab 2).

Effect of Gli on capsaicin responses Perfusion with capsaicin 1 μmol/L shifted FCCB to the left and upward, with increases of PS and PIV, whereas TP and SP were significantly decreased. Preperfusion with Gli 20 μmol/L *per se* did not produce any change in functional parameters of carotid baroreceptor, but markedly blocked the effects of capsaicin (Tab 2).

DISCUSSION

The main finding of the present study is that capsaicin facilitated CBA in a concentration-dependent manner. In our experiment , capsaicin shifted FCCB to the left and upward, with increases of PIV and PS, indicating the excitatory action of capsaicin on CBA. This was in accordance with the facilitatory effect of capsaicin on carotid baroreflex observed in our previous study (to be published). In the present experiment, the technique of perfusing carotid sinus was used and CBA was altered by increasing or decreasing ISP. Under such conditions, administration of capsaicin was restricted to the local sinus area and the indirect effect on carotid sinus secondary to the actions of capsaicin on cardiovascular or central nervous system were avoided.

Recent studies have demonstrated that the effects of capsaicin on sensory neurons were mediated by VR_1, which are present at the cell body, central, and periphery terminals of primary afferent neurons[2,12]. RR was a specific antagonist of VR_1[13]. In our present experiment, the facilitatory effects of capsaicin on CBA appeared within 15 min after acutely perfusing sinus area with different concentrations of capsaicin. In addition, pretreatment with RR could completely block the effects

of capsaicin, thereby implying the involvement of VR_1.

Evidence has been presented that capsaicin was capable of releasing neuropeptides from the peripheral ending of sensory nerves[14-16]. Several studies had shown that calcitonin gene-related peptide (CGRP)-containing nerves were distributed in the internal carotid artery of rat[17,18] and Wellman *et al* demonstrated that CGRP could activate K_{ATP} channel in pig coronary arterial smooth muscle[19]. Brayden[20] recently reported that K_{ATP} channel opening could hyperpolarize the smooth muscle, which led to close of the voltage-dependent Ca^{2+} channels, thereby causing a reduction in intracellular Ca^{2+} and vasodilation. It was also known that distention of carotid sinus could activate mechanosensitive ion channels , which would enhance the activity of the baroreceptor[21]. On the basis of these results, we added glibenclamide, a K_{ATP} channel blocker, to the perfusate and found that it eliminated the capsaicin-induced increase in sinus nerve afferent activity. Since the nerve endings of the carotid sinus are highly sensitive to stretch or distortion, the deformation of the nerve ending by tissue tension (ie wall tension σ) would excite the baroreceptors. According to the Laplace's relation: $\sigma=Pr/h$, where P is the distending pressure, r is the radius of the vessel lumen, and h is the wall thickness, capsaicin might induce arterial dilation through K_{ATP} channel activated by CGRP, and thus increase r, so σ became larger and the sensitivity of baroreceptor was enhanced.

In conclusion, capsaicin may release CGRP from peripheral endings of sensory nerve around carotid sinus via VR_1, and the resultant opening of K_{ATP} channel dilates carotid wall, thus resulting in an increase in sensitivity of carotid baroreceptor.

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