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Genistein inhibits carotid sinus baroreceptor activity in anesthetized male rats[1]

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Key words

genistein; carotid sinus; baroreflex; protein-tyrosine kinase; Bay K8644; *L*-NAME

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Abstract

Aim: To study the effect of genistein (GST) on carotid baroreceptor activity (CBA). **Methods:** The functional curve of carotid baroreceptor (FCCB) was constructed and the functional parameters of carotid baroreceptor were measured by recording sinus nerve afferent discharge in anesthetized male rats with perfused isolated carotid sinus. **Results:** GST at 50, 100, and 200 μmol/L inhibited the CBA, which shifted FCCB to the right and downward, with a marked decrease in peak slope and peak integral value of carotid sinus nerve discharge in a concentration-dependent manner. Pretreatment with 100 μmol/L N^G-nitro-*L*-arginine methyl ester, an inhibitor of nitric oxide synthase, did not affect the effect of GST on CBA. Pretreatment with 500 nmol/L Bay K8644, an agonist of calcium channels, could completely abolish the effect of GST on CBA. A potent inhibitor of tyrosine phosphatase, sodium orthovanadate (1 mmol/L), could attenuate the inhibitory effect of GST. **Conclusion:** GST inhibits CBA, and the effect may be mediated by protein tyrosine kinase inhibition and a decrease in Ca^{2+} influx through the stretch-activated channels.

Introduction

Phytoestrogens are plant-derived diphenolic compounds that are structurally and functionally similar to estradiol. Accumulating evidence indicates that phytoestrogens may confer cardiovascular protection[1–3]. Genistein (GST), one of the most well-known phytoestrogens, is an isoflavone that is also a specific inhibitor of protein tyrosine kinase (PTK)[4]. It has been demonstrated that GST has a hypocholesterolemic effect in animals and humans, and is able to inhibit low density lipoprotein (LDL) oxidation, endothelial cell proliferation and angiogenesis[5], and to enhance the dilator response to acetylcholine of atherosclerotic arteries[6]. All of these effects may predict a favorable impact on the cardiovascular system. Li *et al* reported that GST decreased the contractile response of the aortic artery *in vitro*[7]. Furthermore, our previous studies showed that GST decreased the vascular tone in the femoral, renal and mesenteric vascular beds via protein tyrosine kinase (PTK) inhibition[8], reduced infarct size and apoptosis of myocytes in ischemia/reperfusion rabbit heart[9], and inhibited the voltage-dependent Ca^{2+} channel in isolated guinea pig ventricular myocytes[10]. Whether GST affects carotid baroreceptor activity (CBA) remains to be clarified. The aim of the present study was to observe the effects of GST on CBA in anesthetized male rats with perfusing isolated carotid sinus, and to elucidate the mechanism involved.

Materials and methods

Animals Sprague-Dawley rats (♂ , 350 ± 20 g, Grade II, Certificate No 04036), obtained from the Experimental Animal Center of Hebei Province, were anesthetized with urethane 1.0 g/kg, ip. The trachea was cannulated for ventilation.

Perfusion of left carotid sinus The method of isolating the carotid sinus was described in our previous study[11,12]. The left carotid sinus areas were fully exposed by turning the trachea and the esophagus rostrally. Sternohyoideus muscles and superior laryngeal nerves were sectioned. The bilateral aortic nerves, right carotid sinus nerves, cervical sympathetic 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. A plastic catheter introduced into the left common carotid artery in the anterograde way (serving as an inlet tube) was attached to a peristaltic pump that controlled the intrasinus pressure (ISP). ISP was monitored by a polygragh (RM-6240, Chengdu Instrument Factory, Chengdu, China) connected to the inlet tube. A plastic catheter inserted into the external carotid artery served as an outlet tube. The carotid sinus was then perfused with warm (37 °C) modified Krebs-Henseleit (K-H) solution (NaCl 118.0 mmol/L, $NaHCO_3$ 25.0 mmol/L, KCl 4.7 mmol/L, KH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $CaCl_2$ 2.5 mmol/L, glucose 5.6 mmol/L, pH 7.35–7.45) bubbled with 95% O_2 and 5% CO_2.

Recording of sinus nerve afferent discharge The left carotid sinus nerve was cut near the glossopharyngeal nerve and desheathed carefully. The isolated sinus nerve and surrounding structures were immersed in warm (37 °C) liquid paraffin to avoid drying of the tissues. The sinus nerve was placed on a bipolar platinum electrode and the bioelectrical signal was recorded on a polygragh (RM-6240, Chengdu Instrument Factory), with an integral time of 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 With a computer-controlled program[13], ISP was altered in a stepwise manner by perfusing the 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 the ISP-ISNA relationship was constructed, and the functional parameters of carotid baroreceptor activity, 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 an 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 the 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 GST at 50, 100, and 200 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 N^G-nitro-*L*-arginine methyl ester (*L*-NAME) on the response to GST was examined. After the control parameters of CBA were obtained, the isolated carotid sinus was perfused with K-H solution containing 100 μmol/L *L*-NAME for 20 min, and the above parameters were measured. Then GST 100 μmol/L was added to perfuse the sinus area. The parameters were measured within 15 min, and the drugs were then washed out with K-H solution. To determine whether Ca^{2+} was involved in the effect of GST, one experimental group was treated with 500 nmol/L Bay K8644 for 20 min before GST was added. To further determine the involvement of PTK, pretreatment with a potent inhibitor of tyrosine phosphatase, sodium orthovanadate (1 mmol/L), was carried out.

Drugs Genistein (purity 99%, Sigma, St Louis, MO, USA) was prepared with dimethyl sulphoxide. The final concentration of dimethyl sulphoxide in the perfusing solution was lower than 0.05%. *L*-NAME (Sigma) and sodium orthovanadate (Sigma) were dissolved in saline. Bay K8644 (Sigma) was dissolved in 99% ethyl alcohol. No changes in ISNA were observed during perfusion with ethyl alcohol (1:2000).

Statistical analysis All data are presented as mean±SD. The significance of group differences was determined by ANOVA and *t*-test. Differences were considered significant when $P<0.05$.

Results

Effect of GST on carotid baroreceptor activity By perfusing carotid sinus with K-H solution and elevating ISP from 0 mmHg to 240 mmHg in a stepwise manner, ISNA was increased. There was no difference in CBA parameters among the controls. As compared with control groups, treatment with GST decreased PIV and PS, and increased TP and SP, shifting FCCB to the right and downward (Table 1, Figure 1). The above effects occurred within 5 min of perfusing the carotid sinus with GST, and reached a peak during 8–12 min. Figure 2 is an original tracing showing the effects of GST on ISNA.

Effect of *L*-NAME on GST responses *L*-NAME (100 μmol/L) did not induce any change in the functional parameters of the carotid baroreceptor, and also did not influence the effect of 100 μmol/L GST (Table 2).

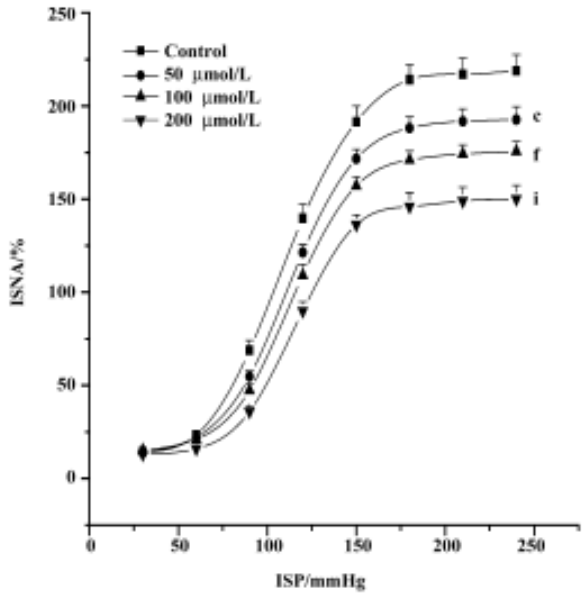

Figure 1. Effect of different concentrations of genistein (GST) on the functional curves of carotid baroreceptor in rats. n=6. Mean±SD. ^{c}P<0.01 *vs* control. ^{f}P<0.01 *vs* GST (50 μmol/L). ^{i}P<0.01 *vs* GST (100 μmol/L). ISP, intrasinus pressure; ISNA, integral of sinus nerve activity.

Effect of Bay K8644 on GST responses Bay K8644 (500 nmol/L) did not produce any change in the functional parameters of the carotid baroreceptor, but completely blocked the action of GST (Table 2).

Effect of sodium orthovanadate on GST responses Sodium orthovanadate (1 mmol/L) did not change CBA, but completely blocked the effect of GST (Table 2).

Discussion

The present study demonstrated that GST inhibited CBA in a concentration-dependent manner. By perfusing the left carotid sinus baroreceptor with GST, the FCCB were shifted to the right and downward, with a reduction in PS and PIV, indicating the inhibitory action of GST on CBA. It is well established that the arterial baroreceptors play an important role in the short-term control of cardiovascular activity. Inhibition of CBA is inclined to cause increased arterial blood pressure that can antagonize hypotensive effects caused by other ingredients.

As nitric oxide synthase (NOS) is present in afferent baroreceptor fibers innervating the carotid sinus[14], and increasing evidence has shown that nitric oxide (NO) may suppress the action potential of baroreceptors[15,16]. Furthermore, our previous study demonstrated that 17β-estradiol inhibited CBA via endothelial NO release. NO suppressed Na^{+} current in baroreceptor neurons and activated the cal-

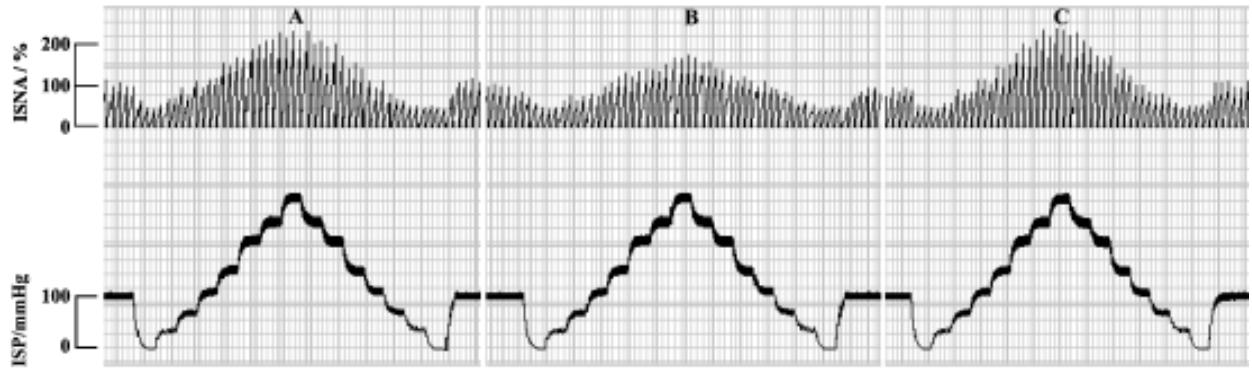

Figure 2. Original recording showing the responses of integral of sinus nerve activity to intrasinus perfusion with genistein (GST). (A) Control; (B) Perfusion with GST (100 μmol/L); (C) Washing out.

Table 1. Effect of genistein on the functional parameters of carotid baroreceptor in anesthetized male rats. n=6. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* control. ^{e}P<0.05, ^{f}P<0.01 *vs* GST (50 μmol/L). ^{h}P<0.05, ^{i}P<0.01 *vs* GST (100 μmol/L).

Genistein (μmol/L)	TP (mmHg)	SP (mmHg)	OR (mmHg)	PS (%/mmHg)	PIV (%)
Control	63.0±3.2	164.7±3.7	101.7±0.5	2.52±0.07	215±8
50	68.9±4.0^{b}	175.7±6.6^{c}	102.3±1.4	2.39±0.03^{c}	191±7^{c}
100	74.7±2.8^{f}	179.3±2.8^{e}	103.0±0.5	2.29±0.04^{f}	177±5^{f}
200	81.9±2.6^{i}	185.0±3.7^{h}	103.9±1.2	2.16±0.03^{i}	155±7^{i}

Table 2. Effect of N^G-nitro-*L*-arginine methyl ester (*L*-NAME) 100 μmol/L, Bay K8644 500 nmol/L, and sodium orthovanadate 1 mmol/L on the responses of carotid baroreceptor to 100 μmol/L genistein (GST). n=6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control. $^eP<0.05$, $^fP<0.01$ *vs* GST (100 μmol/L).

Drug	TP (mmHg)	SP (mmHg)	OR (mmHg)	PS (%/mmHg)	PIV (%)
Control	63.3±4.1	165.0±4.7	101.9±0.6	2.52±0.08	214±10
GST	74.9±3.5^c	178.5±3.5^c	103.8±0.5	2.29±0.05^c	177±6^c
L-NAME	64.2±3.3	166.2±4.3	101.7±0.6	2.50±0.08	212±9
L-NAME+GST	75.4±2.4	180.8±2.9	104.8±0.4	2.28±0.05	175±7
Control	64.5±2.6	166.2±3.4	101.8±0.4	2.49±0.05	211±8
GST	76.0±2.3^c	179.5±2.4^b	103.7±0.5	2.27±0.03^c	174±5^c
Bay K8644	61.8±3.2	163.1±3.0	101.7±0.6	2.50±0.06	217±7
Bay K8644+GST	65.4±2.1^f	167.5±2.8^e	102.0±0.7	2.47±0.07^f	210±6^f
Control	63.0±3.6	164.7±4.1	101.8±0.4	2.52±0.08	214±9
GST	75.5±4.2^c	179.0±3.2^b	103.2±0.9	2.28±0.09^b	176±6^c
Sodium orthovanadate	64.8±3.2	166.7±4.0	102.0±1.0	2.49±0.08	210±9
Sodium orthovanadate+GST	64.1±5.2^f	165.8±2.8^e	101.8±0.4	2.50±0.04^f	212±7^f

cium-dependent K^+ channels localized in vascular smooth muscle, then hyperpolarized baroreceptor neurons[17]. Both of these mechanisms may account for the inhibitory effect of 17β-estradiol on CBA. In the present study, pretreatment with *L*-NAME, a non-selective inhibitor of NOS, did not affect the action of GST, thus suggesting that locally released NO was not involved in the effect of GST on CBA. This result indicated that GST and 17β-estradiol inhibited CBA via different pathways.

It has been reported that a mechanosensitive ion channel is localized on the baroreceptor neurons, and that vascular distention is effectively translated to the deformation of the afferent nerve endings as arterial pressure rises. Deformation then depolarizes the nerve endings by opening non-selective cation channels to create a generator potential that triggers action potential discharge[18,19]. Moreover, it has been demonstrated that stretching of the walls of the carotid sinus may induce an increase in Ca^{2+} influx on baroreceptor neurons, which is mediated by stretch-activated channels[16]. Suppressing Ca^{2+} influx through the stretch-activated channels may be an important pathway in regulating CBA. Studies have shown that agmatine[20], cholecystokinin octapeptide[21] and aminoglycoside antibiotics (such as streptomycin)[22] can inhibit CBA in this way. We have observed that pretreatment with L-type calcium channel agonist Bay K8644 completely blocks the inhibitory effect of GST on CBA. Based on the above observations, it may be concluded that GST inhibits CBA through a decrease in Ca^{2+} influx by blocking the stretch-activated channels.

Genistein has also proved to be a specific inhibitor of PTK. Evidence has been presented to suggest that enhanced tyrosine phosphorylation participates in the mechanisms that regulate the contraction of smooth muscle[5]. Vanadate, an inhibitor of tyrosine phosphatase, can enhance protein tyrosine phosphorylation[6]. PTK has been described as an important modulator regulating the tone of vascular smooth muscle[23]. Our present study showed that the effect of GST on CBA was inhibited by pretreatment with sodium orthovanadate, suggesting that the PTK pathway is involved. From this data together with our previous findings, it may be inferred that GST inhibits PTK, relaxes the vascular smooth muscle, and then attenuates the stretch-activated Ca^{2+} channels on the baroreceptor neurons.

In summary, the present study has revealed that GST inhibits CBA, and the effect may be mediated by PTK inhibition and a decrease in Ca^{2+} influx through the stretch-activated channels.

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