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Research Paper

Ginkgolide B inhibits carotid sinus baroreflex in anesthetized male rats

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Abstract: The effects of ginkgolide B on the carotid sinus baroreflex (CSB) were studied in the perfused isolated carotid sinus of 30 anesthetized Sprague-Dawley male rats. The results were as follows. (1) By perfusing with ginkgolide B (0.1, 1, 10 μmol/L), the functional curve of the baroreflex was shifted to the right and upward. There was a marked decrease in peak slope (PS) and reflex decrease (RD) in mean arterial pressure (P<0.01), while the threshold pressure (TP), equilibrium pressure (EP) and saturation pressure (SP) were significantly increased (P<0.05, P<0.01). Among the functional parameters of CSB, the changes in PS, RD, TP, EP and SP were dose-dependent. (2) Pretreatment with Bay K8644 (500 nmol/L), an agonist of L-type calcium channel, completely eliminated the effects of ginkgolide B (1 μmol/L) on the CSB. (3) Pretreatment with tetraethylammonium (TEA, 1 mmol/L), an inhibitor of potassium channel, completely abolished the above effects of ginkgolide B (1 μmol/L) on the CSB. These results suggest that ginkgolide B inhibits the CSB in anesthetized rats, which is mediated by decreased calcium influx and increased potassium efflux in baroreceptor nerve endings.

Key words: ginkgolide B; carotid sinus; baroreflex; tetraethylammonium

银杏苦内酯 B 抑制麻醉大鼠颈动脉窦压力感受性反射

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摘 要：本研究在 30 只麻醉雄性 Sprague-Dawley 大鼠隔离灌流颈动脉窦区观察了银杏苦内酯 B (ginkgolide B)对颈动脉窦压力感受性反射的影响。结果显示：(1)银杏苦内酯 B (0.1, 1, 10 μmol/L)隔离灌流左侧颈动脉窦区，使压力感受性机能曲线向右上方移位，曲线最大斜率(peak slop, PS)减小，血压反射性下降(reflex decrease, RD)幅度减小(P<0.01)，阈压(threshold pressure, TP)、平衡压(equilibrium pressure, EP)和饱和压(saturation pressure, SP)均升高(P<0.05, P<0.01)。其中 PS、RD、TP、EP 和 SP 呈明显的剂量依赖性；(2)预先应用钙通道开放剂 Bay K8644 (500 nmol/L)，可以完全取消银杏苦内酯 B 的抑制作用。 (3)预先应用钾通道阻断剂四乙铵(tetraethylammonium, TEA, 1 mmol/L)，银杏苦内酯 B 的上述作用也被完全取消。结果表明，银杏苦内酯 B 对大鼠颈动脉窦压力感受性反射有抑制作用，此作用与银杏苦内酯 B 减少颈动脉窦压力感受器神经末梢钙离子内流和增加钾离子外流有关。

关键词：银杏苦内酯 B；颈动脉窦；压力感受性反射；四乙铵
中图分类号：Q463

Ginkgo biloba extract (GBE) is a natural compound derived from the *Ginkgo biloba* and has been extensively used in medical treatment and experimental investigation. The primary chemical constituents of GBE approximately include 6% terpenes (ginkgolide A, ginkgolide B, ginkgolide C, and bilobalide) and 24% flavonol glycosides (kaempferol and quercetin)[1,2]. Ginkgolide B (BN52021), a major active component of GBE and a potent antagonist of platelet-activating factor, exhibits a wide range of biological effects, such as reduction of ischemia-reperfusion injury, memory improvement, antioxidative activity, anti-inflammatory property, and antiarrhythmic effect[3-7].

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Ginkgolide B has been used for cerebrovascular and cardiovascular disease by improving blood flow, reducing ischemia-reperfusion injury or inhibiting platelets[8]. Satoh's group reported that ginkgolide B produces vasorelaxation, but the mechanism of ginkgolide B in vascular smooth muscle function is still essentially unknown[9]. Some recent reports have already demonstrated that ginkgolide B decreases L-type calcium current ($I_{Ca,L}$) in a concentration-dependent manner[10] and partially inhibits calcium overload during ischemia[11]. In addition, ginkgolide B may shorten the action potential duration in a concentration-dependent manner mainly due to the increase of the delayed rectifier potassium current (I_K) in single ventricular myocytes by using patch-clamp techniques[12]. Accordingly ginkgolide B may have a potential clinical value in treatment of cardiovascular disease and it is important to evaluate the effects of ginkgolide B in the modulation of blood pressure (BP). Xu *et al.* have found that ginkgolide had no obvious effects on BP and heart rate after intragastric administration in anesthetized dogs[13]. Nevertheless, Lopes-Martins *et al.* formulated that unloading of the carotid sinus baroreceptor elicited a reflex rise in arterial pressure which was markedly potentiated by pretreating the animals with intravenous injection of ginkgolide B[14]. Among these two experiments, the effects of ginkgolide on BP were different. Possible explanations for these inconsistencies include differences in animal species, empirical method, and pharmaceutical concentration. Moreover, intragastic administration of ginkgolide or intravenous injection of ginkgolide B had direct effects on the cardiovascular and central nervous systems. Thus it was not possible to define the precise location where ginkgolide B acted. In our study, administration of ginkgolide B was restricted to the local sinus area and the direct actions of ginkgolide B on the cardiovascular or central nervous systems were avoided.

As known, baroreflex plays a major role in the BP modulation. The aims of the present paper were to study the actions of ginkgolide B on the carotid sinus baroreflex (CSB) in the perfused isolated carotid sinus of 30 anesthetized Sprague-Dawley male rats and the mechanism involved.

1 MATERIALS AND METHODS

1.1 *General surgical procedure*

Sprague-Dawley male rats [(320±20) g] obtained from the Experimental Animal Center of Hebei Province, were anesthetized with 25% urethane (1.0 g/kg body weight, i.p.). The trachea was cannulated for ventilation. The right femoral artery was cannulated for recording BP with a transducer (MPU-0.5A, Nihon Kohden). Body temperature was maintained at 37-38 ºC throughout the experiment.

1.2 *Perfusion of isolated left carotid sinus*

The perfusion of isolated carotid sinus area was carried out with a previously reported method by our laboratory[15]. Carotid sinus areas were fully exposed by turning rostrally the trachea and esophagus. Sternohyoideus muscles and superior laryngeal nerves were cut. The bilateral aortic nerves, right carotid sinus nerve, 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 decrease in carotid sinus pressure. Plastic catheters were inserted into the left common carotid artery in the anterograde way (served as inlet tube) and the external carotid artery in the retrograde way (served as outlet tube). The carotid sinus was then perfused with warm (37 ºC) oxygenated modified Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6, pH 7.35-7.45) bubbled with 95% O_2 and 5% CO_2. The intrasinus pressure (ISP) was monitored by a pressure transducer (MPU-0.5A, Nihon Kohden) connected with inlet tube. ISP was controlled by a peristaltic pump.

After perfusion of the left carotid sinus, ISP was kept at 100 mmHg for 20 min and then was lowered to 0 mmHg rapidly, from which ISP was elevated to 250 mmHg in the form of pulsatile ramp by regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory[16]. It took 0.5 min for ISP to be increased from 0 to 250 mmHg. ISP and BP were simultaneously recorded on a polygraph (RM-6240, Chengdu Instrument Factory). This process was repeated at an interval of 5 min to check the stability of the baroreflex. Reproducibility of the experimental preparation was documented by the recurrent drop of BP in response to the increase in ISP.

1.3 *Experimental protocols*

By perfusing the left carotid sinus with K-H solution and elevating ISP, the functional curve for the ISP-BP relation was constructed, and the functional parameters of

baroreflex such as threshold pressure (TP), saturation pressure (SP), equilibrium pressure (EP), peak slope (PS), reflex decrease (RD) in BP, and operating range (OR) were determined. TP was the ISP at which BP began to decrease in response to the increase in ISP. SP was the ISP at which BP just showed no further reflex decreases with an increase in ISP. OR was the difference of SP minus TP.

Before administration of the pharmaceuticals, K-H solution was used as a control. The experiments consisted of 3 groups. (1) To test the effects of ginkgolide B on the CSB (n=6): ISP was fixed at 100 mmHg for 20 min with K-H solution as control and the baroreflex parameters were measured. Then K-H solution containing ginkgolide B (0.1, 1, 10 μmol/L) was used to perfuse the isolated carotid sinus for 50 min, followed by measurements of the parameters again. Finally, the carotid sinus was perfused with K-H solution to wash out ginkgolide B. (2) To test the effects of Bay K8644 (500 nmol/L) on the actions of ginkgolide B (n=6): baroreflex parameters were examined following the application of ginkgolide B before and after pretreatment with Bay K8644 for 15 min. (3) To test the effects of tetraethylammonium (TEA, 1 mmol/L) on the actions of ginkgolide B (n=6): parameters were examined following the application of ginkgolide B before and after pretreatment with TEA for 15 min.

1.4 *Pharmaceuticals*

Ginkgolide B was purchased from Fluka. Bay K8644 and TEA were purchased from Sigma. Ginkgolide B was dissolved with dimethylsulphoxide. The final concentration of dimethylsulphoxide in the perfusing solution was 0.1% (*V*/*V*). No change was observed in the CSB during perfusion with the final concentration of dimethylsulphoxide. TEA was dissolved in saline. Bay K8644 was dissolved in 99% ethyl alcohol. No change of CSB was observed during perfusion with ethyl alcohol (1:2 000).

1.5 *Data analysis*

All data were expressed as mean±SD. The differences between groups were assessed by one-way ANOVA and further analyzed using the Student-Newmen-Keuls test. P<0.05 was considered statistically significant.

2 RESULTS

2.1 Effects of ginkgolide B on the CSB

By perfusing the left carotid sinus with K-H solution and elevating the ISP from 0 to 250 mmHg, BP was reflexly decreased. Ginkgolide B induced obvious changes in baroreflex parameters, appeared approximately 5 min after perfusing the isolated carotid sinus with K-H solution containing ginkgolide B, and disappeared at 30-50 min after washout. Compared with the control group, ginkgolide B concentration-dependently decreased RD and PS, whereas increased TP, EP and SP (Table 1), thus shifted the functional curve of the baroreflex to the right and upward in a concentration-dependent manner (Fig.1). These results indicate that ginkgolide B induces an inhibitory effect on the CSB (Fig.2).

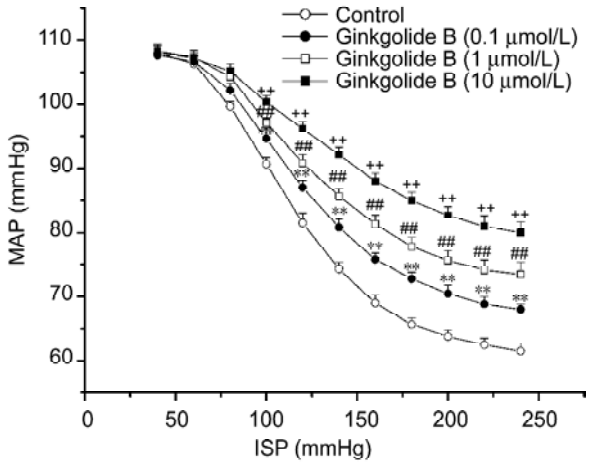

Fig.1. Changes in functional curves of baroreflex during intrasinus perfusion with different doses of ginkgolide B in rats. mean±SD. n=6. $^{**}P$<0.01 *vs* control, $^{\#\#}P$<0.01 *vs* ginkgolide B (0.1 μmol/L), ^{++}P< 0.01 *vs* ginkgolide B (1 μmol/L). ISP, intrasinus pressure; MAP, mean arterial pressure.

Table 1. Effects of ginkgolide B on the functional parameters of carotid sinus baroreceptor in anesthetized male rats

Group	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Control	65.46±1.76	93.94±0.82	186.33±2.73	120.83±2.56	0.46±0.01	46.50±1.38
Ginkgolide B (0.1 μmol/L)	71.66±0.84**	96.28±1.10*	192.00±2.37*	120.50±2.51	0.37±0.01**	40.00±0.89**
Ginkgolide B (1 μmol/L)	75.18±1.56$^{**\#}$	98.29±0.90$^{**\#}$	195.83±2.04$^{**\#}$	120.83±0.98	0.32±0.01$^{**\#\#}$	34.83±1.94$^{**\#\#}$
Ginkgolide B (10 μmol/L)	84.56±1.76$^{**\#\#++}$	100.64±1.01$^{**\#\#+}$	205.17±2.14$^{**\#\#++}$	120.83±2.14	0.23±0.01$^{**\#\#++}$	28.33±1.50$^{**\#\#++}$

TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; OR, operating range; PS, peak slope; RD, reflex decrease. mean±SD. n=6. $^{*}P$<0.05, $^{**}P$<0.01 *vs* control; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* ginkgolide B (0.1 μmol/L); ^{+}P<0.05, ^{++}P<0.01 *vs* ginkgolide B (1 μmol/L).

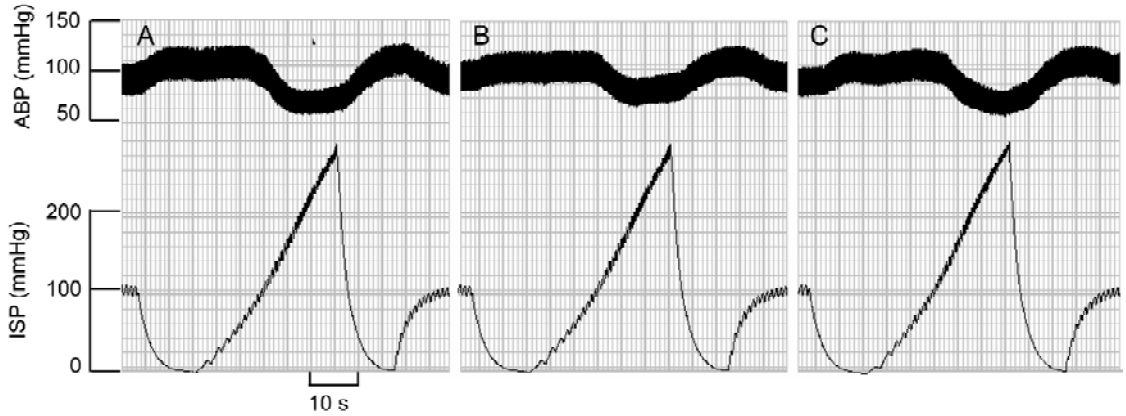

Fig. 2. Original recordings showed the reflex response of arterial blood pressure (ABP) to intrasinus perfusion with ginkgolide B (1 μmol/L). *A*: Control. *B*: Ginkgolide B. *C*: Washout. ISP, intrasinus pressure.

2.2 Effects of Bay K8644 on the actions of ginkgolide B

Bay K8644 (500 nmol/L) alone had no effects on the CSB, but completely blocked the actions of ginkgolide B (1 μmol/L) on the CSB (Table 2).

2.3 Effects of TEA on the actions of ginkgolide B

TEA (1 mmol/L) alone had no effects on the CSB, but completely eliminated the actions of ginkgolide B (1 μmol/L) on the CSB (Table 2).

Table 2. Effects of Bay K8644 and TEA on the reflex response induced by perfusing carotid sinus with ginkgolide B in anesthetized male rats

Group	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Control	65.80±1.64	93.77±0.55	186.67±2.16	121.00±1.90	0.45±0.02	45.33±2.16
Ginkgolide B (1 μmol/L)	74.67±1.05**	97.45±1.34**	195.50±2.88**	121.50±2.45	0.33±0.01**	35.00±2.10**
Bay K8644 (500 nmol/L)	65.63±1.77$^{\#\#}$	93.60±0.82$^{\#\#}$	185.33±2.16$^{\#\#}$	120.33±1.97	0.45±0.01$^{\#\#}$	46.50±1.87$^{\#\#}$
Bay K8644 + ginkgolide B	67.30±1.73$^{\#\#}$	93.77±0.84$^{\#\#}$	187.17±2.40$^{\#\#}$	120.00±2.38	0.45±0.02$^{\#\#}$	45.50±2.70$^{\#\#}$
Control	65.80±1.87	93.60±1.04	187.50±2.17	122.00±1.55	0.46±0.01	46.00±2.76
Ginkgolide B (1 μmol/L)	74.17±0.64**	98.63±1.22**	195.17±2.32**	121.17±2.14	0.33±0.01**	35.55±1.05**
TEA (1 mmol/L)	66.47±1.64$^{\#\#}$	93.94±0.82$^{\#\#}$	186.67±2.16$^{\#\#}$	120.00±1.79	0.45±0.01$^{\#\#}$	45.67±1.75$^{\#\#}$
TEA + ginkgolide B	66.80±1.64$^{\#\#}$	93.94±0.52$^{\#\#}$	186.50±2.43$^{\#\#}$	119.83±2.23	0.45±0.01$^{\#\#}$	45.50±1.76$^{\#\#}$

TEA, tetraethylammonium; TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; OR, operating range; PS, peak slope; RD, reflex decrease. mean±SD. n=6. $^{**}P$<0.01 *vs* control, $^{\#\#}P$<0.01 *vs* ginkgolide B.

3 DISCUSSION

The present study shows that ginkgolide B inhibits the CSB in a concentration-dependent manner. In order to observe the direct actions of ginkgolide B on the CSB, the technique of perfusing was used in the carotid sinus and the activity of baroreceptor restricted to the local carotid sinus area was altered by changing ISP with ginkgolide B. The functional curve of CSB was shifted to the right and upward, with decreases in PS and RD and increases in TP, EP and SP, indicating that ginkgolide B inhibits the CSB.

Sensory nerve endings of baroreceptor are located in the adventitia of artery and transmit electrical signal of arterial distension into the nucleus of the solitary tract in the central nervous system. Previous studies have shown mechanosensitive ion channels present in the baroreceptor neuron membrane[17] and mechanical deformation of vascular stretch activates inward ionic currents[18]. Furthermore, it has been demonstrated that stretching of the wall of carotid sinus may induce an increase in calcium influx in baroreceptor neurons which is mediated by stretch-activated channels[19]. According to the fact that ginkgolide B decreased $I_{Ca,L}$ in a concentration-dependent manner[10], pretreatment with L-type calcium channel agonist Bay K8644 was used in our experiments. Pretreatment with Bay K8644

completely abolished the effects of ginkgolide B on the CSB, suggesting the inhibitory effects of ginkgolide B on the CSB are due to the decreased calcium influx.

Baroreceptor nerve endings possess three potassium channels, including transient outward channel, Ca^{2+}-activated potassium channel and delayed rectifier potassium channel[20]. Ginkgolide B may open the delayed rectifier potassium channel and cause the increase of potassium efflux[12]. We speculate that the activation of potassium channels may hyperpolarize baroreceptor neurons, thereby decreasing CSB activity[21]. In our study, TEA (1 mmol/L) had no effects on the CSB, but it completely eliminated the effects of ginkgolide B on the CSB, which can be ascribed to the increased potassium efflux.

The use of herbal therapies has been increasing rapidly, especially in the past decade[22]. Ginkgolide B is an effective herbal medicine, which indicates protective effects on the cardiovascular system such as against ischemic heart disease and arrhythmia. The arterial baroreceptors play an important role in short-term adjustments of blood volume, cardiac output, or peripheral resistance occurrence. However, long-term control of BP is determined by the fluid balance, namely, the balance between fluid intake and fluid output[23]. In our study, ginkgolide B displays inhibitory effects on the CSB, which inclined to increase arterial BP that can antagonize hypotension when ginkgolide B is used as vasodilator in cardiovascular diseases. In the next step, it is necessary to test the effects of ginkgolide B on cardiovascular center.

In summary, ginkgolide B inhibits the CSB, which may be mediated by decreased calcium influx and increased potassium efflux in baroreceptor nerve endings.

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