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Antihypertensive Action of Geraniin in Rats

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Abstract—The effects of geraniin, one of the ellagitannins purified from the leaves of *Sapium sebiferum*, on blood pressure were investigated in the spontaneously hypertensive rat (SHR). A single intravenous bolus injection of geraniin into anaesthetized SHRs lowered the arterial mean blood pressure in a dose-dependent manner without affecting the heart rate. A similar action was also observed in the normotensive (WKY) rat that received this compound at a higher dose. Geraniin did not modify the baroflex sensitivity in the phenylephrine-challenged SHR. This tannin reduced the plasma noradrenaline in a dose-dependent fashion which was not influenced by adrenalectomy. Failure of the antagonists, idazoxan and yohimbine for α_2-adrenoceptors as well as haloperidol and domperidone for dopamine receptors, to reverse the antihypertensive actions of geraniin ruled out the possible mediation of these receptors. Moreover, geraniin attenuated the pressor responses to exogenous noradrenaline and Bay K 8644 to a similar degree, indicating the direct effect of this compound on vascular activity in rats. These results suggest that geraniin possesses the ability to lower systemic blood pressure through the reduction of noradrenaline release or by direct vasorelaxation.

On the basis that hypertensive patients need to receive long-term medication, development of new substances with less toxic effects for the control of hypertension seems important. In an attempt to pursue this goal from natural products, we have screened the blood pressure-lowering activity of several plants (Cheng & Hsu 1990) and have purified geraniin (Kimura et al 1986), one of the ellagitannins, from the leaves of the effective plant *Sapium sebiferum*. Ellagitannins have been suggested as the active constituents of medical plants (Okuda et al 1989). Thus, the present study was designed as a preliminary investigation for the effect of geraniin on blood pressure using the spontaneously hypertensive rat (SHR). In order to rule out the factors concerning absorption, geraniin was administered by intravenous injection.

Materials and Methods

Animals

Adult male spontaneously hypertensive Wistar rats (SHRs), 250–350 g, were purchased from the animal centre of National Yang-Ming Medical College, while the age-matched male rats of Wistar-Kyoto strain (WKY), the origin of SHRs, served as normotensive controls. Rats were housed four per cage at 23 ± 1°C under a 12-h light-dark cycle, with lights on between 0700 and 1900 h. Food and water were freely available.

Blood pressure recordings

Blood pressure of conscious rats was measured indirectly using a photoelectric sphygmomanometer (IITC) according to our previously used method (Cheng et al 1990a). For direct monitoring of the intravenous vasodepressor effects, rats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.) and the right femoral artery and vein cannulated for the monitoring of blood pressure and for intravenous administration of drugs, respectively. A Gould pressure transducer (model 13-4615-35) was used to detect the pressure responses and heart rate was derived from the arterial pulse signal. Continuous recording of the heart rate and blood pressure was carried out using a Gould 4-channel polygraphic recorder from the rats, which were maintained at a constant temperature of 36 ± 1 °C.

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Baroreflex sensitivity

Effects of geraniin on baroreflex sensitivity were carried out in anaesthetized rats as described previously (Pettersson et al 1984). In brief, the change of heart rate in response to a rapid change in arterial blood pressure induced by phenylephrine (7 $\mu g\ kg^{-1}$, i.v.) was used to indicate the baroreceptor sensitivity. Changes in blood pressure and heart rate were determined 3 s after the initial deviation from the baseline and mean values of the Δheart rate/Δblood pressure ratio from three infusions were calculated (Larson et al 1982). Geraniin was injected into the femoral vein, 10 min before stimulation by phenylephrine. The obtained ratios were compared with those from the vehicle-treated control.

Assay of plasma noradrenaline

Blood samples (2 mL), collected from arteries, were centrifuged at 5000 *g* for 10 min at 4°C and aliquots (200 μL) of plasma were removed for assay. Plasma samples to which had been added 20 ng dihydroxybenzylamine (DHBA) as internal standard, were adsorbed onto activated alumina by continuous shaking for 30 min. The alumina was then washed three times with 1 mL distilled water. The catechols were eluted by 0·1 M perchloric acid with a 10-min shaking. Quantitation of noradrenaline in the clear supernatant was performed (Cheng et al 1990b) using HPLC with an electrochemical detector (BAS200). All values, after correction of recovery (80–82%), were expressed as pmol mL^{-1}.

Adrenalectomized rat

Bilateral adrenalectomy was carried out according to our

previous report (Cheng & Tsai 1986) using the dorsal approach under ether anaesthesia. Sham-operated animals served as control. The animals were allowed to recover from surgery for 24 h before being used for experiments.

Statistical analysis

Results are given as a value of mean ± s.d. from numbers (n) of experiments. The difference was estimated by means of Student's *t*-test for paired or unpaired comparison; a probability of 0·05 or less was considered significant.

Drugs

Geraniin was extracted from the leaves of *Sapium sebiferum*. Chromatographic analysis and other determinations (melting points and spectroscopic studies) indicated that this purification procedure yielded a homogeneous substance (Kimura et al 1986). Commercially-obtained drugs were as follows: domperidone, haloperidol, noradrenaline, phenylephrine, propranolol, and yohimbine from Sigma Chemical Co. (St Louis, MO, USA); idazoxan from Research Biochemicals Inc. (RBI, Natick, MA, USA). Bay K 8644 was kindly supplied by Dr K. Ito of Kanebo Research Institute (Osaka, Japan).

Results

Antihypertensive effects of geraniin

Intravenous injection of geraniin into anaesthetized hypertensive rats produced a marked hypotensive effect in a dose-dependent manner during treatment from 0·5 mg kg^{-1} (Fig. 1). In normotensive (WKY) rats, geraniin lowered the blood pressure at a dose of 10 mg kg^{-1} and higher (Fig. 1). Heart rate was not significantly modified during the geraniin-induced hypotensive state in either SHRs or WKY rats. Actions of geraniin reached a plateau within 20 min and were maintained for 40 min more. Duration of action for geraniin was also prolonged with the increase of injected concentrations but this action was completely reversed within 2 h even at 50 mg kg^{-1}; no irreversible inhibition was observed.

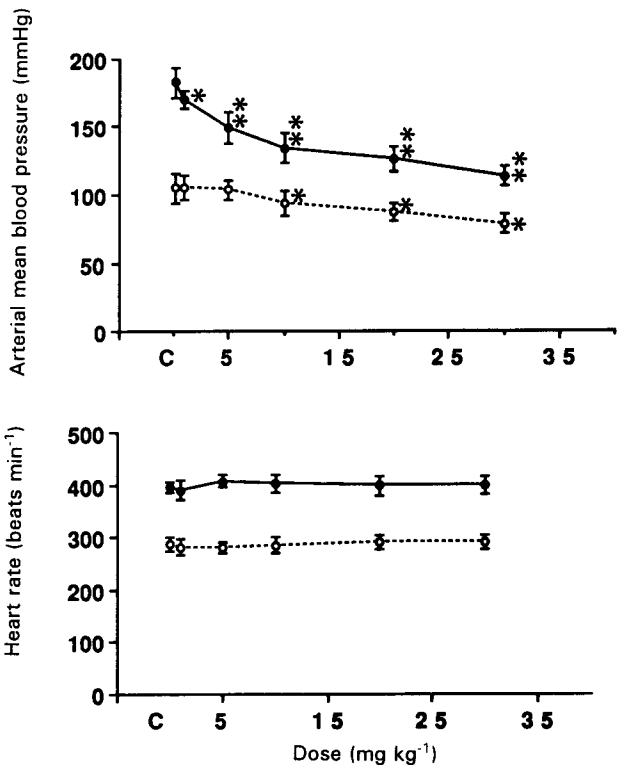

FIG. 1. Effects of geraniin on the arterial mean blood pressure and heart rate of anaesthetized rats. C = vehicle-treated control. ● SHRs, ○ WKY rats. Values (mean ± s.d.) are calculated from eight rats per group after a single bolus injection of geraniin (mg kg^{-1}). * $P < 0.05$ and ** $P < 0.01$ vs control, respectively.

Table 1. Effect of geraniin on baroreflex sensitivity of anaesthetized SHRs.

Experiment	Change in heart rate/change in blood pressure
Control (vehicle)	1·46 ± 0·59
Geraniin (mg kg^{-1})	
2	1·40 ± 0·48
4	1·32 ± 0·19
8	1·37 ± 0·55

Values are means ± s.d. (n = 8).

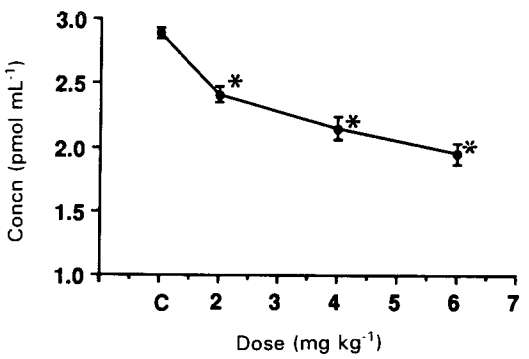

FIG. 2. Effect of geraniin on plasma noradrenaline. C = vehicle-treated control. Each point indicates the mean of eight hypertensive rats with s.d. value shown by vertical bars. * $P < 0.05$ from the value of control.

Effect of geraniin on baroreflex sensitivity

In anaesthetized SHRs, phenylephrine-challenged baroreflex sensitivity, assessed as the ratio of Δheart rate/Δblood pressure, as shown in Table 1, was not markedly affected by geraniin even at higher concentrations.

Effect of geraniin on plasma noradrenaline

Plasma for assay was taken from animals receiving a single bolus intravenous injection of geraniin at 20 min, the time for production of maximal hypotensive action. Plasma from rats which received vehicle only was used as control. Plasma noradrenaline levels in anaesthetized SHRs were markedly reduced by treatment with geraniin at concentrations sufficient to produce antihypertensive action (Fig. 2). Decrease of plasma noradrenaline was parallel to the antihypertensive action in geraniin-treated animals. Geraniin did not interfere with the assay of noradrenaline; addition of geraniin at 1 mmol mL^{-1} into test tubes containing known amounts of noradrenaline gave a recovery of 97·4 ± 3·6% (n = 8).

Table 2. Effect of geraniin on plasma noradrenaline and mean blood pressure in adrenalectomized SHRs.

Geraniin (mg kg^{-1})	Plasma noradrenaline (pmol mL^{-1})		Mean blood pressure (mmHg)	
	Sham-operated	Adrenalectomized	Sham-operated	Adrenalectomized
Control	2·95±0·07	2·43±0·05	183·4±10·6	182·7±11·2
1	2·66±0·14*	2·08±0·11*	152·6±13·9*	153·3±10·1*
5	2·17±0·09**	1·81±0·12**	141·2±11·7**	140·6±14·7**
8	1·85±0·06**	1·49±0·08**	126·6±10·4**	124·3±12·5**

* $P<0·05$, ** $P<0·01$ vs control.

Table 3. Effect of antagonists on the antihypertensive action (changes in mean arterial blood pressure and noradrenaline) of geraniin in SHRs.

Antagonists (mg kg^{-1})	Vehicle		Geraniin (10 mg kg^{-1})	
	Mean arterial blood pressure (mmHg)	Plasma noradrenaline (pmol mL^{-1})	Mean arterial blood pressure (mmHg)	Plasma noradrenaline (pmol mL^{-1})
Control	−5±4	−0·04±0·02	−48±7	−1·72±0·14
Idazoxan				
0·01	5±2	0·09±0·06	−40±6	−1·68±0·11
0·05	7±3	0·11±0·08	−38±7	−1·61±0·18
Yohimbine				
0·05	4±3	0·11±0·05	−42±8	−1·66±0·09
0·1	3±2	0·13±0·08	−45±7	−1·62±0·12
Haloperidol				
0·05	−8±5	−0·06±0·03	−41±3	−1·74±0·18
0·1	−10±7	−0·08±0·06	−46±4	−1·78±0·21
Domperidone				
0·05	−6±3	−0·05±0·04	−46±5	−1·73±0·08
0·1	−8±2	−0·07±0·05	−47±4	−1·76±0·07

Table 4. Effect of geraniin on the vasopressive responses of normotensive rats.

Geraniin (mg kg^{-1})	Change in mean arterial blood pressure (mmHg)	
	Noradrenaline (10 μg kg^{-1})	Bay K 8644 (0·1 mg kg^{-1})
Control	47·2±5·8	43·1±4·7
10	35·4±7·1*	34·8±3·1*
30	28·6±5·2**	26·5±4·3**
50	18·4±4·1**	18·6±3·4**

* $P<0·05$, ** $P<0·01$ from the value of vehicle-treated control, respectively. A positive correlation ($y=41·597-0·477x$, $r=0·972$; $P<0·01$) was obtained between the geraniin-induced inhibition against noradrenaline and Bay K 8644.

Actions of geraniin in adrenalectomized rats

In adrenalectomized SHRs under anaesthesia, arterial mean blood pressure was 182·7±11·2 mmHg (n=8) which was not statistically different from the sham-operated SHRs (183·4±10·6 mmHg, n=8). Geraniin lowered the blood pressure and decreased the plasma noradrenaline in adrenalectomized SHRs in a fashion similar to that on sham-operated animals (Table 2); no statistical difference ($P>0·05$) was found between the samples from adrenalectomized and sham-operated rats.

Effects of antagonists on the geraniin-induced actions

In an attempt to understand the possible participation of receptor-mediated mechanisms in geraniin-induced actions, antagonists at the concentrations sufficient to block the specific receptors were injected 10 min before the administration of geraniin into anaesthetized SHRs. Idazoxan and yohimbine at concentrations sufficient to block α_2-adrenoceptors did not reverse the antihypertensive action. Similar results were also obtained in animals treated with haloperidol or domperidone at concentrations sufficient to block the dopamine receptors (Table 3). Moreover, the presence of these antagonists did not modify the effect of geraniin on plasma noradrenaline (Table 2). The basal level of systemic mean blood pressure of plasma noradrenaline was influenced by the treatment of antagonists, as the values in vehicle-treated samples in Table 2. However, they are not significantly different ($P>0·05$) from the value of control.

Effects of geraniin on the pressor responses in rats

In anaesthetized normotensive rats, a single bolus injection of noradrenaline (10 μg kg^{-1}) or Bay K 8644 (0·1 mg kg^{-1}) raised the mean arterial blood pressure about 47·2±5·8 (n=8) or 43·3±4·2 mmHg (n=8), respectively. Vasoconstrictive response to noradrenaline in rats was markedly reduced by geraniin by a 10-min pre-treatment. The dose-related reduction in vasopressive response elicited by geraniin was also observed in Bay K 8644-stimulated rats (Table 4). Positive correlation (Table 3) can be found between the geraniin-induced vasodepressive action against noradrenaline and Bay K 8644. In addition, this inhibitory effect of geraniin was not reversed by the combined treatment with the antagonist, both the dopamine blockers of haloperidol or domperidone and the α_2-adrenergic antagonists of idazoxan

and yohimbine. Otherwise, single bolus injection of propranolol (50 $\mu g\ kg^{-1}$) lowered the mean blood pressure about 17 ± 4 mmHg (n = 6). Combined treatment of geraniin (10 $mg\ kg^{-1}$) with propranolol (50 $\mu g\ kg^{-1}$) produced a hypotensive action of about 28 ± 6 mmHg (n = 6); an additive effect can be observed.

Discussion

In the present study, we found that geraniin had the ability to lower the blood pressure of SHRs. In the anaesthetized normotensive rat, geraniin produced a similar hypotensive effect at the higher concentrations. Geraniin belongs to the family of ellagitannins. These hydrolysable tannins are known for their inability to enter the central nervous system (Okuda et al 1989) and participation of central nervous factors in the geraniin-induced antihypertension seems unlikely. This view is in agreement with our findings that geraniin did not influence the baroreflex sensitivity as challenged by phenylephrine.

A marked reduction in plasma noradrenaline is associated with the antihypertensive activity of geraniin in anaesthetized rats. An inhibitory effect of geraniin on the release of noradrenaline, either the rate or the amount, can be concluded. Similar results obtained from the adrenalectomized animals exclude the involvement of the adrenal gland, the other source of endogenous catecholamine (Ikeda et al 1966). Thus, the noradrenergic nervous terminal seems responsible for the action of geraniin. Interference with sympathetic conduction or function may also account, in part, for the absence of tachycardia in geraniin-treated animals. Tachycardiac responses would be provoked by a fall in blood pressure and reflex stimulation of sympathetic nerve activity (Vander 1967; Koch-Weser 1974).

Inhibition of noradrenaline release has been attributed to the presynaptic actions of α_2-adrenoceptor (Armstrong & Boura 1973), dopamine receptors (Kaiser & Jain 1985), and others (Langer 1981). We employed the antagonists specific for these receptors, such as idazoxan and yohimbine for α_2-adrenoceptors (Dabire 1986; Yamamoto & Cline 1988), haloperidol and domperidone for dopamine receptors (Lokhandwala & Barrett 1982; Goldberg & Kohli 1983), to identify the possible mediation of these receptors. However, the antagonists at concentrations sufficient to block the presynaptic receptors did not reverse the geraniin-induced actions (Table 3). Moreover, geraniin has been characterized as lacking any effect on β-adrenoceptors using radioligand binding techniques (Cheng et al 1991). Mediation through these endogenous receptors can thus be ruled out.

Geraniin attenuated the pressor responses to exogenous noradrenaline in normotensive rats. At the same concentrations, geraniin suppressed the blood pressure elevation in Bay K 8644-treated rats to a similar degree, resulting in a positive correlation (Table 4). Bay K 8644 and noradrenaline induce vasoconstriction through different mechanisms, either by opening calcium L-channels (Lefer et al 1986) or by activation of α-adrenoceptors (Kenakin 1984), to mobilize calcium ions. Therefore, a direct effect of geraniin on the vascular activity seems to be responsible for this inhibition.

In conclusion, the data suggest that geraniin possesses the ability to lower the systemic blood pressure of SHRs through the blockade of noradrenaline release or by the attenuation of vascular responses.

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