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THE EFFECT OF STEVIOSIDE ON BLOOD PRESSURE AND PLASMA CATECHOLAMINES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Summary

Stevioside is a sweet-tasting glycoside, composed of stevia, a diterpenic carboxylic alcohol with three glucose molecules, mainly used as a substitute for non-alcoholic sweetener. It has previously been shown to reduce blood pressure in studies in animals and human. The effect of intravenous stevioside on the blood pressure was studied in spontaneously hypertensive rats (SHR). The hypotensive effect on both systolic and diastolic blood pressure was dose-dependent for intravenous doses of 50, 100 and 200 mg/kg in conscious SHR. The maximum reductions in systolic and diastolic blood pressure were 31.4 ± 4.2 % and 40.8 ± 5.6 % (mean ± SEM) respectively and the hypotensive effect lasted for more than 60 min with a dose of 200 mg/kg. Serum dopamine, norepinephrine and epinephrine levels were not changed significantly 60 min after intravenous injection of stevioside 100 mg/kg in anesthetized SHR. The present data show that stevioside given intravenously to conscious SHR was effective in blood pressure reduction and there was no change in serum catecholamines in anaesthetized animals with this natural compound.

Key Words: stevioside, spontaneously hypertensive rat, blood pressure, plasma catecholamines

Stevioside, a glycoside isolated from the plant *Stevia rebaudiana Bertoni*, which is widely used as sweetener (1).

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There are a few reports of the effects of stevioside and other natural products from *S. rebaudiana* on the cardiovascular system. Melis and Sainati have shown that purified stevioside induces hypotension, diuresis, and natriuresis in rats and these effects are probably related to changes in prostaglandin activity (2). These pharmacological effects appears similar to those of verapamil, a calcium channel antagonist active in cardiac conducting tissue and vascular smooth muscle. As studies in humans, demonstrated stevioside caused bradycardia and hypotension (3). A slight hypotensive effect was noted in human subjects who received a tea prepared from *Stevia rebaudiana* daily for 30 days (4).

Arterial hypertension is a pathologic state involving an inappropriate relationship between vascular resistance and blood volume. Studies in spontaneously hypertensive rats (SHR) have shown that vascular resistance is increased in every organ system (5). As with the calcium antagonists, stevioside should be effective in reducing blood pressure by reducing the total peripheral resistance.

Abnormalities of sympathetic neural activity, norepinephrine removal, or vascular smooth muscule responsiveness to released norepinephine have long been thought likely to play a pathophysiologic role in the development of increased total peripheral resistance, which is the hall mark of the SHR, and has also been demonstrated in some studies of human hypertension. Increased rates of directly recorded sympathetic activity have been found in SHR (6,7), and plasma levels of norepinephine have also been reported to be significantly, increased especially in young SHR (8,9).

This study was undertaken to investigate: (i) whether stevioside administered intravenously would decrease the blood pressure in conscious SHR, and if the hypotensive effect would be dose-dependent; (ii) whether stevioside administered intravenously could decrease plasma catecholamines in anesthetized SHR.

Materials and Methods

Preparation of stevioside: Stevioside was prepared from dried *S. rebaudiana* leaves by the method of Alvarez et al., which has been reported to provide a product which is 95% pure (10). The remaining impurity (about 5%) mainly includes other sweetening principles like rebaudioside A, B, C, D and E, mucilage and pigments from *stevia* leaves. These impurities can easily be removed by recrystallization but the less pure stevioside is more soluble in water and thus becomes easier to manipulate.

The measurement of arterial blood pressure in conscious SHR: Experiments were performed on male spontaneously hypertensive rats (SHR). Eight rats per group were studied, each weighing 230 to 300 g. The animals were maintained individually in metabolic cages. Water, food consumption and body weight gain were recorded daily. Before the experiment, carotid artery and jugular vein catheters (PE50) were implanted respectively under ether anesthesia. The catheters were passed subcutaneously, brought out at the back of the neck and fixed on the skin. When not in use, they were filled with heparinized saline and plugged with a stainless-steel stylet. Each animal was placed in a separate cage (25×15×15 cm) and allowed to recover from the surgery for at least 10 hr. The animals were left unrestrained and allowed 15 to 30 min in their home cage for blood pressure and heart rate to stabilize before initiating an experimental protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the implanted arterial catheter connected to a Statham P23 pressure transducer (Gould Inc., Oxnard, Calif., USA) with the display on a Gould RS-3200 Physiological Recorder (Gould Inc., Cleveland, Ohio, USA).

Assay of plasma catecholamines: SHR were anesthetized with sodium pentobarbitone (40 mg/kg, intraperitoneal) and the right femoral artery and vein were cannulated for monitoring of blood pressure and for intravenous administration of drugs. Continuous recording of heart rate and blood pressure was carried out using a polygraphic recorder. Animals were placed on a temperature-regulated table to maintain body temperature between 37.0 and 37.5℃.

Blood samples (1 ml), collected from the female artery, were centrifuged at 5000 g for 10 min at 4℃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 with 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 catecholamines in the clear supernatant was performed using HPLC with an electrochemical detector (BAS200; Bioanalytical Systems Inc., West Lafayette, Indiana, USA). All values, after correction for recovery (80-82%), were expressed as pmol/ml.

Statistics: Results are expressed as mean ± SEM of at least 8 separate experiments. Statistical significance was evaluated using the Kruskal-Wallis test when multiple groups were compared; where only two groups were compared, Student's t-test was used. The level of significance was considered to be $p < 0.05$.

Results

The study showed that the hypotensive effect was maximum when using 200 mg/kg stevioside (Fig. 1). The maximal decrease of mean systolic blood pressure was 31% (200 to 137 mmHg) whereas the maximal decrease of mean diastolic blood pressure was 33% (149 to 100 mmHg). The hypotensive effect was sustained almost throughout the 60 min study period. As for 50 mg/kg, although the hypotensive effect was statistically significant, the fall in blood pressure was small. From Fig. 1, the maximal hypotensive effect was noticed at 10 min after the injection of stevioside. Heart rate was not changed significantly throughout the observation period with these different dosages studied.

The plasma catecholamine levels were unchanged at 60 min after the dosage of 100 mg/kg (Table 1). Although the plasma levels of norepinephrine tended to decrease, it did not reach statistical significance ($p>0.05$).

Table 1

The effect of 100 mg/kg stevioside, administered intravenously on plasma catecholamines in anesthetized SHR

	0 min	60 min
Norepinephrine	2.78 ± 0.20	2.48 ± 0.12
Epinephrine	3.94 ± 0.41	4.05 ± 0.62
Dopamine	2.20 ± 0.12	2.23 ± 0.43

Values are mean ± SEM of mean in pmol/ml; n = 8.
Statistics: *T*-test.

Discussion

The present data show that intravenous stevioside is an effective hypotensive agent in conscious SHR, and the effect is dose-dependent. Previous studies have also shown that the Stevia extract is an effective hypotensive agent (3,12); and Melis et al. have shown that intravenous

administration of stevioside in pure form (8, 12, and 16 mg/kg/h) resulted in a significant dose-dependent decrease in mean arterial pressure from 121 mmHg to 72 mmHg in anesthetized Wistar rats. Our experiment also showed that pure stevioside administered intravenously was also very effective in lowering blood pressure in conscious SHR which were ambulatory in cages.

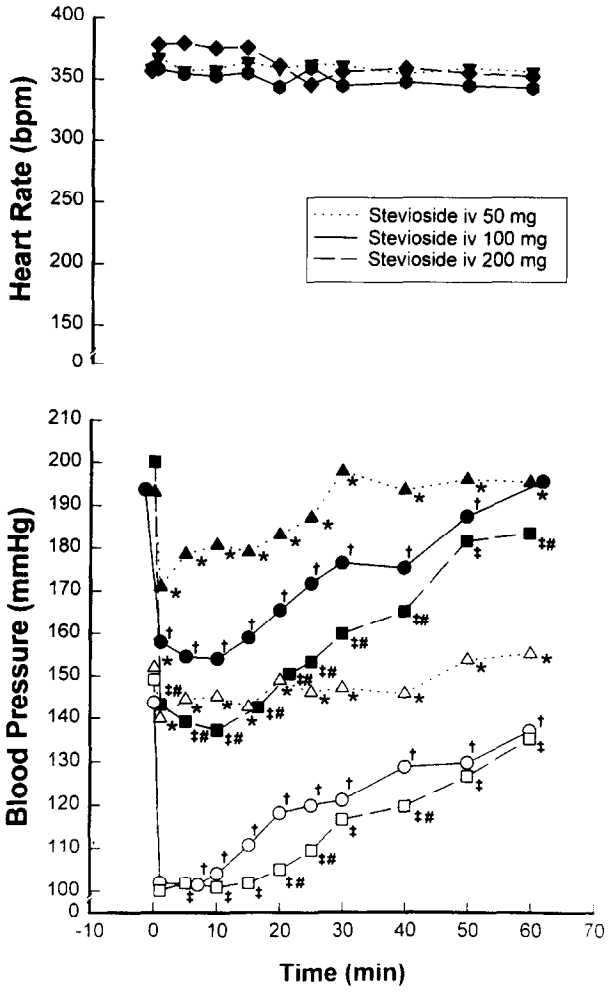

Fig. 1

The dose-response effect of intravenous stevioside (50, 100, 200 mg/kg) on blood pressure and heart rate in conscious spontaneously hypertensive rats. ■●▲ = systolic blood pressure, □○△ = diastolic blood pressure. *$p < 0.05$, †$p<0.01$, ‡$p<0.001$, ‡#$p<0.0001$.

In contrast to previous studies, stevioside was used at higher doses (50, 100, 200 mg/kg) in our experiments and no significant abnormalities were observed in the experimental animals. Previous toxicology studies with stevioside have been extensive, and have shown that stevioside presents low toxicity in mammals (13).

The mechanism of the hypotensive action of stevioside has also been investigated. Previous studies have shown that the hypotensive response to stevioside appears to occur through a calcium antagonist mechanism similar to that with verapamil (14,15). These investigators also showed that the hypotension induced by stevioside in the rat is almost completely blocked by indomethacin, which is a potent inhibitor of prostaglandin synthesis (1,16). Thus, the blood pressure lowering effect of stevioside probably depends on prostaglandin activity.

Previous reports have shown that plasma norepinephrine or epinephrine are increased in the SHR (6-9). A similar phenomenon has also been reported in human essential hypertension (17,18). Epinephrine could enhance norepinephrine release from sympathetic nerve endings by stimulating presynaptic beta-adrenoceptors and circulating epinephrine also could enhance alpha-adrenoceptor-mediated vasoconstriction by a postsynaptic effect (19), which could result in an increase in peripheral arterial resistance and blood pressure elevation. Although stevioside administered intravenously did not decrease plasma catecholamine levels, the present data show that it did not enhance reflex sympathetic activity which might result in an increase in heart rate and catecholamine levels as shown with other antihypertensive drugs such as nifedipine, a widely used dihydropyridine calcium channel blocker.

With pharmacological treatment of hypertension the patient's quality of life should be considered since different antihypertensive drugs may cause different side effects.

The treatment of hypertensive patients creates special problems related to the nature of the disease. Many patients are largely unaware of the significance of blood pressure levels, possible causes, sequelae, and therapeutic needs of hypertension. Being a symptomatic, patients often have little motivation to seek or follow treatment.

The results of various well-designed studies confirm the general impression that about 10 to 20% of patients will experience bothersome side effects from virtually any antihypertensive drug (20,21). Interference with the quality of life by therapy is undoubtedly one of the major factors responsible for the finding that fewer than half of hypertensive patients put on treatment have their blood pressure well controlled (22).

Since stevioside has been widely used in Japan for about 20 years as a sweetening agent, taste modifier and sugar substitution (23), its safety in humans has been well-established. There could be considerable benefit for hypertensive patients if this natural chemical could lower blood pressure effectively with negligable side effects.

The present study found that stevioside has antihypertensive activity through mechanism unrelated to changes of sympathetic tone.

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