行政院國家科學委員會專題研究計畫成果報告

中文題目: 中藥純化成份 isosteviol 降血壓作用機轉之研究

英文題目: The mechanism of antihypertensive effect of isosteviol (ST-1)

計畫編號：NSC 90-2314-B-038-016

執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

主持人：陳保羅

執行機構及單位名稱:台北醫學大學萬芳醫院心臟內科

VASODILATIVE ACTION OF ISOSTEVIOL TO LOWER BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS

[1]Paul Chan, [2]Juei-Tang Cheng,

[1] Department of Medicine and Cardiovascular Medicine, Taipei Medical University- Wan Fang Hospital, Taipei 117, Taiwan;
[2]Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan 700, Taiwan;

Author for correspondence: Dr. Paul Chan, MD, PhD
Department of Medicine, Taipei Medical University-Taipei Wan-Fang Hospital, Taipei 117, Taiwan;
#No. 111, Hsin Lung Road, Section 3, Wen Shan, Taipei, Taiwan (117)
Fax: +886-2-2933-9378
E-mail: chanpaul@wanfang.gov.tw
Running Title: Antihypertensive effect of isosteviol

SUMMARY

1. Isosteviol (IST) is the derivative from stevioside, a sweet-tasting glycoside existed in the leaves of *Stevia rebaudiana* that has been using widely in Japan as sugar substitute.

2. IST (25 mg kg^{-1}) given intraperitoneally into spontaneously hypertensive rats (SHRs) decreased systolic blood pressure 20 mmHg. In isolated aortic rings from rats, IST could dose-dependently relax vasopressin-induced vasoconstriction. IST had no effect on phenylephrine- or KCl-induced vasoconstriction that produced mainly due to intracellular calcium (Ca^{2+}) release. Also, IST failed to modify the vasopressin-induced vasoconstriction in Ca^{2+}-free medium. The results indicate that IST had vasorelaxation effect via an inhibition of Ca^{2+} influx.

3. Relaxation of the vasopressin-induced vasoconstriction by IST was obtained in the absence of endothelium, showing this action of IST was not related to nitric oxide and/or endothelium. Isosteviol-induced Ca^{2+} influx inhibition was blocked by specific K^+ channel blockers glibenclamide (K_{ATP} channel blocker), 4-aminiopyridine (Kv channel blocker) and apamin (Ca^{2+}-sensitive small conductance K^+ channel blocker) but not by charybdotoxin (K_{Ca} channel blocker).

4. Thus, open of the K^+ channel especially Ca^{2+}-sensitive small conductance to inhibit the Ca^{2+} influx by IST can be considered. These results suggest that IST can open the K^+ channel to inhibit Ca^{2+} influx to induce vasodilation in SHRs.

Key words: Isosteviol (IST), Spontaneously hypertensive rats, Calcium influx, Potassium

channel blockers

INTRODUCTION

Hypertension is one of the most common cardiovascular disease.[1] Inadequate blood pressure control still persists as a major public health problem.[2] Compliance of patients to antihypertensive treatment may be an important barrier to improve blood pressure control, since antihypertensive drugs commonly have a negative effect on quality of life.[3] Diuretics are most commonly prescribed initial therapy and have been proven to reduce cardiovascular mortality.[2] Beta-blockers are another important class of antihypertensive drugs commonly used as first-line monotherapy or in combination therapy.[2] Unfortunately, these two classes of the drugs usually have side effects especially on sexual function.[4] So, these could be of considerable benefits for patients with hypertension if some natural products could lower blood pressure effectively with lesser side effects.

Stevioside is a glycoside isolated from the plant *Stevia rebaudiana Bertoni* (Compositae), which is widely used as a non-caloric sweetener in Japan and Brazil for about 20 years.[5] Its safety in human usage has been well established. Recent data from animal study show that stevioside is an effective antihypertensive agent.[6] The action mechanism is still unknown except the calcium antagonistic effect was proposed.[7] In order to have the active principle, we prepared isosteviol (Fig. 1) from stevioside. This study was performed to evaluate the antihypertensive effect of isosteviol (IST) and to know its possible action mechanism.

MATERIALS AND METHODS

Animals

Male spontaneously hypertensive rats (SHRs, 350-450 g) and male Wistar-Kyoto rats (WKY) or Wistar rats (350-450 g) were used. They were obtained from the animal center of the National Cheng Kung University Medical College. Rats were housed in a temperature-controlled room (25±1℃) and kept on a 12hr : 12hr light-dark cycle (light on at 0600 h). Food (Purina Rat Chow) and water were available *ad labitum* throughout the experiment.

Measurement of systemic blood pressure

As described previously,[8] the systemic blood pressure was measured by a noninvasive tail-cuff monitor (UR-5000, Ueda Company, Japan) in conscious SHRs. The systolic blood pressure and heart rate of SHRs can be recorded simultaneously. The SHRs (n=8), which had mean blood pressure higher than 160 mmHg were used to evaluate the antihypertensive activity of drugs. When the blood pressure was stable (varied less than 5 mmHg between 20 minute intervals), 25 mg kg^{-1} IST (dissolved in normal saline) were administered intraperitoneally into SHRs. Other group of SHRs (n=8) received the same volume of normal saline as control. The systolic blood pressure was measured repeatedly 30 minutes and 60 minutes after the injection of IST.

Determination of vasoconstriction of isolated aortic rings

Change of vascular tone was determined on the thoracic aortic ring isolated from Wistar rat.

Each rat was anaesthetized with 35 mg kg^{-1} pentobarbital and sacrificed by decapitation. A medial laparotomy was then performed immediately to excise the thoracic aorta. The vessel was sectioned into four 3mm rings after being gently dissected free of fat and connective tissue. Rings were then mounted into 10-ml organ baths filled with oxygenated Krebs buffer (95% O_2, 5% CO_2) and warmed to 37℃. The preparations were connected to strain gauges (Grass FT03) and isometric tension was recorded on a Grass polygraph (model 79). After being mounted, the rings were allowed to stabilize for 2 h. Each preparation was then stretched gradually to an optimal 1g tension.

Experimental protocols for IST in isolated aortic rings

After the stabilization, 10^{-8} M vasopressin, 10^{-8} M phenylephrine and 40 mM KCl were administered into Ca^{2+}-containing buffer to induce vasoconstriction. Similar responses were obtained in aortic rings prepared from Wistar rats and WKY in the preliminary experiments. Thus, we employed the aortic rings from Wistar rats in the present study. Treatment groups was administered IST from concentrations of 10^{-8} to 10^{-5} M to observe the effect of IST on constricting aortic rings. Otherwise, the above 3 vasoconstrictors (vasopresin, phenylephrine, KCl) were administered into Ca^{2+}-free buffer to know the role of Ca^{2+} influx. Moreover, 10^{-5} M methylene blue was administered 15 min before the addition of 10^{-8} M vasopressin to observe whether IST still can relax this vasopressin–induced vasoconstriction or not.

Culture of rat aortic smooth muscle cells (A7r5)

The A7r5 aortic smooth muscle cell line,[9] obtained from the Food Industry Institute (Hsin-Chu, Taiwan), were cultured as described previously.[10]

Measurement of cytosolic Ca^{2+} in A7r5 with Fura-2

Measurements of Ca^{2+} in aortic smooth muscle cells (A7r5) were performed at room temperature using the calcium-sensitive dye fura 2-acetoxymethyl ester (Fura-2, Molecular Probes), as previously described.[11,12] The cells were kept on ice for 15 min before incubation with 4 μmol Fura-2 in PBS for 60 min in the dark at room temperature. Then, the solution was centrifuged for 2-3 min to remove Fura-2. The pellet of cells was put on ice for 10-15 min and 300 μl of physiological salt solution (PSS) were then added slowly back to the cells over 2-3 min. Harvested cells were suspended in Ca^{2+}-containing PSS for 30 min up to 4 h before Fura-2 determinations. The cells were maintained on ice until immediately before an experiment.

For measurements of Ca^{2+}, drug at the desired concentration was then added into the 10 μl of cell solution during the stable state of fluorescence recorded in Hitachi F-2000 spectrophotometer; an excitation and emission wavelength of 340 and 380 nm was used, respectively. The value of Ca^{2+} was calculated based on the ratio at 340/380 nm, as described previously.[11]

The role of Ca^{2+} influx in the responses to stimulating agents (vasopressin, phenylephrine

and KCl) was evaluated using normal PSS containing Ca^{2+} and Ca^{2+}-free PSS. Then IST was administered to observe its Ca^{2+} antagonistic effect. The K^+ channel blockers, glibenclamide (K_{ATP} channel blocker), 4-aminopyridine (K_V channel blocker), charybdotoxin(Kca channel blocker) and apamin (Ca^{2+} -sensitive small conductance K^+ channel blocker) were also used to evaluate whether the inhibition of Ca^{2+} influx by IST was through K^+ channel opening.[13]

Materials and solutions

Drugs used in this study were: 4-aminopyridine, apamin, charybdotoxin, HEPES, L-phenylephrine hydrochloride (Sigma, USA); fetal bovine serum, FBS (Hyclone, Utah, USA); Fura-2 (Molecular Probes Inc., Eugene, USA); glibenclamide (RBI, MA, USA); arginine vasopressin (Parke-Davis Co., USA) and methylene blue (Fisher Scientific Limited, Montrèal, Canada). IST was prepared from stevioside that was supplied by Professor Die-Yu Xu (Nanjing Railway College of Medicine, Nanjing City, China). The standard PSS contained (in mmol/l): 140 NaCl, 5.9 KCl, 1.2 NaH_2PO_4, 5 $NaHCO_3$, 1.4 $MgCl_2$, 1.8 $CaCl_2$, 11.5 glucose, and 10 HEPES (titrated to PH 7.4 with NaOH). In the preparation of Ca^{2+}-free solution, $CaCl_2$ was replaced by 1.8 mmol/l $MgCl_2$ (total 3.2 mmol/l) with an addition of 0.5 mmol/l EGTA. The ionic composition of the Krebs solution was as follows (mmol/l): NaCl 135, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 20, D-glucose 10 and EDTA-2Na 0.026. The solution was aerated with 95% O_2 and 5% CO_2, and the pH of the solution was maintained at 7.4.

Statistics:

All values were presented as mean ± standard error of mean. All experimental animal groups or experiments were 8 unless specified. ANOVA and Dunnetts post-hoc test was used to evaluate data between different experimental groups. A p value less than 0.05 was regarded as significant.

RESULTS

The effect of IST on blood pressure of SHRs

In conscious SHRs, the maximal hypotensive effect was observed by an intraperitoneal injection of 25 mg kg^{-1} IST. After 60 minutes, the mean arterial blood pressure was decreased from 166.2±3.6 to 157±3 mmHg (Fig. 2, $p<0.05$).

Stimulating agents on Ca^{2+} influx

Figure 3a indicates the response of isolated aortic rings in Ca^{2+}-free solution. The contractile response to 10^{-8} M vasopressin was 67.5±3.4% of that in Ca^{2+}-containing solution (100 %). Thus, vasoconstriction caused by vasopressin in thoracic aorta was acted in part through the Ca^{2+} influx. Phenylephrine at the concentration of 10^{-8} M caused the increase of vascular tone similar in either Ca^{2+}-free or Ca^{2+}-containing solution (Fig. 3b), implicating that the vasoconstricting effect of phenylephrine was acting through intracellular Ca^{2+} release. Moreover, the response to

40 mM KCl was also the same in Ca^{2+}-free or Ca^{2+}-containing solution; no significant difference ($P>0.05$) was found. Intracellular Ca^{2+} release was indicated (Fig. 3c).

In Ca^{2+}-containing solution, IST can relax the constricting aortic rings caused by 10^{-8} M vasopressin in a concentration-dependent manner (Table 1). The maximal effect by 10^{-5} M IST was 54.9±6.5 % of the vascular tone raised by 10^{-8} M vasopressin. In Ca^{2+}-free solution, even at the concentration of 10^{-5} M, IST had no effect on 10^{-8} M vasopressin-induced vasoconstriction. Despite Ca^{2+}-free or Ca^{2+}-containing solution, even at the concentration of 10^{-5} M, IST failed to produce effect on 10^{-8} M phenylephrine-induced vasoconstriction. Similar phenomenon was also observed in the aortic rings response to 40 mM KCl ; no vasorelaxation can be induced by 10^{-5} M IST .

Effect of IST on aortic rings under methylene blue

After treatment with methylene blue, IST still produced vasorelaxation in aortic rings response to 10^{-8} M vasopressin (Table 1). The vascular tone decreased to 55.6 ± 9.3 % of the control, implicating that this action of IST was not methylene blue-sensitive.

Effect of K^+ channel blockers on the action of IST in aortic rings

Glibenclamide (K_{ATP} channel blocker) was administered 20 min before the increase of vascular tone by 10^{-8} M vasopressin. Table 1 showed that the vasorelaxation effect of IST was reversed by glibenclamide at the concentration of 10^{-5} M.

After a 20-min preincubation of the aortic rings with charybdotoxin, a K_{Ca} channel blocker, the action of IST was not modified; the relaxation effect was still at 51.7 ± 4.3% (Table 1). Otherwise, a 10-min pretreatment with apamin, Ca^{2+}-sensitive small conductance K^+ channel blocker, reversed the vasorelaxation of IST in a concentration-related manner.

Effect of IST on Ca^{2+} influx in A7r5

In the presence of Ca^{2+} in medium containing A7r5 cells, vasopressin increased the intracellular Ca^{2+} concentration from 184.8 ±18 nM to 427.2±60 nM (Table 2). In Ca^{2+}-free medium, vasopressin failed to modify the intracellular Ca^{2+} amount. An increase of Ca^{2+} influx into A7r5 cells by vasopressin seems responsible for the raise of intracellular Ca^{2+}.

In Ca^{2+}-containing medium, IST attenuated the intracellular Ca^{2+} increasing effect of 10^{-6} M vasopressin (Table 2). This inhibitory effect of IST was in a concentration-dependent manner; the intracellular Ca^{2+} was decreased from 339.6 ±12.4 nM to 102.2±13.2 nM. Phenylephrine increased the intracellular Ca^{2+} in A7r5 regardless the medium containing Ca^{2+} or not (Table 2). However, the intracellular Ca^{2+} concentration increased by 10^{-6} M phenylephrine was from 185.2±22.8 nM to 814.9±33.8 nM in Ca^{2+}-containing medium but only to 401.1±15.5 nM in Ca^{2+}-free medium. An increase of Ca^{2+} influx into A7r5 cells by phenylephrine can thus be considered. In Ca^{2+}-containing medium, IST inhibited the raise of intracellular Ca^{2+} by 10^{-7} M phenylephrine (Table 2). However, this inhibition by IST disappeared in Ca^{2+}-free medium.

Table 2 also showed the concentration-dependent inhibition by IST on the increase of intracellular Ca^{2+} by 10^{-6} M phenylephrine in Ca^{2+}-containing medium and no difference in Ca^{2+}-free medium.

A23187 can increase intracellular Ca^{2+} concentration without depolarization. Data showed that IST had no inhibitory effect on the increase of Ca^{2+} influx by A23187; the intracellular Ca^{2+} was still at the concentration of 1038.0±103.3 nM.

In the presence of K^+ channel blocker, glibenclamide (K_{ATP} channel blocker) or 4-aminopyridine (K_V channel blocker), IST failed to inhibit Ca^{2+} influx. Intracellular Ca^{2+} concentration recovered from 245.8±5.9 nM and 174.08±23.6 nM to 501.27±26.13 nM and 480.9±13.8 nM respectively (Fig. 4a & 4b)

However, charybdotoxin (K_{Ca} channel blocker) at the concentration of 5×10^{-8} M failed to modify the action of 10^{-6} M IST; the intracellular Ca^{2+} was still decreased by IST from 2207±55.1 nM to 1406.36±53.6 nM (Fig. 4c). Even at the concentration of 10^{-7} M, charybdotoxin was not effective to the inhibition of Ca^{2+} influx by IST; the concentration was decreased from 2024.5±91.5 nM to 1444.7±44.5 nM. Furthermore, in the presence of apamin (Ca^{2+}-sensitive small conductance K^+ channel blocker), inhibition of Ca^{2+} influx by IST was reversed significantly (Fig. 4d).

DISCUSSION

In the present study, we found that IST has the ability to lower blood pressure in conscious SHRs. Like the previous report,[6] stevioside has an effective blood pressure lowering action in SHRs. This action was probably due to calcium antagonism.[7] In consistent to this view, our data indicate that the mechanism for antihypertensive action of IST, one derivative of stevioside, may be due to inhibit Ca^{2+} influx in smooth muscle cells of blood vessels.

First, we evaluated various vascular stimulating agents. Under Ca^{2+}-free solution, vasoconstriction induced by vasopressin attenuated significantly, implicating the involvement of Ca^{2+} influx. Then, IST could dose-dependently inhibit this vasoconstriction by vasopressin only in Ca^{2+}-containing solution but not in Ca^{2+}-free solution. Inhibition of Ca^{2+} influx by IST can thus be considered.

Furthermore, IST was not effective to relax the vasoconstriction induced mainly due to the release of Ca^{2+} such as the responses to phenylephrine (α_1 receptor agonist) or KCl. In thoracic aorta, increase of vascular tone by phenylephrine was introduced to dependent on the release of Ca^{2+} in smooth muscle cells.[14] Our data are in agreement with this view because there was no difference of vasoconstriction by phenylephrine in organ bath containing Ca^{2+} or not. Similar results were observed in the response to KCl. Data show that KCl had no difference in inducing vasoconstriction despite the solution was Ca^{2+}-free or Ca^{2+}-containing, implicating that vasoconstriction induced by KCl in thoracic aorta was mainly due to intracellular Ca^{2+} release. Therefore, the vasorelaxation effect of IST was probably due to inhibition of Ca^{2+} influx.

Then, the role of endothelium and/or nitric oxide (NO) was investigated. It is well-known that NO can stimulate cGMP synthesis to induce vasorelaxation.[15] After pretreatment with methylene blue for 15 min, IST could still relax the vasoconstriction induced by vasopressin which is similar to vehicle control. Also, the activity of IST was not modified by the removal of endothelium. Mediation of NO and/or endothelium-derived substance(s) in the vasodilative action of IST can thus be ruled out.

Moreover, inhibition of Ca^{2+} influx by IST was furthered identified in cultured vascular smooth muscle cell line (A7r5) using Fura-2 as indicator. However, we found that IST had no effect on the increase of intracellular Ca^{2+} by A23187. It has been documented that A23187 can induce Ca^{2+} influx without depolarization.[16] Thus, the inhibitory effect of IST on Ca^{2+} influx seems not due to the direct blockade of Ca^{2+} channels. Otherwise, K^+ channels play an important role in the control of vascular tone.[17] Drug that can open K^+ channels may induce hyperpolarization to close the Ca^{2+} channels and resulted in vasodilatation. In an attempt to meet this possibility, we employed the K^+ channel blockers. In the presence of glibenclamide, K_{ATP} channel blocker, decrease of Ca^{2+} influx by IST was totally reversed. Similar results were observed in the vasorelaxation of aortic rings. Relation of K^+ channels in the vasodilatation of IST can thus be considered. Then, apamin, Ca^{2+}-sensitive small conductance K^+ channel blocker, reversed the decrease of Ca^{2+} influx and vasorelaxation of IST but charybdotoxin, K_{Ca} channel blocker, was not effective. Selective open of K^+ channel by IST can thus be considered. It is reasonable to suggest that the blood pressure lowering action of IST is related to the vasodilatation due to the opening of K^+ channels to inhibit the Ca^{2+} influx into vascular smooth muscles.

In conclusion, IST derived from stevioside is an effective agent to lower blood pressure and its mechanism is probably due to K^+ channel opening (except the charybdotoxin-sensitive K^+ channel) resulting in an inhibition of Ca^{2+} influx and the subsequent vasorelaxation.

We acknowledge Professor Brian Tomlinson for editing and Professor Die-Yu Xu (Nanjing Railway College of Medicine, Nanjing City, China) for kindly supply of stevioside. The present study is supported in part by a grant from National Science Council of the Republic of China (NSC88-2314-B038-130).

REFERENCES

1. Frohlich ED: *Hypertension, Evaluation and Treatment*. pp. 1-21, Williams and Wilkins, Ltd. Baltimore. 1998
2. Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. The 1996 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VI). *Arch. Intern. Med.* 1997; **157**: 2413-46.
3. Jachuck SJ, Brierley H, Jachuck S, Willzox PM. The effect of hypotensive drugs on the quality of life. *J. R. Coll. Gen. Pract.* 1982; **32**:103-5.
4. Williams GH, Croog SH, Levine S, Testa MA, Sudilovsky A. Impact of antihypertensive

therapy on quality of life. Effect of hydrochlorothiazide. *J. Hypertens.* 1987; **5**: 29-35.

5. Soejarto DD, Kinghorn AD, Farnsworth NR. Potential sweetening agents of plant origin. III. Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. *J. Nat. Prod.* 1982; **45**: 590-9.
6. Chan P, Xu DY, Liu JC, Chen YJ, Tomlinson B, Huang WP, Cheng JT. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. *Life Sci.* 1998; **63**: 1679-84.
7. Melis MS. Influence of calcium on the blood pressure and renal effects of stevioside. *Brai. J. Med. Biol. Res.* 1992; **25**: 943-9.
8. Zatz R. A low-cost tail cuff method for the estimation of mean arterial pressure in conscious rats. *Lab. Anim. Sci.,* 1990; **42**: 198-201.
9. Kimes BW, Brandt BI. Characterization of two putative smooth muscle cell lines from rat thoracic aorta. Exp. Cell. Res. 1976; **98**: 349-66.
10. Goldman WF, Bova S, Blaustein MP. Measurement of intracellular Ca in cultured arterial smooth muscle cells using fura-2 and digital imaging microscopy. *Cell Calcium.* 1990; **11:** 221-31.
11. Grynkiewicz G, Poenie GM, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 1985; **260:** 3440-50.
12. Zhu Z, Arendshortst WJ. Angiotensin II receptor stimulation of cytosolic calcium concentration in cultured renal resistance arterioles. *Am. J. Physiol.* 1996; **271**: 1239-47.
13. Hashitani H, Suzuki H. K^+ channels which contribute to the acetylcholine-induced hyperpolarization in smooth muscle of the guinea-pig submucosal arteriole. *J. Physiol.* 1997; **501:** 319-29.
14. Ford WR, Broadleg KJ. Effect of adenosine receptor agonists on induction of contractions to phenylephine of guinea-pig aorta mediated via intra-or extracellular calcium. *Gen. Pharmacol.* 1999; **33**:143-50.
15. Vila-Petroff MG, Yonues A, Egan J, Lakatta EG, Sollott SJ. Activation of distinct cAMP-dependent and cGMP-dependent pathways by nitrtic oxide in cardiac myocytes. *Circ. Res.* 1999; **84**: 1021-31.
16. Ferrante J, Triggle DJ, Rutledge A. The effects of chronic depolarization on L-type 1,4-dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels in chick neural retina and rat cardiac cells. *Can. J. Physiol. Pharmacol.* 1991; **69:** 914-20.
17. Huang Y, Lau CW, Chan FL, Yao XQ. Contribution of nitric oxide and K^+ channel activation to vasorelaxation of isolated rat aorta induced by procaine. *Eur. J. Pharmacol.* 1999; **367:** 231-7.

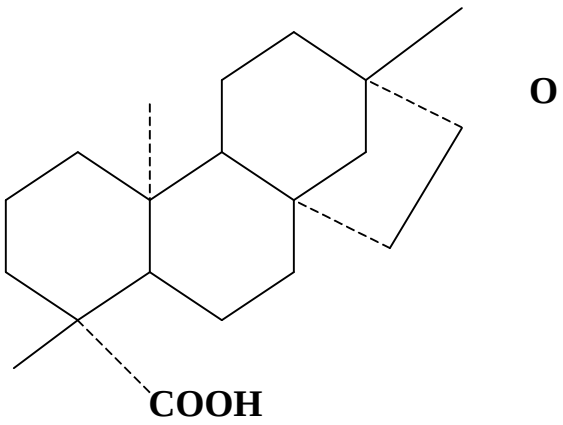
Legends of Figures

Figure 1 The chemical structure of isosteviol.

Figure 2 The mean arterial blood pressure changes at 60 min after an intraperitoneal injection of IST (25 mg kg^{-1}) into conscious SHRs (n=8). *p<0.05.

Figure 3 Amplitude of vasoconstriction in isolated aortic rings. A: 10^{-8} M vasopressin in Ca^{2+}-free [Ca^{2+} (-)] or Ca^{2+}-containing [Ca^{2+} (+)] solution. **p<0.01. B: 10^{-8} M phenylephrine in Ca^{2+}-free [Ca^{2+} (-)] or Ca^{2+}-containing [Ca^{2+} (+)] solution. C: 40 mM KCl in Ca^{2+}-free [Ca^{2+} (-)] or Ca^{2+}-containing [Ca^{2+} (+)] solution.

Figure 4 IST could inhibit intracellular Ca^{2+} increase induced by vasopressin. A: Glibenclamide could reverse the action of IST dose-dependently. *p<0.05, **p<0.01. B: 4-aminopyridine could reverse the action of IST dose-dependently. **p<0.01. C: Charybdotoxin could not reverse the effect of IST. □ = 5×10^{-8} M charybdotoxin; ▨ =10^{-7} M charybdotoxin. **p<0.01. (IST vs Vehicle). D: Apamin could reverse the action of IST dose-dependently. ***p<0.001.



Isosteviol

Figure 1

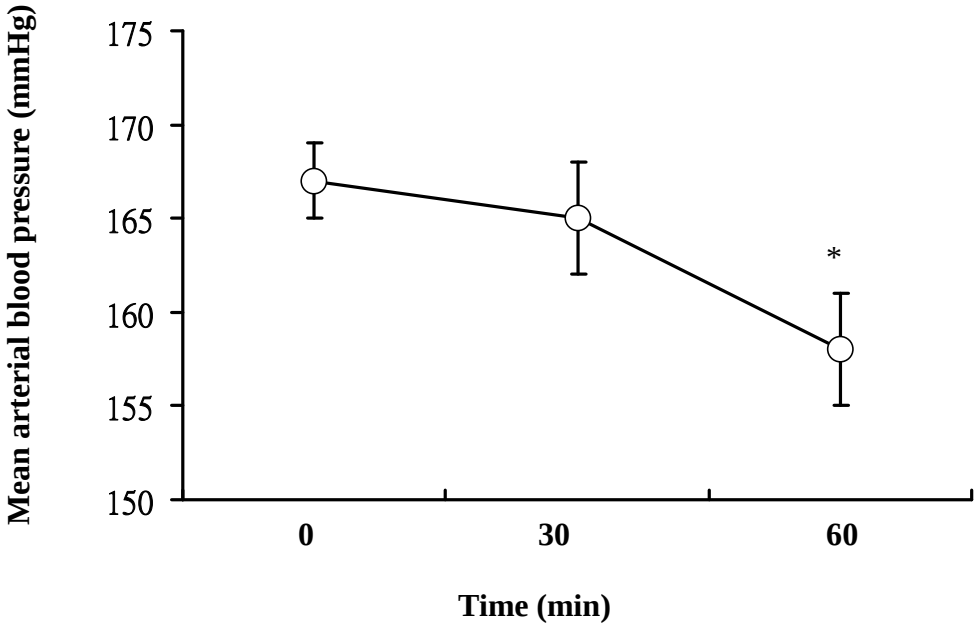


Figure 2

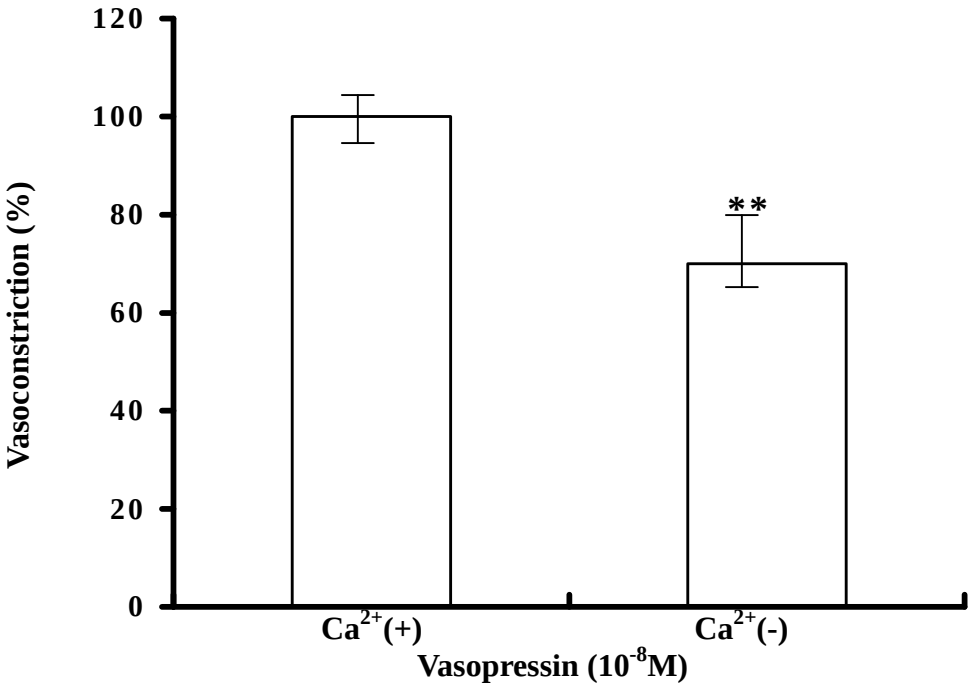


Figure 3 A)

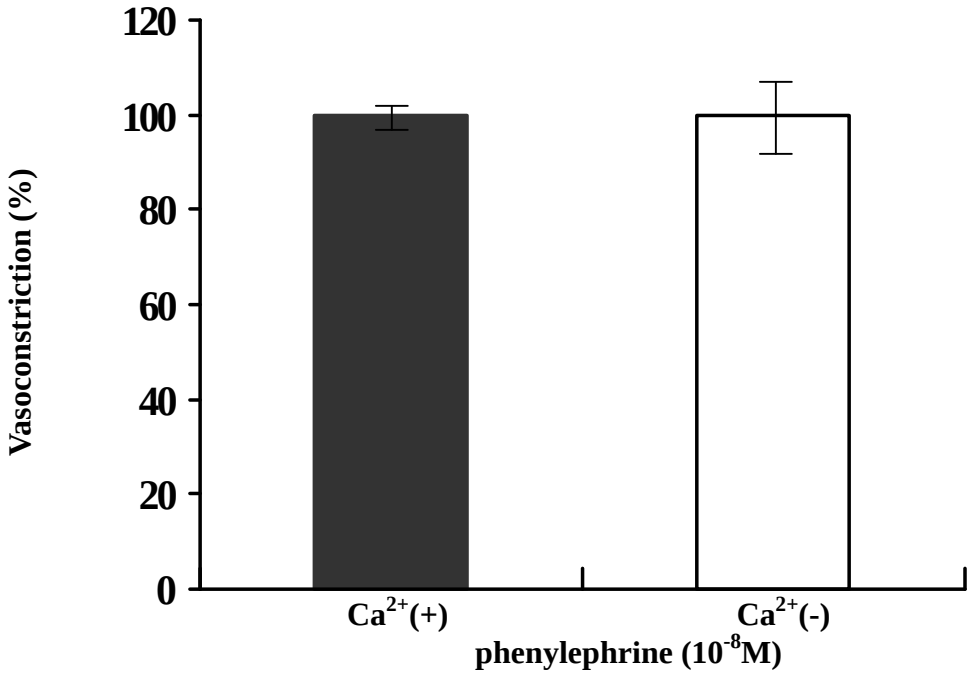


Figure 3 B)

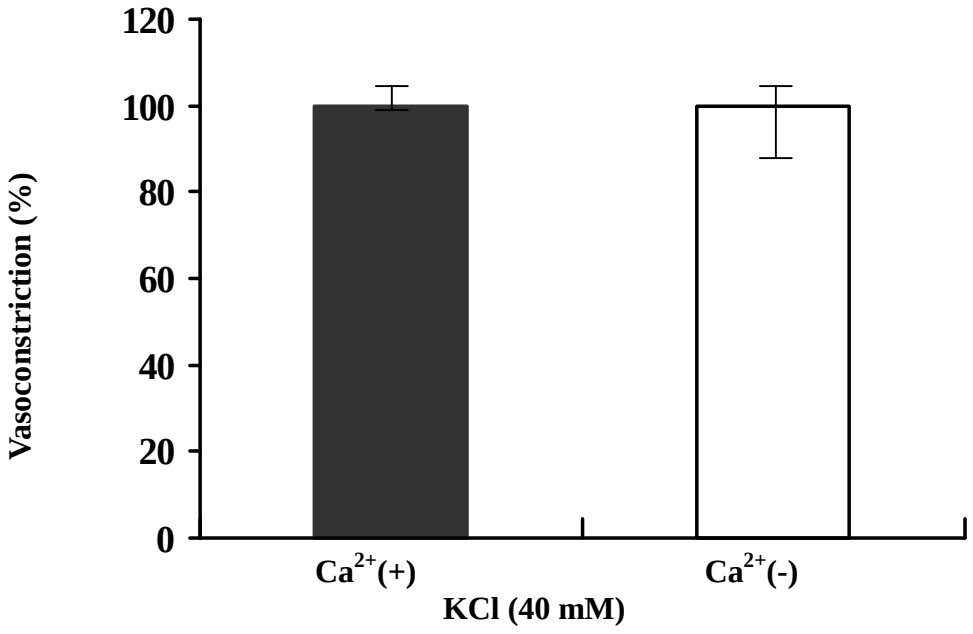


Figure 3 C)

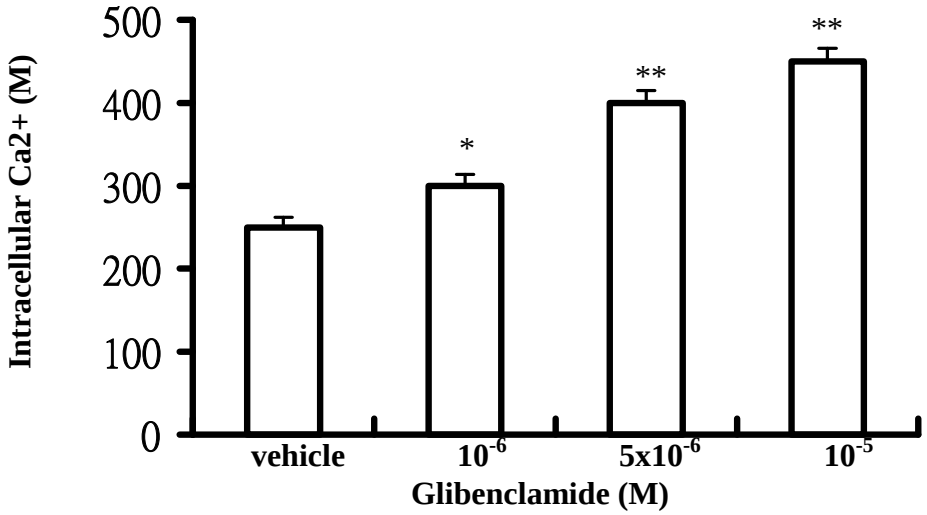


Figure 4 A) -

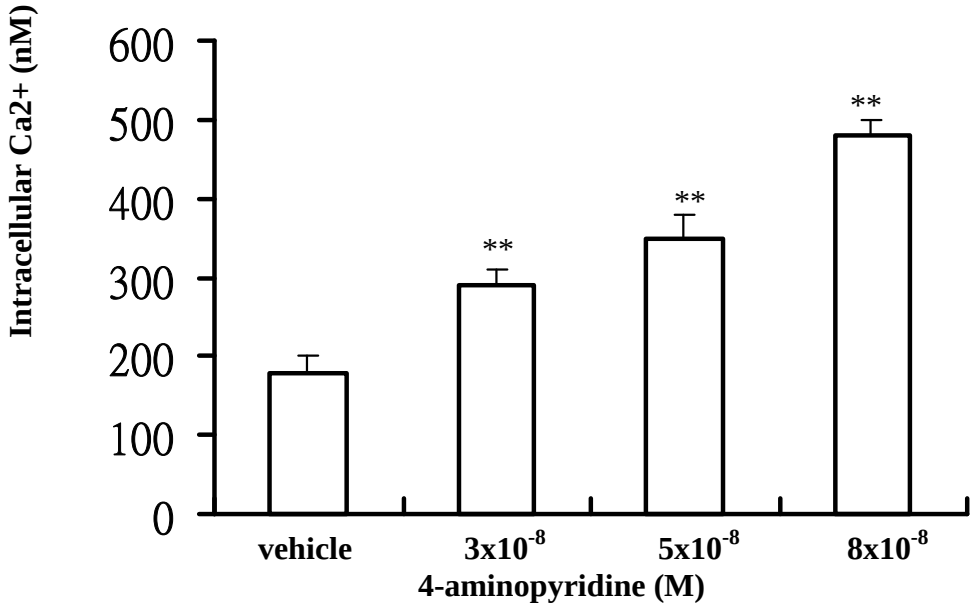


Figure 4 B)

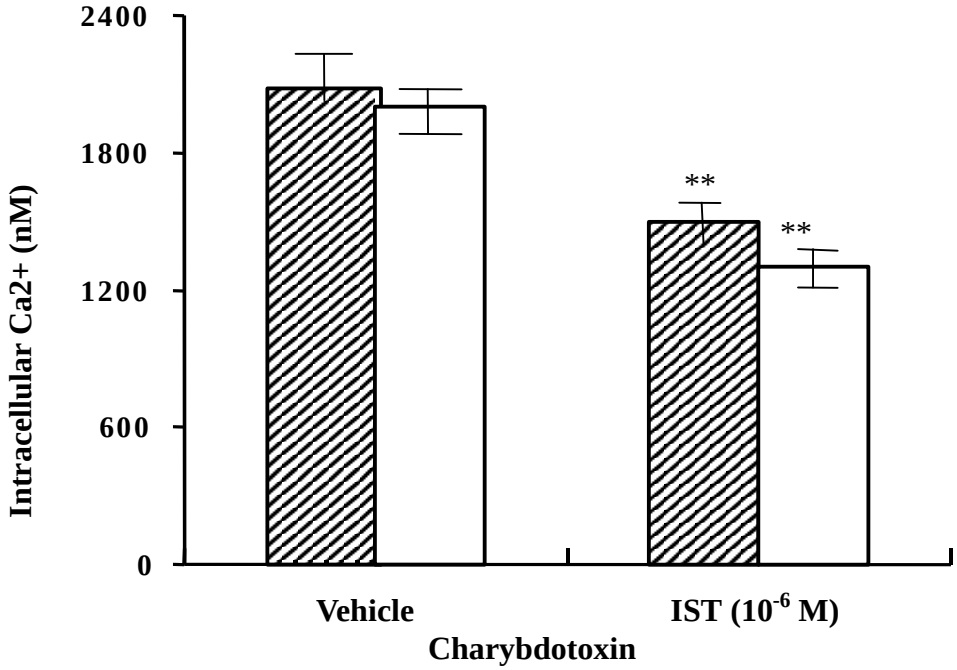


Figure 4 C)

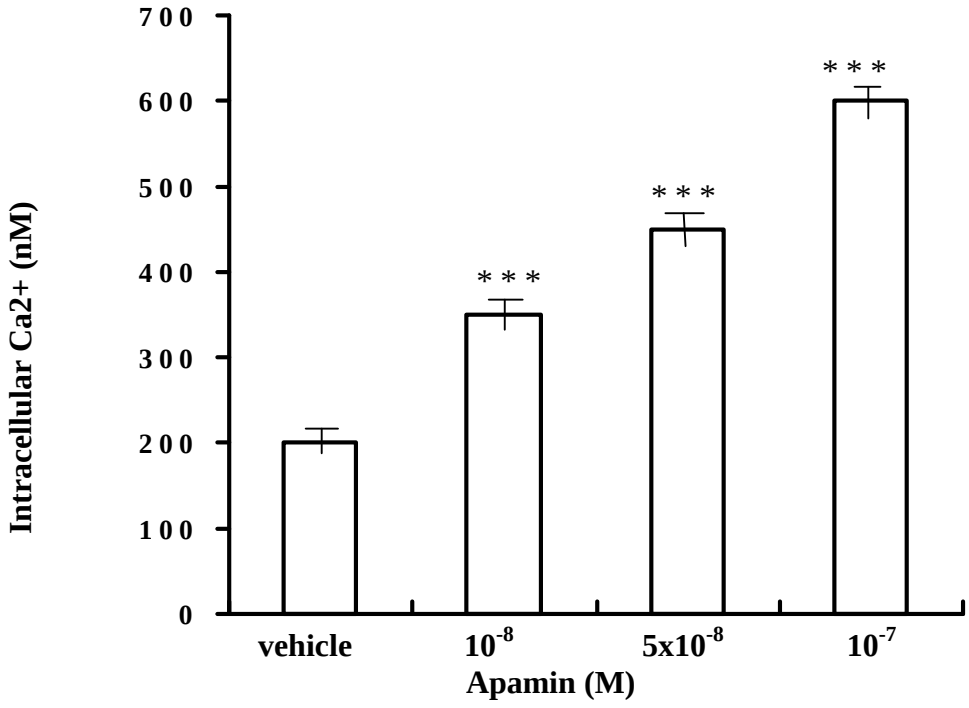


Figure 4 D)

Table 1 The vasorelaxtion effect of IST on 10^{-8} M vasopressin-induced vasoconstriction in aortic rings.

IST level(M) / %	0	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Baseline	100±0	94.6±2.0*	86.1±4.3**	73.2±4.4**	54.9±6.5**
Methylene blue	100±0	91.1±7.2*	80.4±8.9**	67.1±8.4**	55.6±9.3**
Glibenclamide					
5×10^{-6} M	100±0	101.2±1.5	98.4±6.5	85.5±5.1*	71.9±4.9*
7×10^{-6} M	100±0	100.1±5.0	96.9±5.0	94.5±6.0	87.4±5.6*
10^{-5} M	100±0	101.8±5.5	103.4±7.2	101.2±6.7	98.2±4.1
Charybdotoxin	100±0	99.8±6.8	82.4±6.0**	69.8±11.3**	51.7±4.3**
Apamin					
5×10^{-8} M	100±0	100.4±6.7	99.2±6.8	93.8±5.8*	83.9±6.2**
10^{-7} M	100±0	101.9±5.2	102.0±6.1	101.5±4.0	105.2±10.5

Values are given as mean ± SEM. Number of experiments = 10.
*p<0.05; **p<0.01; ***p<0.001 (vs 0 M)

Table 2. Using Fura-2 to evaluate different drug effect on intracellular Ca^{++} level (nM) in A7r5 cells.

Drug level (M)	Vehicle	10^{-8}	10^{-7}	10^{-6}
Ca (-) solution				
Vasopression	174.3±19	199.2±36	180.9±21	172.8±22
Phenylephrine	143.4±5.0	204.7±13.3*	296.4±29.5*	401.1±15.5**
Phenylephrine +IST	334.1±27	342.4±26	315.1±17**	229.5±13.2***
Ca (+) solution				
Vasopressin	184.8±18	289.2±30*	353.7±63**	427.2±60**
Vasopressin + IST	339.6±12.4	274.8±18.4*	193.9±6.6**	102.2±13.2***
Phenylephrine	185.2±22.8	329.0±24.4**	608.6±41.1**	814.9±33.8**
Phenylephrine + IST	651.0±13.4	557.8±18.4*	315.1±6.6**	328.8±47

Values are given as mean ± SEM. Number of experiments = 10.
*p<0.05; **p<0.01; ***p<0.001. (vs Vehicle)