1UNIVERSIDADE ESTADUAL DE MARINGÁ – UEM
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

VALÉRIA DO AMARAL

L-NOARG E ODQ ANTAGONIZAM A HIPOTENSÃO E A REDUÇÃO DO TÔNUS VASCULAR PRODUZIDAS POR ESTEVIOSÍDEO

Maringá
2006

VALÉRIA DO AMARAL

L-NOARG E ODQ ANTAGONIZAM A HIPERTENSÃO E A REDUÇÃO DO TÔNUS VASCULAR PRODUZIDAS POR ESTEVIOSÍDEO

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas (Área de Concentração - Produtos Naturais Biologicamente Ativos) da Universidade Estadual de Maringá, para obtenção do título de mestre em Ciências Farmacêuticas.

Orientador: Wilson Alves- do- Prado

Maringá
2006

VALÉRIA DO AMARAL

L-NOARG E ODQ ANTAGONIZAM A HIPERTENSÃO E A REDUÇÃO DO TÔNUS VASCULAR PRODUZIDAS POR ESTEVIOSÍDEO

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas (Área de Concentração - Produtos Naturais Biologicamente Ativos) da Universidade Estadual de Maringá, para obtenção do título de mestre em Ciências Farmacêuticas.

Aprovada em 14 de setembro de 2006.

Prof. Dr Wilson Alves-Do-Prado
Universidade Estadual de Maringá – UEM

Dedico este trabalho

Ao meu pai pela alegria, exemplo de vida e por todas as lembranças maravilhosas que deixou em meu coração.

Ao meu marido por acreditar em meus sonhos, por acompanhar-me em todos os momentos desta jornada, com amor e dedicação.

Aos meus dois anjos enviados por Deus, que trazem alegria e orgulho para minha vida, Ana Clara e Bárbara.

AGRADECIMENTOS

Ao Senhor meu Deus por capacitar-me e ser sempre a rocha firme sobre meus pés.

Ao Professor Wilson Alves-Do-Prado, meu muitíssimo obrigado, pela oportunidade e honra de me deixar fazer parte de sua equipe, poder compartilhar e conviver com sua sabedoria, brilhantismo, paciência e amizade constantes. Um exemplo a seguir.....

A Irani Lopes dos Santos por sua presença diária e colaboração nos dias difíceis, por todos os sorrisos de alegria que me presenteou com minhas vitórias.

A amiga, irmã de todas as horas, Elaine Campana Sanches Bornia, pelo exemplo de dedicação, solidariedade e companheirismo. Às vezes encontramos anjos em forma de amigos, você é um deles.

As amigas Célia Regina Ambiel e Simone Marques Bolonheis, que sempre estiveram presentes em meu crescimento científico, incentivando, auxiliando e compartilhando suas experiências e conhecimento comigo.Sem dúvida sinto-me honrada e agradecida por tê-las em minha vida.

Aos amigos Edivan, Monalisa, Maurício e Eliane pela amizade, respeito e carinho desenvolvidos por tantos dias de agradável convivência.

Aos técnicos Jailson, Célia, Carlos, Soledalva e Marcos, meus sinceros agradecimentos, por colaborarem inúmeras vezes comigo na elaboração desta pesquisa, pelo carinho e amizade que sempre me dedicaram.

Aos professores do programa de pós-graduação por compartilharem seus conhecimentos conosco e por apostarem em nosso sucesso acadêmico.

A todos que colaboraram direta ou indiretamente por mais esta conquista em minha vida, meu muito obrigado.

“Daí-me Senhor, a perseverança das ondas do mar, que fazem de cada recuo um ponto de partida para um novo avanço”. (Gabriela Mistral)

ABSTRACT

Finding new agents for the treatment of hypertension has been one of several targets of pharmacology. The current research was undertaken to investigate the effects of stevioside, a natural sweetener, on arterial blood pressure of normotensive rats treated with inhibitors of the L-arginine-nitric oxide (NO) synthase (NOS) - guanylate cyclase (CG) - cGMP pathway. The effects of stevioside on spontaneous cardiac beats of isolated atria preparations of rats and on aortic rings preparations of rats treated with L-NOARG or ODQ were also investigated. Intravenous injection of L-NOARG (1 mg/Kg) or ODQ (2.5 μg/Kg) alone did not produce any variations on arterial blood pressure, but antagonized the hypotensive effect induced by stevioside (32 to 64 mg/Kg). Stevioside (0.4 to 24 mM), L-NOARG (0.1 to 1 mM) or ODQ (10 to 50 μM) did not change the spontaneous cardiac beats in isolated atria preparations of rats, but L-NOARG (0, 1 mM) and ODQ (10 μM) antagonized the reduction of vascular tension induced by stevioside (10 μM and 1 mM) in the intact aortic rings preparations of rats pre-contracted by norepinephrine (0,01 μM) or KCl (20 mM), respectively. Such data indicate that the hypotensive effect induced by stevioside is not dependent of an action of the sweetener on cardiac tissue, but it is influenced by endogenous activity of the L-arginine- NOS-NO- GC- cGMP pathway in vascular tissue. Additionally, it was recorded that L-NOARG (0, 1 mM) or ODQ (10 μM) increased the pre-contractions produced by norepinephrine (0,01 μM) in preparations with and without intact endothelium. L-NOARG and ODQ also increased the pre-contraction induced by 20 mM KCl, but such effect occurred only in preparations with intact endothelium. The percentage of increase produced by L-NOARG or ODQ was similar in preparations with and without intact endothelium pre-contracted by norepinephrine. L-NOARG (0, 1 mM) and ODQ (10 μM) antagonized the reduction of vascular tonus induced by stevioside in the intact-endothelium aortic rings preparations pre-contracted by norepinephrine or KCl, but did not modify the reduction of vascular tonus induced by the sweetener in the denuded aortic rings pre-contracted by those agents. Since the increase produced by L-NOARG or ODQ on pre-contraction induced by norepinephrine was not able to impair the effect of stevioside in the denuded aortic rings preparations, the data indicate that the stevioside-induced vasodilatation is not dependent of an intact endothelium, but is strongly influenced by NO synthesized in such tissue.

Keywords: *Stevia rebaudiana (Bert)* Bertoni – Compositae – stevioside - nitric oxide - blood pressure - aortic ring.

RESUMO

Encontrar novos agentes para o tratamento da hipertensão tem sido um entre vários alvos da farmacologia. O presente trabalho foi realizado para investigar os efeitos do esteviosídeo, um adoçante natural, na pressão arterial de ratos normotensos tratados com inibidores da via L-arginina – óxido nítrico sintase – GC (guanilato ciclase) – GMPc. Os efeitos do esteviosídeo na freqüência cardíaca espontânea de preparações de átrio isolados de ratos e preparações de anéis de aorta de ratos tratados com L-NOARG ou ODQ também foram investigados. Injeção intravenosa de L-NOARG (1 mg /Kg) ou ODQ (2,5 µg /Kg) não produziram nenhuma variação na pressão arterial mas antagonizaram o efeito hipotensivo induzido por esteviosídeo (32 a 64 mg /Kg). Esteviosídeo (0,4 a 24 mM), L-NOARG (0,1 a 1 mM) ou ODQ (10 a 50 µM) não mudaram os batimentos cardíacos espontâneos em preparações de átrio isolado de ratos, mas L-NOARG (0,1 mM) ou ODQ (10 µM) antagonizaram a redução da tensão vascular induzida por esteviosídeo (10 µM e 1 mM) em preparações de anéis de aorta isolados de ratos, pré-contraídos por norepinefrina (0,01 µM) ou KCl (20 mM), respectivamente. Os dados indicam que o efeito hipotensivo induzido por esteviosídeo não é dependente de uma ação do adoçante no tecido cardíaco, mas é influenciada pela atividade endógena da via L-arginina – óxido nítrico sintase (NOS) – guanilato ciclase (GC) – GMPc no tecido vascular. Adicionalmente, foi registrado que L-NOARG (0,1 mM) ou ODQ (10 µM) aumentaram a pré-contração produzida por norepinefrina (0,01 µM) em preparações com e sem endotélio. L-NOARG e ODQ também aumentaram a pré-contração induzida por 20 mM de KCl, mas este efeito ocorreu somente em preparações com endotélio intacto. A porcentagem de aumento produzida por L-NOARG ou ODQ foi similar em preparações com e sem endotélio intacto pré-contraídos por norepinefrina. L-NOARG (0,1 mM) e ODQ (10 µM) antagonizaram a redução no tônus vascular induzida por esteviosídeo em preparações de anéis de aorta com endotélio intacto pré-contraídos por norepinefrina ou KCl, mas não modificou a redução no tônus vascular induzida pelo adoçante em preparações de anéis de aorta com endotélio pré contraídos por estes agentes. Desde que o aumento induzido por L-NOARG ou ODQ na pré-contração induzida por norepinefrina não foi capaz de prejudicar o efeito de esteviosídeo nas preparações de anéis de aorta, os dados indicam que a vasodilatação induzida por esteviosídeo não depende de endotélio intacto, mas é fortemente influenciada pelo NO sintetizado em tal tecido.

Palavras chaves: *Stevia rebaudiana* (Bert) Bertoni – Compositae – esteviosídeo – óxido nítrico – pressão arterial – anéis de aorta.

SUMMARY

- Introduction

The hypotensive effect induced by different agents may be mediated or influenced by activation of synthesis of a factor dependent on intact endothelium (Furchgott and Zawadzki, 1980; Welch and Loscalzo, 1994) known as nitric oxide (NO) (Ignarro et al., 1987; Palmer et al., 1987). Such factor is synthesized from L-arginine by the action of NO-synthase (NOS) (Schmidt et al., 1992), and N^G-nitro L-arginine (L-NOARG) is an agent used as pharmacological tool to inhibit the activity of such enzyme (Moncada et al., 1991; Chinellato et al., 1998). Since the biological effects induced by NO may also be mediated by activation of the guanylate cyclase increasing intracellular level of cGMP (Moncada et al., 1997), 1H-[1, 2, 4] oxidiazolo [4,3- a] quinoxalin-1-one (ODQ) has been used as pharmacological tool to reduce the activation of the guanylate cyclase by NO (Boulton et al. ,1995; Schrammel et al., 1996).

Stevioside, a sweet-tasting glycoside isolated from the herb *Stevia rebaudiana* (*Bert*) Bertoni (Compositae), is a sugar substitute (Soejarto et al., 1982). However, it has been showed that intravenous injection of stevioside produces a dose-dependent reduction on arterial blood pressure in spontaneously hypertensive rats (Chan et al., 1998). Similar effect is observed when the sweeter is orally administered to human hypertensive patients (Chan et al., 2000; Hsieh et al., 2003) or intraperitoneally in normotensive rats (Hsu et al., 2002). As it is accepted that the hypotensive effect produced by stevioside has origin in peripheral vessels reducing Ca^{++} influx (Lee et al., 2001), its oral administration has been proposed as able to produce clinical benefit in the control of hypertension (Melis, 1992; Chan et al., 1998; Liu et al., 2003). Despite the oral administration of drugs to offer clinical practicability to treatment of hypertension, the oral ingestion of stevioside was not able to reduce the arterial blood pressure in untreated essential hypertension patients with mean systolic blood pressure ≤ 159 mmHg and diastolic blood pressure ≤ 99 mmHg (Ferri et al., 2006). The absence of hypotensive effect after oral ingestion of stevioside has been attributed to its low gastro intestinal absorption (Yamamoto et al., 1985; Bracht et al., 1985; Geuns et al., 2003). Although exist controversies about clinical benefices of oral administration of stevioside to treatment of hypertension (Chan et al., 2000; Hsieh et al., 2003; Ferri et al., 2006), there is not any conflict about the hypotensive effect of sweeter in studies performed

with intravenous administration of sweeter in rats (Melis and Sainati, 1991; Melis, 1992; Lee et al., 2001; Liu et al., 2003). Since it is not possible to negligence the efficiency of stevioside reducing the arterial blood pressure during its intravenously administration , and considering that in hypertensive clinical emergence such via of administration is used to injections of agents that block Ca^{++} channels (Wallin et al., 1988; Halpern et al., 1992; Kovac et al., 2001; Levy, 2005), the current study was undertaken to obtain data those might be useful to clinical research using the intravenous administration of stevioside. Therefore, the influence of L-NOARG and ODQ on hypotensive effect induced by intravenous administration of stevioside in normotensive rats (in vivo experiments) was investigated. The effects of sweeter (pure= 99.9 %, mixture = 70 %) alone or in combinations with L-NOARG or ODQ were also investigated in the atria isolated and aortic rings preparations of rats (in vitro).

2- Material and Methods

2.1- Arterial Blood Pressure (in vivo)

The experiments were performed on normotensive male Wistar rats (250-280 g), with free access to food and water. Animals were anesthetized with intraperitoneal injection of sodium thiopental (36 mg/Kg) and urethane (600 mg/Kg). After tracheotomy, the carotid artery was catheterized and connected to BP transducer (PowerLab® AD Instruments, MLT0380) and arterial blood pressure was recorded on the computer equipped with Chart Software of Powerlab® (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia). The jugular vein was also catheterized (dead space 0.1 ml) to permit injection of different agents. When the stabilization of arterial blood pressure (110 to 120 mmHg) was reached, saline (0.1 ml) was administered to verify possible mechanical effects of the injections.

The lowest dose of stevioside able to reduce the arterial blood pressure was established and administered in additive form at each 15-min interval. The injections of stevioside were always followed by administration of saline (0.1 ml). The lowest doses of ODQ or L-NOARG able to modify the reduction on arterial blood pressure induced by stevioside were also researched (2.5 µg/Kg and 1 mg/Kg), respectively. When the experiments were performed with associations of drugs, the first agent (ODQ or L-NOARG) was always administered 15 minutes before stevioside injection.

The values of arterial blood pressure recorded after administration of stevioside were taken as a percentage of that obtained without administration of any agent (control). Data were submitted to ANOVA, followed by the Bonferroni test with the level of significance set at $P< 0.05$.

2.2- Isolated Atria

After all detectable blood was washed from the heart, entire right atria was isolated and allowed to beat spontaneously in a temperature-controlled (37℃) tissue bath cont aining the Tyrode solution (in mmol/L: NaCl, 134,8; KCl, 496; $MgCl_2$, 1,0; $CaCl_2$, 1,97; NaH_2PO_4, 0,86; glucose, 11 and $NaHCO_3$, 15).The preparation was connected to strain gauges (Grass, FT 03) and heart rate was recorded on the computer equipped with Chart Software of Powerlab (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia). The preparations were stretched gradually to an optimal 0.5 g resting tension. After being mounted, the right atria were allowed to stabilize for 30 min. During this period, the medium was replaced every 15 min. Pacemaker frequency was directly monitored via computer (Chart Software of Powerlab). The mean of spontaneously beating atria obtained after addition of drugs was every 15 min taken as percentage of that recorded in free drugs Tyrode solution (control frequency). Data were submitted to ANOVA, followed by the Bonferroni test with the level of significance set at $P< 0.05$.

2.3- Isolated Aortic rings ("in vitro" preparations)

Rats were previously heparinized and killed by decapitation after intraperitoneal injection of sodium thiopental (36 mg/Kg) and urethane (600 mg/Kg). A medial laparotomy was then performed immediately to excise the thoracic aorta. The vessel was sectioned into 3-mm rings and after gently dissected free of fat and connective tissue. Rings were then mounted into 30 ml organ baths filled with oxygenated (95% O_2, 5% CO_2) Krebs buffer (in mmol/L: NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; EDTA 0.03, and glucose, 11) at 37 ºC, and the pH of the solution was maintained at 7.4.

The preparation was connected to strain gauges (Grass, FT 03) and isometric tension was recorded on the computer equipped with Chart Software of Powerlab (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia). After being mounted, the rings were allowed to stabilize for 2 h. The preparations were then stretched gradually to an optimal 1g resting tension.

The presence of intact endothelium was considered when the addition of acetylcholine (1.0 μM to 10 nM) in the bath was able to produce 100% relaxing of the preparations pre-contracted with norepinephrine (0.01 μM). After the resting tension was stabilized, KCl (20 mM) or norepinephrine (0.01 μM) was administered into the bathing buffer to induce a rapid increase of vascular tone followed by a stable vasoconstriction (tonic contraction). Norepinephrine was the drug chosen to produce pre- contraction of vessels because is naturally produced in the organism and, in contrast with epinephrine, has higher affinity for alpha-adrenoceptors (Westfall and Westfall, 2006).

The concentration of norepinephrine used was that proposed in literature as able to produce about 50% of maximal tension in isolated aortic rings preparations of rats deprived of the endothelium (Testai et al., 2005). The concentration of KCl used was that proposed in literature as able to produce contraction of the aortic rings preparations without, however, to impair the action of agents considered as a possible K^+ channel opener (Nelson and Quayle, 1995) as such mechanism of action and/or the blockage of Ca^{++} channels might explain the vessel relaxing induced by stevioside (Lee et al., 2001, Wong et al., 2004a, b). The lowest concentration of stevioside able to produce a

reduction on vascular tonus induced by KCl or norepinephrine was researched and then added in the bath. The moment when stevioside produced its maximal relaxing effect was also set (10 min after its addition in the bath) and used for all the experiments. When the assays were performed with association of drugs, the different agents were administered in the bath 20 min before stevioside addition. The tension recorded at t= 10 min was taken as the percentage of that (control) obtained after addition of KCl or norepinephrine in the bath. Concentrations of ODQ (10 μM) (Schrammel et al., 1996; Crauwels et al., 2000) and L-NOARG (0, 1 mM) (Moore et al, 1990) proposed in literature as able to block the guanylate cyclase or NOS, respectively, were used in the current studies. Data were submitted to ANOVA, followed by the Bonferroni test with the level of significance set at $P< 0.05$.

2.4- Stevioside and Others Agents

Standardized stevioside (Fig. 1) was obtained from dried leaves by a method developed by Alvarez (1987). The method produces a mixture of stevioside (70%) and rebaudioside (20%). Since the effects produced by a mixture of stevioside might be influenced by rebaudioside, pure stevioside (100%), according to Dacome (2005) was also utilized to confront of data. N^G- nitro L-arginine (L-NOARG), 1H - [1, 2, 4] oxidiazolo [4, 3-a] quinoxalin-1-one (ODQ), acetylcholine, norepinephrine and urethane were purchased from Sigma (St. Louis, MO, USA), and sodium thiopental from Cristália (SP, Brazil).

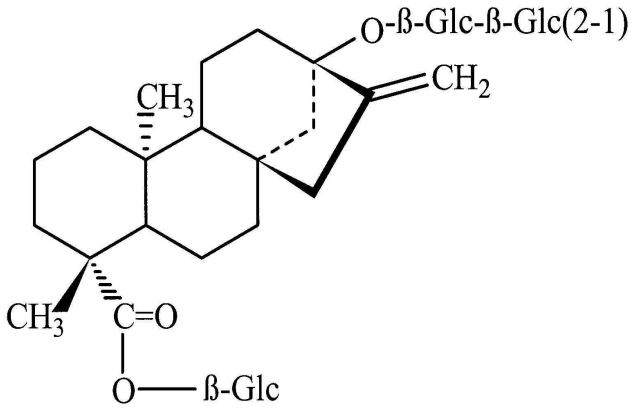

Fig.1. Chemical structure of stevioside

3- Results

Intravenous injection of L-NOARG (1 mg/Kg) or ODQ (2.5 μg/Kg) alones did not produce any variations on arterial blood pressure, but antagonized the hypotensive effect induced by pure or mixture of stevioside (32 to 64 mg/Kg) (Fig.2). Pure or mixture of stevioside (0.4 to 24 mM), L-NOARG (0.1 to 1 mM) or ODQ (10 to 50 μM) did not change the spontaneous frequency of beats in preparations atria isolated of rats (Fig.3), but L-NOARG (0,1 mM) and ODQ (10 μM) antagonized the reduction of vascular tension induced by pure (1mM and 10 μM) or mixture (1 mM and 0,1 mM) of stevioside in the intact aortic rings preparations of rats pre-contracted by norepinephrine (0,01 μM) or KCl (20 mM), respectively (Fig.4A and 4B).

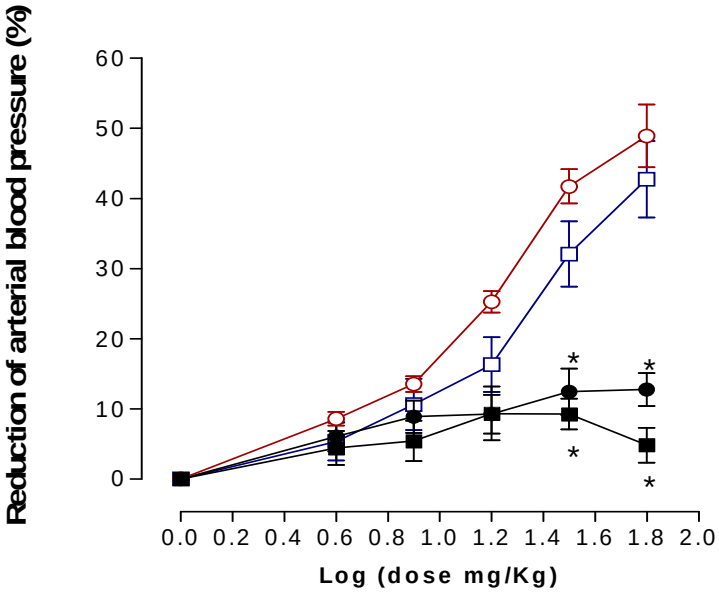

Fig.2 Antagonism by L-NOARG (1mg/Kg; ■) and ODQ (2,5µg/Kg; ●) of hypotensive effect induced by intravenous injections of stevioside (pure , mixture O) in normotensive rats. Abscissas indicate Log dose (mg/Kg) of stevioside. On the ordinate, reduction (%) of arterial blood pressure. Points represent mean (± SEM) of 6 experiments. * P< 0, 05 compared to stevioside (ANOVA; Bonferroni post hoc test).

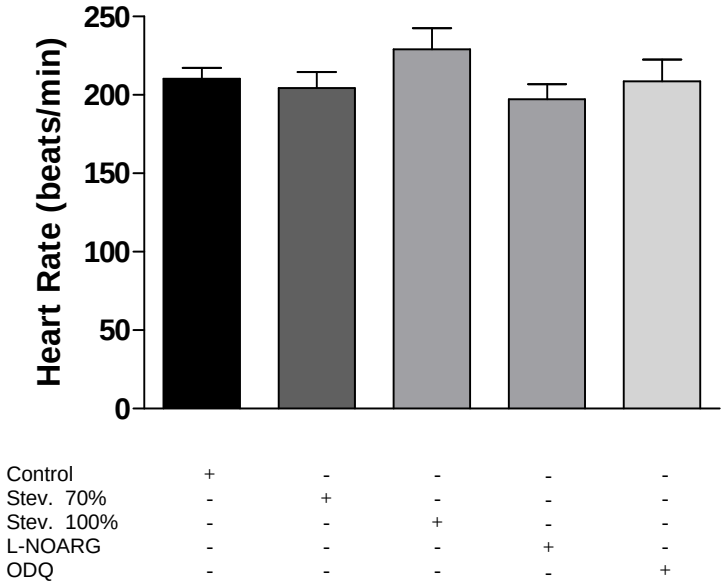

Fig.3 Absence of effect of stevioside (Stev, 0, 4 to 24 mM) pure (100%) or mixed (70%), L-NOARG (0, 1 to 1 mM) or ODQ (10 to 50 µM) on spontaneous heart activity (beats/min) of the right atrium isolated from rats. Abscissas indicate absence (-) or presence (+) of drugs in the bath. Height of columns represent mean of spontaneous hearts beats ± S.E.M. of 6 to 8 preparations (ANOVA).

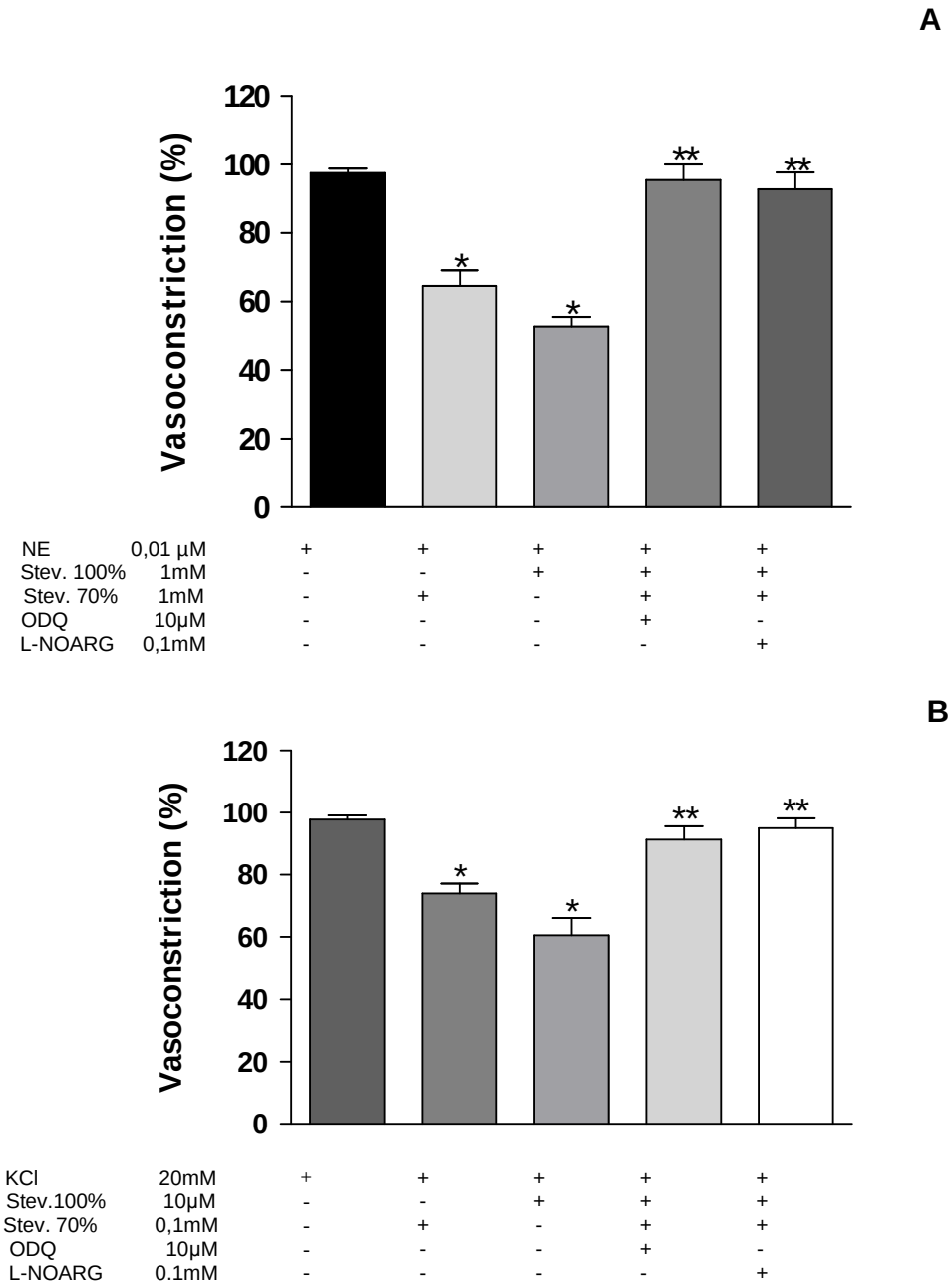

Fig. 4 Antagonism by L-NOARG (0.1 mM) and ODQ (10 µM) of reduction of tension (vasoconstriction) induced by stevioside (Stev) pure (100%, 1 mM and 10 µM) or mixed (70%, 1 mM and 0,1 mM) in the isolated aortic rings preparations of rats pre-contracted by 0,01 µM norepinephrine (NE, A) or 20 mM KCl (B). Abscissas indicate presence (+) or absence (-) of drugs in the bath. Ordinate indicates percentage (%) of reduction of tension induced by stevioside alone, or in combination with others agents. Heights of columns show mean ± SEM of 6 to 8 experiments. * P< 0, 05 compared to control (NE or KCl alone taken as 100 %, ANOVA; Bonferroni post hoc test). ** P< 0, 05 compared to stevioside (100% and 70 %) alone (ANOVA; Bonferroni post hoc test).

4- Discussion

Current research confirms that stevioside produces a dose-dependent reduction on arterial blood pressure if experiments are performed with “in vivo” preparations (Melis and Sainati, 1991a; Melis and Sainati, 1991b; Chan et al., 1998; Hsu et al., 2002; Liu et al., 2003). Additionally, data show that such effect of pure stevioside or of that obtained according to Alvarez (1987) is antagonized by previous intravenous administration of L-NOARG or ODQ. Although previous report indicates that the reduction on arterial blood pressure induced by intravenous administration of stevioside is not determined by an action of sweeter in the central nervous system (Liu et al., 2003), the antagonism by L-NOARG or ODQ of hypotensive effect induced by stevioside might has origin on inhibition of the L-arginine- NOS- NO- GC- cGMP pathway activity in several biological systems regulator of arterial blood pressure (Ignarro,1989; Jover and Mimran, 2001) However, the current work permitted to verify that the antagonisms by L-NOARG or ODQ of hypotensive effect induced by stevioside has not origin in the cardiac pacemaker, as the blockers of the L-arginine- NOS- NO- GC- cGMP pathway activity or stevioside did not modify the spontaneous cardiac activity in atria isolated preparations. In contrast, it was verified that the inhibitor of NOS, L-NOARG, or the inhibitor of guanylate cyclase, ODQ, antagonized the reduction of vascular tension induced by stevioside in intact endothelium aortic rings preparations of rats pre- contracted by norepinephrine or KCl. Although can not be disregard the actions of L-NOARG or ODQ in others tissues than cardiac or vascular, data indicate that exits a strong influence of NO synthesized in vascular tissues on hypotensive effect induced by stevioside. It is unlikely that the antagonisms by L-NOARG or ODQ of stevioside- induced reduction of arterial blood pressure had been determined by inhibition of metabolism of stevioside by NOS or by reduction of activation by stevioside of GC, as NOS is an enzyme highly stereospecific to L isomer of arginine (Palmer et al., 1988) and GC is sensitive to agents that contain Fe^{+3} in their molecules (Martin et al., 1985). It is also unlikely that the antagonism by L-NOARG or ODQ of vasodilatation induced by stevioside had been determined by a previous reduction of vasodilatation mediate by NO, thereby inducing

an acute vasoconstriction, as the intravenous injections of 1 mg/Kg L-NOARG or 2,5 μM/Kg ODQ alones did not produce any variations on arterial blood pressure.

Although NOS exist in endothelium and arterial smooth muscle of rats (Gurdal et al., 2005), the literature offers evidences indicating that reduction of vascular tension induced by stevioside is not dependent on intact endothelium in the aortic rings preparations of rats (Lee et al., 2001). Nevertheless, the vasoconstriction induced by norepinephrine may be depressed by NO released through stimulation of alpha-adrenoceptors (Kaneko and Sunano, 1993; Lamb and Barna, 1998). Thus, the antagonism by L-NOARG or ODQ of reduction of vascular tension induced by stevioside in aortic rings preparations of rats pre- contracted by norepinephrine or KCl might be a functional antagonism produced by reduction of synthesis of NO by L-NOARG or by decrease the activity of guanylate cyclase by ODQ determining a decrease on inhibitory action of NO and/or cGMP on vasoconstriction mediated by alpha-adrenoceptors or KCl.

Since stevioside pure, or that obtained from method of Alvarez (1987), produced similar qualitative and quantitative results, is possible to admit that the reduction of arterial blood pressure induced by mixture of stevioside (70%) and rebaudioside (20%) is not determined by action of other agents in mixture than the stevioside. However, have to be considered that the results obtained with the intravenous injection of stevioside, in contrast with those obtained from “in vitro” preparations, might be produced by a metabolic of stevioside, as it has been shown that such sweeter is degraded to steviol after its intravenous administration in rats (Cardoso et al., 1996; Wong et al., 2004).

As whole, data indicate that there is not important difference between the reduction of arterial blood pressure induced by intravenous administration of pure stevioside and that obtained from method of Alvarez (1987). The hypotensive effect induced by stevioside it is strongly influenced by endogenous activity of the L-arginine- NOS- NO-GC- cGMP pathway, and is, at least in part, mediated by actions of sweeter and it’s metabolic in vascular tissue.

Acknowledgements

We are also grateful to Prof. Dr. S. C. Costa for the generous gift of pure stevioside, and Mrs. I. L. Santos for technical support. This work was supported by CNPq (Grant number 400075/2002-0) and Steviafarma that prepared the standardized stevioside.

References

Alvarez M, Kusumoto IT.1987. Análise quantitativa dos glicosídeos edulcorantes da stevia rebaudiana e dos seus produtos de hidrólise através de cromatografia líquida de alta performance (HPLC). *Arq Biol Tecnol* **30(2):** 337-348

Boulton CL, Southam E, Gartwaite J.1995. Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. *Neurosci* **69:** 699-703.

Bracht KAM, Kemmelmeier, FS, Ishii, EL, Alvarez M, Bracht A. 1985. Effect of *Stevia rebaudiana* natural products on cellular and sub-cellular metabolism. *Arq. Biol. Tecnol*. **28:** 431-455

Cardoso VN, Barbosa MF, Muramoto E, Mesquita CH , Almeida MA. 1996. Pharmacokinetic Studies of I-Stevioside and its Metabolites. *Nuclear Medicine & Biology* **23:** 97-100.

Chan P, Tomlinson B, Chen YJ, Liu JC, Hsieh MH, Cheng JT. 2000.A double- blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. *Br. Med. J. Clin. Pharmacol* **50:** 215-220.

Chan P, Xu DJ, Liu JC, Chen YJ, Tomlinson B, Huang WP, et al. 1998.The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. *Life Sci* **63:** 1679-1684

Chinellato A, Froldi G, Caparrota L, Ragazzi E.1998.Pharmacological characterization of endothelial cell nitric oxide synthase inhibitors in isolated rabbit aorta. *Life Sci.* **62(6):** 479-490.

Crauwels HM, Van Hove CE, Herman AG, Bult H. 2000. Heterogeneity in relaxation mechanisms in the carotid and the femoral artery of the mouse. *Eur J Pharmacol* **404:** 341-351.

Dacome AS, Silva CC, Costa CEM, Fontana JD, Adelman J, Costa SC. 2005. Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni: Isolation and quantitative distribution by chromatographic, spectroscopic, and eletrophoretic methods. *Process Biochem* **40:** 3587-3594.

Ferri LA, Alves-Do-Prado W, Yamada SS, Gazola S, Batista MR, Bazotte RB. 2006. Investigation of the antihypertensive effect of oral crude stevioside in patients with mild essential hypertension. *Phytother Res.* [Epub ahead of print].

Furchgott RF, Zawadzki JV.1980.The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288:** 373-376.

Geuns JMC, Augustijins A, Mols R, Buyse JG, Driessen B. 2003. Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. *Food and Chemi. Toxicol* **41:** 1599-1607.

González-Fernández F, López-Farré A, Rodríguez-Feo JA, Farré J, Guerra J, Fortes J, Millás I, García-Durán M, Rico P, Mata P, Miguel LS, Casado S. 1998. Expression of Inducible Nitric Oxide Synthase after Endothelial Denudation of the Rat Carotid Artery. *Circ Res.* **83:**1080-1087.

Gurdal H, Can A, Ugur M. 2005. The role of nitric oxide synthase in reduced vasocontractile responsiveness induced by prolonged alpha1-adrenergic receptor stimulation in rat thoracic aorta. *Br J Pharmacol* **145(2):** 203-210.

Halpern NA, Goldberg M, Neely C, Sladen RN, Goldberg JS, Floyd J, Gabrielson G, Greenstein RJ. 1992. Postoperative hypertension: a multicenter, perspective, randomized comparison between intravenous nicardipine and sodium nitroprusside. *Crit Care Med*. **20(12):** 1637-1643.

Hsieh MH, Chan P, Sue YM, Liu JC, Liang TH, Huang TY, et al. 2003. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. *Clin Ther* **25:** 2797-2808.

Hsu YH, Liu JC, Kao PF, Lee CN, Chen YJ, Hsieh MH, et al. 2002. Antihypertensive effect of stevioside in different strains of hypertensive rats. *Chin Méd J* **65:** 1-6.

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G.1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci* **84:** 9265-9269.

Ignarro LJ. 1989. Endothelium-derived nitric oxide: actions and properties. *FASEB J*. **3**: 31-36.

Joly GA, Schini VB, Vanhoutte PM. 1992. Balloon injury and interleukin-1 beta induced nitric oxide synthase activity in rat carotid arteries. *Circ Res* **71:** 331-338.

Jover B, Mimran A. 2001. Nitric oxide inhibition and renal alterations. *J Cardiovasc Pharmacol* **38 (2):** S65-70.

Kaneko K, Sunano S. 1993. Involvement of α-adrenoceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. *Eur. J. Pharmacol* **240:** 195-200.

Kovac AL, McKinley C, Tebbe CJ, Williams C, 2001. Comparison of nicardipine versus placebo to control hemodynamic responses during emergence and extubation. *Cardiothorac Vasc Anesth* **15(6):** 704-709.

Lamb FS, Barna TJ. 1998. The endothelium modulates the contribution of chloride currents to norepinephrine-induced vascular contraction. *Am J Physiol Heart Circ Physiol* **275:** H161-H168.

Lee CN, Wong KL, Liu JC, Chen YJ, Cheng JT, Chan P. 2001.Inhibitory effect of stevioside on calcium influx to produce antihypertension. *Planta Med* 67**:** 796-799.

Levy JH. 2005. Management of systemic and pulmonary hypertension. *Tex Heart Inst. J.* **32(4):** 467-471.

Liu JC, Kao PF, Chan P, Hsu YH, Hou CC, Lien GS, et al. 2003. Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. *Pharmacology* 67**:** 14-20.

Martin W, Villani GM, Jothianandan D, Furchgott RF. 1985. Blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation of rabbit aorta by certain ferrous hemoproteins. *Pharmacol Exp Ther* **233 (3):** 679-685.

Melis MS. Stevioside effect on renal function of normal and hypertensive rats. 1992. *J Ethnopharmacol* **36:** 213-217.

Melis MS, Sainati AR. 1991a.Participation of prostaglandins in the effect of stevioside on rat renal function and arterial pressure. *Braz J Med Biol Res* **24:** 1269-1276.

Melis MS, Sainati AR. 1991b. Effect of calcium and verapamil on renal function of rats during treatment with stevioside. *J Ethnopharmacol* **33:** 257-262.

Moncada S, Higgs A, Furchgott R.1997. XIV. International Union of Pharmacology Nomenclature in Nitric Oxide *Research. Pharmacol Rev* **49 (2):** 137-142.

Moncada S, Palmer RMJ, Higgs EA. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* **43:** 109-142.

Moore PK, al-Swayeh OA, Chong NW, Evans RA, Gibson A. 1990. L-NG-nitro arginine(L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br J Pharmacol* **99(2):** 408-412.

Nelson MT, Quayle JM. 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* **268:** C799- C822.

Palmer RM, Ferrige AG, Moncada S. 1987.Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327:** 524-526.
Palmer RMJ, Ashton DS, Moncada S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333:** 664-666.

Schmidt HHHW, Pollock JS, Nakane M, Forstermann U, Murad F.1992. Ca^{2+}-calmodulin-regulated nitric oxide synthases. *Cell Calcium* **13:** 427-434.

Schrammel A, Behrends S, Schmidt K, Koesling D, Mayer B. 1996. Characterization of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one as a heme-site inhibitor of nitric oxide-sensitive guanylyl cyclase. *Mol Pharmacol* **50(1):** 1-5.

Soejarto DD, Kinghorn AD, Farnsworth NR. 1982. Potential sweetening agents of plant origin.III.Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. *J Nat Prod* **45:** 590- 599.

Testai L, Chericoni S, Bader A, Luisa P, Vincenzo C, Martinotti E. 2005. Vasorelaxant effects of the chloroformic crude extract of *Bupleurum fruticosum* L. (Umbelliferae) roots on rat thoracic aorta. *J Ethnopharmacol* **96:** 93-97.

Wallin JD, Cook ME, Blaski L, Bienvenu GS, Clifton GG, Langford H, Turlapaty P, Laddu A.1988. Intranenous nicardipine for the treatment of severe hyprttension. *Am J Med* **85(3):** 331-338.

Welch G, Loscalzo J. 1994. Nitric Oxide and the Cardiovascular System. *J Card Surg* **9:** 361-371.

Westfall TC, Westfall DP. 2006. Adrenergic agonists and Antagonists. In: Goodman & Gilman. The Pharmacological Basis of Therapeutics, McGrawHill (11ed). New York :USA; 237-295.

Wong KL, Chan P, Yang HY, Hsu FL, Liu IM. Cheng YW, Cheng JT. 2004. Isosteviol acts on potassium channels to relax isolated aortic strips of Wistar rat. *Life Sciences* **74:** 2379- 2387.

Yamamoto NS, Bracht KAM, Ishii EL, Kemmelmeier FS, Alvarez M, Bracht A. 1985. Effect of steviol and its structural analogues on glucose production and oxygen uptake in rat renal tubules. *Experientia* **41:** 55-57.

1- Introduction

The vascular relaxation induced by different agents may be mediated, or influenced, by nitric oxide (NO) [1, 2]. NO is a gas synthesized from L-arginine (L-Arg)

by the action of NO-synthase (NOS) [3] and N^G-nitro L-arginine (L-NOARG), an analog of L-arginine [2], may inhibit the activity of such enzyme in different tissues. As the vascular relaxation produced by NO may also be mediated by activation of the guanylate cyclase (GC), increasing the intracellular level of cGMP, [4], 1H-[1,2,4] oxidiazolo [4,3- a] quinoxalin-1-one (ODQ), a selective blocker of guanylate cyclase, has been used to investigate if the vascular effects produced by different agents might depend on the activity of such enzyme [5]

Stevioside, a sweet-tasting glycoside isolated from the herb *Stevia rebaudiana* (*Bert*) Bertoni (Compositae), has been used as a sugar substitute [6]. However, it has been shown that such sweetener is also able to produce significant reduction on arterial blood pressure when administered intraperitoneally, orally or intravenously [7, 8, 9 and 10]. Despite the oral administration of stevioside being practical for the treatment of hypertension, clinical studies have shown that stevioside does not reduce the arterial blood pressure when it is orally administered in untreated patients with essential hypertension with mean systolic blood pressure ≤ 159 mmHg and diastolic blood pressure ≤ 99 mmHg [11].The absence of hypotensive effect of stevioside in clinical studies using the oral administration has been attributed to a low gastrointestinal absorption of the compound [12]. However, it is not possible to underestimate the efficiency of stevioside in reducing the arterial blood pressure during intravenous administration [7, 13, 14, 15 and 16]. Since the hypotensive effect produced by stevioside depends on its action in peripheral vessels [13] reducing the Ca^{++} influx [17], and considering that the intravenous administration of agents that block Ca^{++} channels is useful in clinical emergency [18], the current study was undertaken to verify the influence of L-NOARG and ODQ on the reduction of vascular tension produced by stevioside in aortic rings preparations of rats pre-contracted by norepinephrine or KCl.

2- Material and Methods

2.1- Isolated Aortic Rings

Rats were killed by decapitation after intraperitoneal injection of sodium thiopental (36 mg/Kg) and urethane (600 mg/Kg). A medial laparotomy was then performed immediately to excise the thoracic aorta. The vessel was sectioned into 3-mm rings and gently dissected free of fat and connective tissue. Rings were then mounted into 30-ml organ baths filled with oxygenated (95% O_2, 5% CO_2) Krebs buffer (in mmol/L: NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; EDTA 0.03, and glucose, 11) at 37 ºC, and the pH of the solution was maintained at 7.4. The Ethic Committee of the State University of Maringá approved the procedures used in the current study.

The preparation was connected to strain gauges (Grass, FT 03) and isometric tension was recorded on the computer equipped with Chart Software of Powerlab (AD Instruments, Pty, Ltd., Castle Hill, NSW, Australia). After being mounted, the rings were allowed to stabilize for 2 h. The preparations were then stretched gradually to an optimal 1g resting tension. The presence of intact endothelium was considered when the addition of acetylcholine (1.0 μM) to the bath was able to produce 100 % relaxation of the preparations pre-contracted by norepinephrine (0.01 μM). When the experiments were performed in the preparation without endothelium, this was removed by gentle rubbing. The successful removal of the endothelium was confirmed by the failure of 1.0 μM acetylcholine to relax the rings pre-contracted by norepinephrine (0.01 μM). After the resting tension was stabilized, KCl (20 mM) or norepinephrine (0.01 μM) was administered to the bathing buffer to induce a rapid increase of vascular tone followed by a stable vasoconstriction (tonic contraction). Norepinephrine was the drug chosen to produce pre-contraction of vessels because it is naturally produced in the organism and, in contrast to epinephrine; it has higher affinity for alpha-adrenoceptors [19]. The concentration of norepinephrine used was that proposed in the literature as able to produce about 50% of maximal tension in isolated aortic rings preparations of rats deprived of the endothelium [20]. The concentration of KCl used was that proposed in the literature as able to produce contraction of the aortic rings preparations without, however, impairing the action of agents considered as possible K^+ channel openers [21], as such mechanism of action and/or the blockade of Ca^{++} channels might explain the vessel relaxation induced by stevioside [17]. The lowest concentration of stevioside able

to produce a reduction of vascular tonus induced by KCl or norepinephrine was researched and then added to the bath. The moment when stevioside produced its maximal relaxing effect was also set (10 min after its addition to the bath) and used for all the experiments. When the assays were performed with association of drugs, the different agents were administered to the bath 20 min before stevioside addition. The tension recorded at t = 10 min was taken as the percentage of that obtained after addition of KCl or norepinephrine to the bath (control). Concentrations of ODQ (10 μM) [22] and L-NOARG (0.1 mM) [23], proposed in the literature as able to block the guanylate cyclase or NOS, respectively, were used in the current studies. Data were submitted to ANOVA, followed by the Bonferroni test with the level of significance set at $P< 0.05$.

2.2- Stevioside and others agents

Standardized stevioside (Fig. 1) was obtained and purified (100%) according to Dacome et al. [24]. Other agents, N^G-nitro L-arginine (L-NOARG), 1H-[1, 2, 4] oxidiazolo

[4, 3-a] quinoxalin-1-one (ODQ), norepinephrine and urethane were purchased from Sigma (St. Louis, MO, USA) and sodium thiopental from Cristália (SP, Brazil).

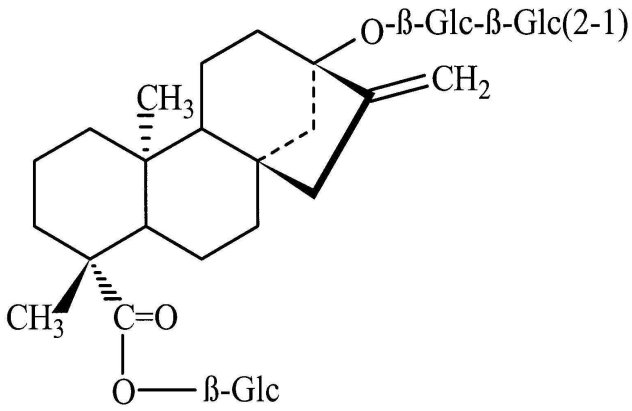

Fig.1 The chemical structure of stevioside

3- Results

The lowest concentration of stevioside able to reduce the increase of tension produced by 0.01 μM norepinephrine was 1 mM in preparations with or without intact endothelium (Fig.**2**). In contrast, the lowest concentrations of the sweetener able to reduce the increase of tension produced by 20 mM KCl were 0.01 mM and 1 mM in preparations with and without intact endothelium, respectively (Fig **3**). The percentage of increase recorded with L-NOARG (0.1 mM) or ODQ (10 μM) on tension produced by 0.01 μM norepinephrine was similar in preparations with or without intact endothelium (Fig. **2**). On the other hand, L-NOARG or ODQ increased the tension produced by 20 mM KCl in preparations with intact endothelium, but did not modify the values of tension produced by 20 mM KCl in preparations without intact endothelium (Fig. **3**). L-NOARG (0.1 mM) and ODQ (10 μM) antagonized the reduction on vascular tonus induced by stevioside in the intact-endothelium aortic rings preparations of rats pre-contracted by norepinephrine or KCl, but such agents did not modify the reduction on vascular tonus induced by stevioside in the denuded aortic rings preparations of rats pre-contracted by norepinephrine or KCl (Fig. **3**).

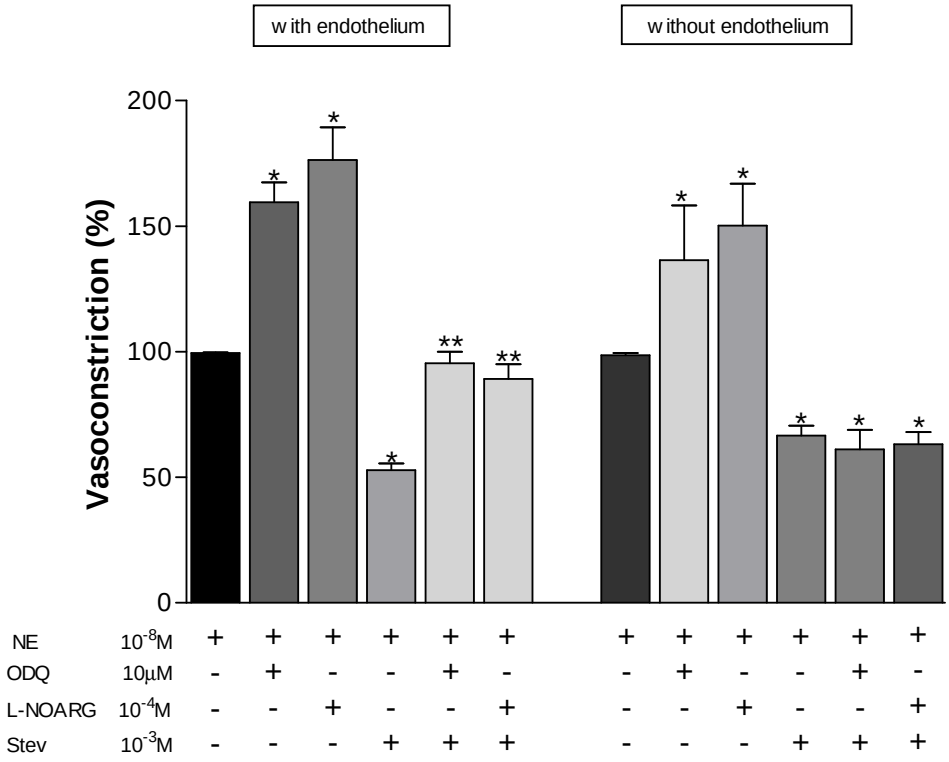

Fig. 2 Effects produced by administration of stevioside (Stev) alone or combined with L-NOARG or ODQ in the aortic rings preparations of rats with and without intact endothelium pre-contracted by norepinephrine (NE). The increment by L-NOARG and ODQ of the pre-contraction produced by NE is also shown. Heights of columns indicate percentage of vasoconstriction (mean ± SEM) of 6 to 8 experiments. * $P<0.05$ different of control (NE alone) taken as 100 %. ** $P<0.05$ different of vasoconstriction induced by combined administration of NE and Stev (ANOVA; Bonferroni post hoc test).

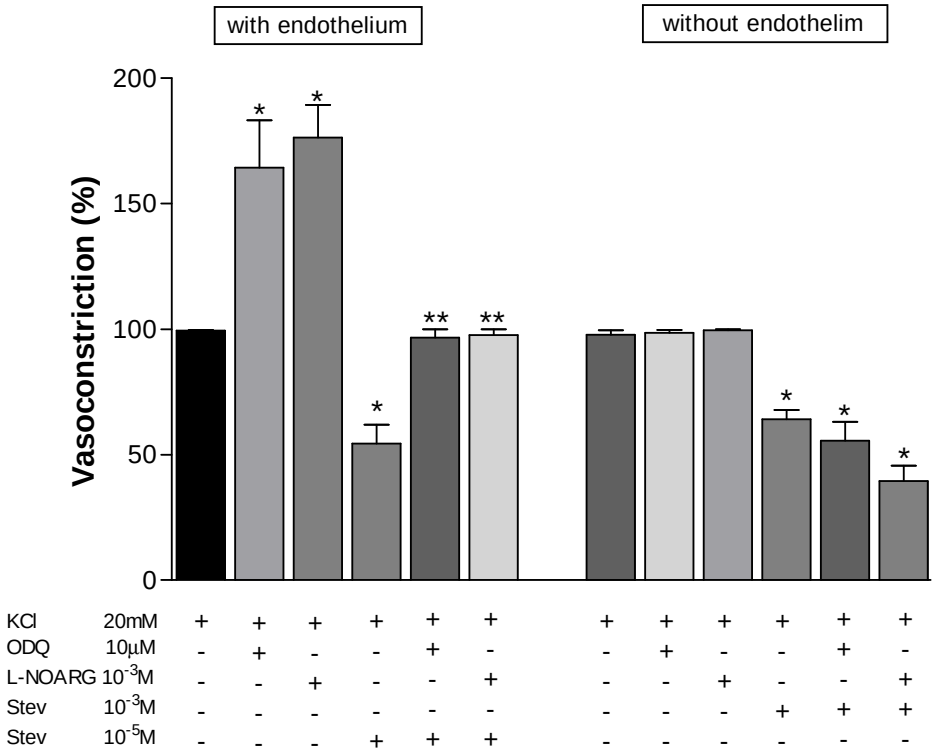

Fig. 3 Effects produced by administration of stevioside (Stev) alone or combined with L-NOARG or ODQ in the aortic rings preparations of rats with and without intact endothelium pre-contracted by KCl. The increment by L-NOARG and ODQ of the pre-contraction produced by KCl in preparations with intact endothelium is also shown. Heights of columns indicate percentage of vasoconstriction (mean ± SEM) of 8 to 10 experiments. * P<0.05 different of control (KCl alone) taken as 100 %. ** P<0.05 different of vasoconstriction induced by combined administration of KCl and Stev (ANOVA; Bonferroni post hoc test).

4- Discussion

Stevioside reduces the vascular tonus in the aortic rings preparations of rats pre-contracted by norepinephrine or KCl (0.01 µM and 20mM). Since the reduction on vascular tonus induced by stevioside was observed in preparations with and without endothelium pre-contracted by norepinephrine or KCl, the current study confirms that the vessel relaxing effect of stevioside does not depend on an intact endothelium [17]. Nevertheless, it was verified that the treatment of preparations with L-NOARG or ODQ antagonized the vessel relaxing effect of stevioside in the endothelium-intact aortic rings preparations of rats pre-contracted by norepinephrine or KCl, but such treatment was ineffective to change the effect of the sweetener in denuded aortic rings preparations of rats pre-contracted by norepinephrine or KCl. Although such data are partially according to Lee et al (2001), that showed that methylene blue, another blocker of guanylate cyclase, did not antagonize the vessel relaxation induced by stevioside in the denuded aortic rings preparations of rats pre-contracted by norepinephrine (0.01 µM) or 40mM KCl, they show for the first time that the reduction of vascular tonus induced by stevioside is strongly influenced by the L-Arg-NOS-GC-cGMP pathway activity in vascular tissues. It is unlikely that the antagonisms by L-NOARG or ODQ of vasodilatation induced by stevioside had been determined by inhibition of the metabolism by NOS of the sweetener or by reduction of the activation of the GC by stevioside, as NOS is an enzyme highly stereospecific for the L isomer of arginine [25] and GC is sensitive to agents that contain Fe^{+3} in their molecules [26]. It is also unlikely that the antagonism by L-NOARG or ODQ of vasodilatation induced by stevioside in preparations with intact endothelium had only been produced by strong vasoconstriction induced by combined administration of L-NOARG or ODQ with norepinephrine or KCl, as such intense vasoconstriction induced by combined administration of L-NOARG or ODQ with norepinephrine was not able to impair the vasodilatation induced by stevioside in denuded aortic rings preparations. It has been shown that NOS exist in endothelium and arterial smooth muscle of rats [27], and that the vasoconstriction induced by norepinephrine may be depressed by NO released through stimulation of alpha- adrenoceptors [27, 28]. Thus, the increment

of tension produced by L-NOARG or ODQ of vasoconstriction induced by norepinephrine in preparations with or without intact endothelium might be determined by reduction of synthesis of NO by L-NOARG or decrease of the guanylate cyclase activity by ODQ, determining a reduction of the inhibitory action of the gas and/or cGMP on vasoconstriction mediated by alpha-adrenoceptors. On the other hand, it was observed that the increment of vasoconstriction induced by norepinephrine was similar in preparations with and without intact endothelium. These observations indicate that the activation of alpha-adrenoceptors by norepinephrine stimulates the synthesis of NO in endothelial and muscular cells. In contrast, the cellular depolarization by 20 mM KCl seems to be able only of activating NOS in endothelial cells, as was recorded that the vasoconstriction induced by KCl was solely incremented by L- NOARG or ODQ in preparations with intact endothelium. Additionally, it was verified that the lowest concentration of stevioside able of reducing the increase of tension induced by KCl in preparations with intact endothelium was lower than that found in aortic rings preparations pre-contracted by norepinephrine. These results indicate that the NO synthesized in the endothelial cells has synergic action with stevioside when the preparations are depolarized by 20 mM KCl. Such synergism of action between NO synthesized in endothelial cells and stevioside might be the main determinant factor of the lowest concentration of stevioside to be lower in preparations with intact endothelium than in preparations without it pre-contracted by KCl. Such synergism might also have occurred in preparations with intact endothelium pre-contracted by norepinephrine, since L-NOARG and ODQ antagonized the reduction of vascular tension induced by stevioside in such experimental conditions. However, it is possible that such synergism had been masked by production of NO and cGMP in arterial smooth muscle, as there were not differences between the increase of tension induced by norepinephrine in preparations with and without endothelium.

Several insights have been proposed to explain the cellular mechanism by which NO acts on membrane ion channels to produce smooth muscle relaxation. There is data indicating that NO activates K^+ channels determining membrane hyperpolarization, but the inhibition of voltage-dependent Ca^{++} current is another

mechanism of action proposed of NO-induced vasodilatation [4]. NO may also inhibit Ca^{++} - channel activity via cGMP [3,4, 29] , but recently it has been proposed that the vasodilatation induced by NO might be supplemented by an ability of the gas to interfere with agonist-induced activation of Cl^{-} current, thereby interfering with depolarization and preventing muscular contraction [30]. Thus, it is possible to suppose that such mechanisms of actions of NO combined or alone, might be those determinants of synergism found between NO and stevioside in preparations with intact endothelium, as stevioside is an agent that blocks Ca^{++} channels on vascular tissues to produce arterial vasodilatation [17].

As a whole, the data indicate the vascular effect produced by stevioside is not dependent of an intact endothelium, but its action of reducing the vascular Ca^{++} influx [17] is strongly influenced by NO synthesized in vascular endothelium.

Acknowledgements

We are grateful to Prof. Dr. M. C. Salgado for suggestions about agents used for pre-contraction of the aortic ring preparation. We are also grateful to Prof. Dr. S. C. Costa for the generous gift of pure stevioside, and Mrs. I. L. Santos for technical support. This work was supported by CNPq (Grant number 400075/2002-0).

References

[1]Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-76.

[2]Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Natl. Acad. Sci. 1987; 84: 9265-69.

[3]Schmidt HHHW, Pollock JS, Nakane M, Forstermann U, Murad F. Ca^{2+}-calmodulin-regulated nitric oxide synthases. Cell Calcium 1992; 13: 427-34.

[4]Fagni L, Bockaert J. Effects of nitric oxide on glutamate-gated channels and other ionic channels. J Chem Neuroanat 1996;10: 231-40.

[5]Boulton CL, Southam E, Gartwaite J. Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. Neurosci 1995; 69: 699-703.

[6]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: 590-99.

[7]Chan P, Xu DJ, Liu JC, Chen YJ, Tomlinson B, Huang WP, et al. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. Life Sci 1998; 63: 1679-84

[8]Hsieh MH, Chan P, Sue YM, Liu JC, Liang TH, Huang TY, et al. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clin Ther 2003; 25: 2797-808

[9]Chan P, Tomlinson B, Chen YJ, Liu JC, Hsieh MH, Cheng JT. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br. Med. J. Clin. Pharmacol**.** 2000; 50: 215-20.

[10]Hsu YH, Liu JC, Kao PF, Lee CN, Chen YJ, Hsieh MH, et al. Antihypertensive effect of stevioside in different strains of hypertensive rats. Chin Méd J 2002; 65: 1-6.

[11]Ferri LA, Alves- Do-Prado W, Yamada SS, Gazola S, Batista MR, Bazotte RB.Investigation of the antihypertensive effect of oral crude stevioside in patients with mild essential hypertension. Phytother Res 2006;[Epub ahead of print]

[12]Geuns JMC, Augustijins P, Mols R, Buyse JG, Bert D. Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. Food Chem Toxicol 2003; 41: 1599-607.

[13]Liu JC, Kao PF, Chan P, Hsu YH, Hou CC, Lien GS, et al. Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. Pharmacology 2003; 67: 14-20.

[14]Melis MS. Stevioside effect on renal function of normal and hypertensive rats. J Ethnopharmacol 1992; 36: 213-17.

[15]Melis MS, Sainati AR. Participation of prostaglandins in the effect of stevioside on rat renal function and arterial pressure. Braz J Med Biol Res 1991a; 24: 1269-76.

[16]Melis MS, Sainati AR. Effect of calcium and verapamil on renal function of rats during treatment with stevioside. J Ethnopharmacol 1991b; 33: 257-62.

[17]Lee CN, Wong KL, Liu JC, Chen YJ, Cheng JT, Chan P. Inhibitory effect of stevioside on calcium influx to produce antihypertension. Planta Med 2001; 67: 796-99.

[18]Levy JH. Management of systemic and pulmonary hypertension. Tex Heart Inst J. 2005; 32(4): 467-71.

[19] Westfall TC, Westfall DP. Adrenergic agonists and Antagonists. In: Goodman & Gilman, editors. The Pharmacological Basis of Therapeutics. New York: MacGrawHill; 2006: 237-95.

[20]Testai L, Silvio C, Ammar B, Luisa P, Vincenzo C, Martinotti E. Vasorelaxant effects of the cloroformic crude extract of *Bupleurm fruticosum* L. (Umbelliferae) roots on rat thoracic aorta. J ethnopharmacol 2005; 96: 93-7.

[21]Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995; 268: C799-C822.

[22]Crauwels HM, Van Hove CE, Herman AG, Bult H. Heterogeneity in relaxation mechanisms in the carotid and the femoral artery of the mouse. Eur J Pharmacol 2000; 404: 341-51.

[23]Moore PK, al-Swayeh OA, Chong NW, Evans RA, Gibson A. L-NG-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitod of endothelium-dependent vasodilatation in vitro. Br J Pharmacol 1990; 99(2): 408-12.

[24]Dacome AS, Silva CC, Costa CEM, Fontana JD, Adelman J, Costa SC. Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni: Isolation and quantitative distribution by chromatographic spectroscopic, and electrophoretic methods. Process Biochem 2005; 40: 3587-94.

[25]Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988; 333: 664-66.

[26]Martin W, Villani GM, Jothianandan D, Furchgot RF. Blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation of rabbit aorta by certain ferrous hemoproteins. Pharmacol Exp Ther. 1985; 233(3): 679-85.

[27]Gurdal H., Can A.,Ugur M. The role of nitric oxide synthase in reduced vasocontractile responsiveness induced by prolonged alpha1-adrenergic receptor stimulation in rat thoracic aorta. Br J Pharmacol. 2005; 145(2): 203-10.

[28]Kaneko K, Sunano S. Involvement of α-adrenoreceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. Eur J Pharmacol 1993; 240:195-200.

[29]Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43: 109- 42.

[30]Lamb FS, Barna TJ. The endothelium modulates the contribution of chloride currents to norepinephrine-induced vascular contraction. Am J Physiol 1998; 275:H161-8.

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo