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Acute administration of nicotine impairs the hypotensive responses to bradykinin in rats

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Abstract

Nicotine may contribute to smoking-induced endothelial dysfunction because of its ability to impair endothelium-dependent vasodilatation. We investigated whether the acute administration of nicotine changes the hypotensive responses to bradykinin in rats. The effects of pre-treatment with losartan or enalapril on the nicotine-induced changes in the responses to bradykinin were also evaluated. In study 1, anesthetized rats were cannulated via carotid artery for the measurement of mean arterial pressure. Dose–response curves to bradykinin (0.1, 0.4, 1.6, 6.4, 25 and 100 μg/kg) were generated before and 10 min after the injection of nicotine (200 μg/kg, i.v.) or saline. The individual dose–response curves were fitted to a four-parameter logistic equation using the ALLFIT program, which provided an estimate of the maximal response (E_{max}) and of the dose of bradykinin producing the half-maximal response (ED_{50}). In study 2, rats were pre-treated orally with losartan (10 mg/kg/day) or enalapril maleate (25 mg/kg/day) for 2 weeks. Control rats received tap water alone. After pre-treatment, the rats were anesthetized and used as described in study 1. Nicotine decreased the E_{max} (from 73.0 ± 7.5 to 65.7 ± 3.3 mm Hg; $P < 0.05$) but did not affect the ED_{50}. In study 2, losartan or enalapril did not affect nicotine-induced decrease in responses to bradykinin; E_{max} decreased in both groups (from 68.7 ± 6.3 to 62.8 ± 4.2 mm Hg, and from 53.8 ± 13.0 to 43.1 ± 7.1 mm Hg, respectively; $P < 0.05$) without significantly changing the ED_{50}. These results suggest that nicotine impairs the endothelium-dependent hypotensive responses to bradykinin. This effect is not influenced by inhibition of the angiotensin-converting enzyme or by blockade of the angiotensin AT_1 receptors.

Keywords: Blood pressure; Bradykinin; Endothelial dysfunction; Enalapril; Losartan; Nicotine

1. Introduction

Smoking accelerates atherosclerosis and increases the risk of cardiovascular diseases and sudden death (Joseph et al., 1996; Lakier, 1992; Jonas et al., 1992). It is not clear which compounds of cigarette smoke contribute to the pathogenesis of cardiovascular diseases. Nicotine apparently has a major role because it impairs endothelium-dependent vasodilatation (Mayhan and Patel, 1997), regulates the production of growth factors by endothelial cells (Cucina et al., 1999) and may influence smoking-induced endothelial dysfunction (Moreno et al., 1998; Chalon et al., 1999) and atherosclerosis (Jonas et al., 1992).

Bradykinin acts via specific receptors (bradykinin B_1 and B_2 receptors) (Regoli et al., 1998). The bradykinin B_1 receptors are mainly expressed under pathologic conditions. The bradykinin B_2 receptors, however, are thought to mediate most of the effects of bradykinin, including vasodilation. Specifically, bradykinin stimulates bradykinin B_2 receptors on endothelial cells causing the release of vasodilators such as nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) (Linz et al., 1999). Thus, intravenous (i.v.) injections of bradykinin produce transient decreases in arterial pressure. In this study, we investigated the influence of an acute administration of nicotine on the hypotensive responses to bradykinin in rats. Since the renin–angiotensin–aldosterone system is activated in smokers (Laustiola et al., 1988; Sugiyama et al., 1988) and inhibition of this system attenuates the impairment of endothelium-dependent relaxation caused by cigarette smoke (Ota et al., 1997) we also assessed

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whether pre-treatment with losartan, an angiotensin II AT_1 receptor antagonist, or enalapril, an angiotensin-converting enzyme inhibitor, affected the nicotine-induced changes in the hypotensive responses to bradykinin.

2. Material and methods

2.1. Study 1: effect of nicotine on the hypotensive responses to bradykinin

2.1.1. General procedures

Male Wistar rats (250–300 g) provided by CEMIB-UNICAMP were anesthetized with sodium pentobarbital (Hypnol, Cristália, SP, Brazil; 40 mg/kg, i.p.). Controlled ventilation with a Harvard ventilator for small animals (model 683) was initiated after cannulation of the trachea with a Gelco tube. The right carotid artery and left femoral vein were cannulated for the measurement of arterial blood pressure and drug administration, respectively. The arterial catheter was connected to a COBE transducer (Arvada, CO), and the signal was amplified with a GP4A-general purpose amplifier (Stemtech, MacLab/PowerLab, Milford, MA). The amplifier outputs were connected to an A/D board and this to a computer loaded with CODAS data acquisition software (AT-CODAS; DATAQ Instruments, Akron, OH). The pulsatile arterial pressure was recorded continuously at a sample rate of 200 Hz throughout the experiment. The experiments were initiated after at least 20 min of stabilization.

2.1.2. Drug administration

All drugs were dissolved in saline and were given in a 100 μl i.v. bolus, then washed in with a further 100 μl of saline. All rats received saline followed by bradykinin at doses of 0.1, 0.4, 1.6, 6.4, 25 and 100 μg/kg (Van Den Buuse and Kerkhoff, 1991; Tanus-Santos et al., 2000a). Each dose of bradykinin was given when the arterial pressure had returned to baseline after the previous injection (usually 2–7 min). Forty-five minutes after the last dose of bradykinin, rats received nicotine (200 μg/kg i.v., Cruz and Vidrio, 1997; Tanus-Santos et al., 2000b) or saline. Ten minutes after nicotine (or saline) injection, the rats received saline followed by the same doses of bradykinin described above.

2.2. Study 2: effect of nicotine on the hypotensive responses to bradykinin in rats pre-treated with losartan or enalapril

2.2.1. Pre-treatment with losartan or enalapril and blood pressure measurements

Male Wistar rats (250–300 g) provided by CEMIB-UNICAMP were maintained on a 12 h light/dark cycle at a

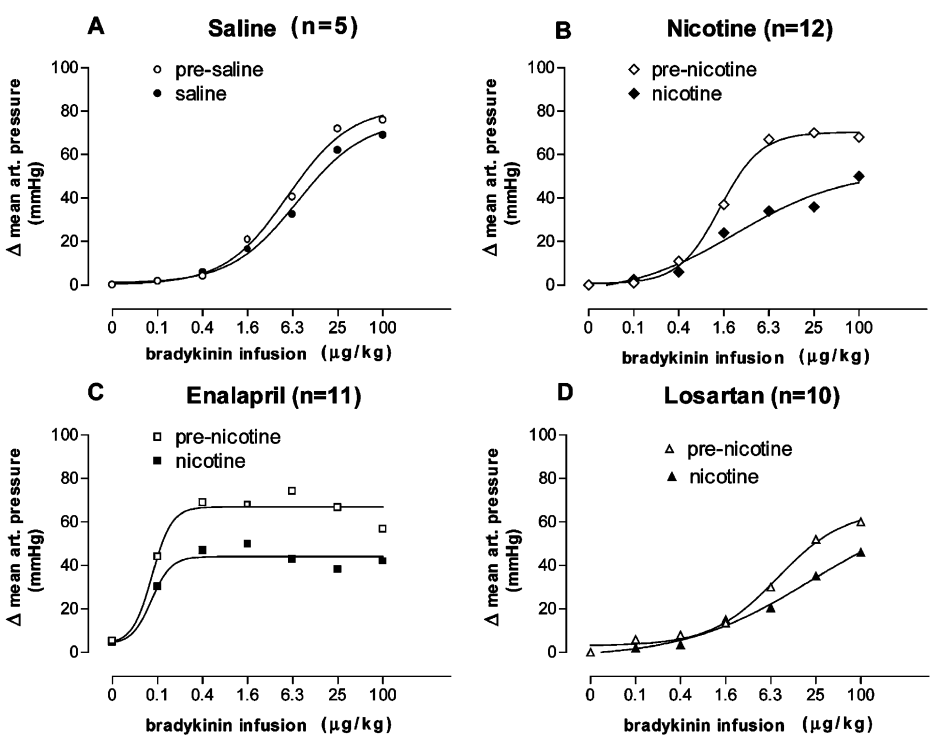

Fig. 1. Effects of saline (A) and nicotine (200 μg/kg, B) on bradykinin-induced hypotension in anesthetized rats. The administration of nicotine decreased the maximal response (E_{max}) to bradykinin without significantly changing the ED_{50} (B). Similarly, nicotine decreased the E_{max} to bradykinin in rats pre-treated with oral enalapril (25 mg/kg/day; C) or losartan (10 mg/kg/day; D) but did not change the ED_{50} values. A 45-min interval was allowed between curves generated before and after the intravenous administration of nicotine. The data shown are the results from representative experiments in each group. A total of 5–12 experiments were done per group.

room temperature (22–25°C) with free access to standard rat chow and water for 2 weeks for the measurement of blood pressure by the tail-cuff method. Thereafter, two groups of rats were treated for two additional weeks either with losartan (10 mg/kg/day; Rodrigo et al., 1997; Tanus-Santos et al., 2000a) or enalapril maleate (25 mg/kg/day; Moreno et al., 1995; Tanus-Santos et al., 2000a) administered via the drinking water. Another group of rats that received tap water served as a control.

2.2.2. Bradykinin and nicotine administrations

Rats pre-treated with losartan or enalapril were anesthetized and underwent the same general procedures described in Study 1. After an injection of saline, the hypotensive responses to bradykinin (0.1, 0.4, 1.6, 6.4, 25 and 100 μg/kg) were registered before and after administration of the same dose of nicotine (or saline) described in Study 1.

2.3. Drugs

Bradykinin and nicotine were purchased from Sigma (St. Louis, MO, USA). Losartan and enalapril maleate were provided by Biosintetica (São Paulo, SP, Brazil).

2.4. Statistical analysis

The results were expressed as means ± S.E.M. as appropriate. The changes in mean arterial pressure were calculated as the difference between the baseline value and those recorded at the lowest values of mean arterial pressure after each dose of bradykinin. Individual dose–response curves to bradykinin generated in each experiment were fitted to a four-parameter logistic equation using the ALLFIT software (Mackay, 1988). This iterative curve-fitting program provides an estimate of the maximal response (E_{max}) and of the dose producing the half-maximal response (ED_{50}). The differences in E_{max} and in ED_{50} values were calculated as the difference between the value "after" and the value "before" nicotine (or saline). The statistical analysis of differences was done by a two-way ANOVA (one way: no pretretment, losartan or enalapril; second way: saline or nicotine). If a significant F value was obtained, the Fisher's test was used to assess specific differences between groups. A probability (P) value < 0.05 was considered the minimum level for statistical significance.

3. Results

3.1. Study 1: effect of nicotine on the hypotensive responses to bradykinin

Bradykinin injections induced dose-dependent transient hypotensive responses (Fig. 1A) that were not affected by treatment with saline, as shown by the unaltered E_{max} and ED_{50} values (Table 1). The administration of nicotine decreased the maximal response to bradykinin (Fig. 1B) without significantly changing the ED_{50} (Table 1). The dose of nicotine used in this study produced only transient increases in arterial blood pressure. The mean arterial pressure returned to basal levels 8 to 10 minutes after nicotine injection (data not shown).

3.2. Study 2:

3.2.1. Effects of pre-treatment with losartan or enalapril

There were no significant differences in the baseline systolic blood pressure among the experimental groups (Table 2). A mild but significant reduction in the tail cuff pressure was observed in the first week of pre-treatment with losartan or enalapril ($P < 0.05$) but not in control rats (Table 2). The pre-treatment with enalapril reduced E_{max} ($P < 0.05$) by 25% and ED_{50} ($P < 0.05$) from 4.3 to 0.2 μg/kg.

3.2.2. Effect of nicotine on the hypotensive responses to bradykinin in rats pre-treated with losartan or enalapril

Bradykinin injections induced dose-dependent hypotensive responses in rats pre-treated with losartan or enalapril. These responses were not affected by saline, as revealed

Table 1
Maximal response (E_{max}, mm Hg) and the dose of bradykinin producing a half-maximal response (ED_{50}) to bradykinin in the first and second studies

Study 1

	Acute treatment with			
	Saline ($n = 5$)		Nicotine ($n = 12$)	
	Before	After	Before	After
E_{max} (mm Hg)	80.5 ± 5.3	76.4 ± 3.3	73 ± 7.5	65.7 ± 3.3[a]
ED_{50} (μg/kg)	4.3 ± 0.9	5.5 ± 2.6	3.1 ± 1.5	2.4 ± 0.9

Study 2

Two-week pre-treatment with losartan (10 mg / kg / day)

	Acute treatment with			
	Saline ($n = 5$)		Nicotine ($n = 10$)	
	Before	After	Before	After
E_{max} (mm Hg)	79.8 ± 14.4	76.2 ± 19.4	68.7 ± 6.3	62.8 ± 4.2[a]
ED_{50} (μg/kg)	4.2 ± 1.3	4.5 ± 0.9	4.2 ± 2.7	4.5 ± 2.5

Two-week pre-treatment with enalapril (25 mg / kg / day)

	Acute treatment with			
	Saline ($n = 5$)		Nicotine ($n = 11$)	
	Before	After	Before	After
E_{max} (mm Hg)	60.7 ± 12.0	64.0 ± 9.6	53.8 ± 13.0	43.1 ± 7.1[a]
ED_{50} (μg/kg)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.9

Values are the mean ± S.E.M.
[a] $P < 0.05$ value before nicotine vs. the respective value after nicotine.

Table 2
Effect of the treatment with losartan (10 mg/kg/day) or enalapril maleate (25 mg/kg/day) on the systolic blood pressure (SBP) of male rats. Control rats received tap water

Group	SBP (mm Hg)
Control (n = 17)	
Basal	111 ± 5
1-week treatment	109 ± 2
2-week treatment	108 ± 4
Losartan (n = 15)	
Basal	115 ± 3
1-week treatment	107 ± 5[a]
2-week treatment	112 ± 2
Enalapril (n = 16)	
Basal	113 ± 5
1-week treatment	106 ± 6[a]
2-week treatment	110 ± 6

Values are the mean ± S.E.M.
[a] $P < 0.05$ versus the respective basal values.

by the unaltered E_{max} and ED_{50} values in both groups (Table 1). Pre-treatment with enalapril potentiated the hypotensive responses to bradykinin compared with the other groups (Fig. 1C, Table 1). The administration of nicotine, however, decreased the maximal response to bradykinin in rats pre-treated with losartan ($P < 0.05$) or enalapril ($P < 0.05$) but did not significantly change the ED_{50} values (Fig. 1C and D; Table 1).

4. Discussion

The present study demonstrates that the acute administration of nicotine reduces the hypotension induced by bradykinin, an endothelium-dependent vasodilator. Impairment of the response to bradykinin was not affected by treatment with enalapril or losartan.

Several investigations have shown that smoking is associated with endothelial dysfunction (Heitzer et al., 1996a; Heitzer et al., 1996b; Jacobs et al., 1993; McVeigh et al., 1996) Although the precise mechanisms involved in such dysfunction are not well understood, the decreased vascular response to endothelium-dependent vasodilator substances strongly suggests that smoke impairs the functional integrity of the endothelium (Moreno et al., 1998). In this regard, nicotine apparently has a major role in the cardiovascular effects of smoking because it interacts with endothelium (Macklin et al., 1998; Villablanca, 1998) to decrease endothelium-dependent vasodilatation (Mayhan and Patel, 1997; Chalon et al., 2000). The decreased response to bradykinin seen after nicotine administration agrees with a previous study showing comparably blunted hypotensive responses to bradykinin after the acute administration of nicotine (Türker et al., 1982). Other studies have also shown that exposure to nicotine (Mayhan and Sharpe, 1998) or to a smoke extract (Ota et al., 1997; Rubenstein et al., 1991) impairs endothelium-dependent relaxation. Whether nicotine is the only component in cigarette of smoke that impairs endothelial function remains to be elucidated.

The mechanism by which nicotine impairs vascular reactivity or the responses to bradykinin is controversial. Bradykinin causes vasodilation by acting on bradykinin B_2 receptors on endothelial cells, thereby stimulating the release of prostacyclin, nitric oxide and EDHF (Linz et al., 1999; Regoli et al., 1998). Thus, nicotine may have decreased the response to bradykinin by impairing the bradykinin-stimulated release of, or response to, vasodilator substances. Indeed, the incubation of cultured human endothelial cells with a cigarette smoke extract decreased the production of prostacyclin (Reinders et al., 1986). Furthermore, nicotine markedly inhibited the release of nitric oxide in response to acetylcholine and ADP in hamsters (Mayhan et al., 1999). Interestingly, decreased basal nitric oxide activity occurs in smokers and is at least partly responsible for the impaired endothelium-dependent relaxation in these subjects (McVeigh et al., 1996; Su et al., 1998; Higman et al., 1996; Kiowski et al., 1994). Exogenous L-arginine restores the impaired bradykinin-induced venorelaxation in smokers, thus strengthening the suggestion that impaired endothelium-dependent relaxation in these subjects is likely attributable to a disturbance of the L-arginine/nitric oxide pathway (Moreno et al., 1998). Alternatively, exposure to nicotine may increase the production of oxygen-derived free radicals, which could scavenge nitric oxide and contribute to impaired endothelium-dependent vasodilatation (Mayhan and Sharpe, 1998; Mayhan et al., 1999).

Pre-treating rats with enalapril or losartan did not attenuate the blunted response to bradykinin observed with nicotine injection. In contrast to this result, captopril attenuated the impairment of endothelium-dependent relaxations caused by a cigarette smoke extract (Ota et al., 1997). This discrepancy may reflect (1) the lack of a sulfhydryl moiety in enalapril, which could scavenge oxygen-derived free radicals (Goldschmidt and Tallarida, 1991), (2) the presence of components other than nicotine in the cigarette smoke extract, and (3) differences in the animal models used. Although angiotensin converting enzyme inhibitors improve endothelial function in hypertension (Clozel et al., 1990), atherosclerotic coronary artery disease (Mancini et al., 1996), and even in smokers (Chalon et al., 1999), our results suggest that enalapril cannot restore the nicotine-induced attenuation of the response to bradykinin. Also, in the present study the pre-treatment with enalapril significantly reduced E_{max} and ED_{50}. This effect of enalapril is probably a consequence of the inhibition of angiotensin-converting enzyme, which is also the enzyme responsible for metabolizing and inactivating bradykinin.

The renin–angiotensin–aldosterone system is activated in smokers (Laustiola et al., 1988; Sugiyama et al., 1988).

Since increased levels of angiotensin II may cause endothelial dysfunction (Mombouli and Vanhoutte, 1999), which can be reduced by blocking angiotensin II AT_1 receptors (De las Heras et al., 1999), we hypothesized that angiotensin II could be involved in nicotine-induced endothelial dysfunction. However, pre-treatment with losartan had no effect on the nicotine-induced decrease in the hypotensive responses to bradykinin, suggesting that nicotine attenuates the responses to bradykinin through a mechanism not involving angiotensin II AT_1 receptors. Finally, we do not know to which extent the use of anesthesia and artificial ventilation may have affected the responses to bradykinin.

In conclusion, our results suggest that the administration of nicotine impairs endothelium-dependent hypotensive responses to bradykinin. This acute effect of nicotine is not influenced by inhibition of angiotensin converting enzyme or by the blockade of angiotensin II AT_1 receptors.

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