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Endothelin ET_A receptor antagonism attenuates the pressor effects of nicotine in rats

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Abstract

The increased endothelin-1 levels observed after smoking may result from nicotine-stimulated endothelin-1 production by endothelial cells. In this study, we investigated the effects of selective endothelin ET_A receptors antagonist Cycle D-*a*-aspartyl-L-prolyl-D-isoleucyl-D-tryptophyl (JKC 301) and of endothelin ET_B receptors antagonist *N-cis*-2,6-dimethylpiperidino-carbonyl-L-gamma-methyl-leucyl-D-L-*m* ethoxycarbonyl-tryptophanyl-norleucine (BQ 788) on the changes in mean arterial pressure, heart rate, and plasma thromboxane B_2 (the stable product of thromboxane A_2) levels caused by increasing doses of nicotine (0.6, 2, 6, and 20 μmol/kg) in anesthetised rats. Nicotine (0.6, 2, and 6 μmol/kg) significantly increased the mean arterial pressure in control and BQ 788-pretreated rats, while only a nicotine dose of 2 μmol/kg) increased the mean arterial pressure in JKC 301-pretreated animals. There were no differences in the nicotine-induced changes in heart rate or in the increases in thromboxane B_2 levels among the groups treated with saline, JKC 301 and BQ 788. These results demonstrate that whereas the antagonism of endothelin ET_A receptors attenuated the increase in blood pressure after nicotine injections, endothelin ET_B receptor antagonism had no such effect. In addition, the antagonism of endothelin ET_A or ET_B receptors did not affect thromboxane A_2 production after nicotine administration. These findings suggest that endothelin-1 may have a role in the acute effects of nicotine.

Keywords: Blood pressure; Endothelin receptor antagonist; Nicotine; (Rat); Thromboxane

1. Introduction

Smoking is an important cause of cardiovascular morbidity and mortality. Although some studies have shown a smoking-induced endothelial dysfunction (Moreno et al., 1998; Chalon et al., 1999), it is not clear which compounds of cigarette smoke contribute to the pathogenesis of vascular disease. Apparently, nicotine has a major role in the cardiovascular effects of smoking because it impairs the endothelium-dependent vasodilatation (Mayhan and Patel, 1997), regulates the production of growth factors by endothelial cells (Cucina et al. 1999) and may thus contribute to the atherosclerosis associated with smoking (Jonas et al.,1992).

Increased arterial blood pressure, heart rate and endothelin-1 levels were observed in healthy smokers after smoking high-tar cigarettes (Haak et al., 1994; Goerre et al., 1995), suggesting that endothelin-1, a potent vasoconstrictor peptide with mitogenic activity (Levin, 1995), may be involved in this condition. In support of this hypothesis, nicotine-stimulated endothelin production by cultured human endothelial cells was maximal after 5 min of exposure to this drug (Lee and Wright, 1999). The vasoconstriction caused by endothelin-1 is mediated by two types of receptors (ET_A and ET_B) located on vascular smooth muscle cells (Sumner et al., 1992). In addition, endothelin ET_B receptors expressed by endothelial cells mediate vasodilatation through the release of nitric oxide and prostacyclin (De Nucci et al., 1988).

In this study, we investigated the effects of endothelin ET_A and ET_B receptor antagonism on the changes in arterial pressure and heart rate caused by nicotine in rats. In addition, since increased urinary excretion of thromboxane B_2 (the stable breakdown product of thromboxane A_2) has been described after cigarette smoking (Benowitz et al., 1993), we also measured the serum concentrations of thromboxane B_2 to assess the effects of selective endothe-

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lin receptor antagonism on the production of thromboxane A_2 after nicotine administration.

2. Material and methods

2.1. Arterial blood pressure measurements

Male Wistar rats (250–280 g) provided by CEMIB–UNICAMP were anesthetized with sodium pentobarbital (Hypnol®, 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), and the signal was amplified with a GP4A general purpose amplifier (Stemtech). 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). The pulsatile arterial pressure was continuously recorded at a sample rate of 200 Hz throughout the experiment. The experiments were initiated after at least 15 min of stabilization, at which point the mean arterial pressure was generally 100–130 mm Hg. Heart rate was obtained by the number of beats in a 2-s interval before and during the peak values of mean arterial pressure after each dose of nicotine.

2.2. Experimental design

All drugs were dissolved in saline and were administered in a 100-μl intravenous (i.v.) bolus, and then washed in with a further 100 μl of saline. Rats were randomly assigned to one of three experimental groups: control group ($n = 10$), which received saline; group ET_A ($n = 9$), which received the selective endothelin ET_A receptor antagonist cycle D-*a*-aspartyl-L-prolyl-D-isoleucyl-D-tryptophy (JKC 301, 200 nmol/kg); and group ET_B ($n = 9$), which received the selective endothelin ET_B receptor antagonist *N-cis*-2,6-dimethylpiperidino-carbonyl-L-gamma-methyl-leucyl-D-L-*m* ethoxycarbonyl-tryptophanyl-norleucine (BQ 788, 200 nmol/kg). This equimolar dose of JKC 301 and BQ 788 was chosen because it significantly inhibits endothelin ET_A (Cahill et al., 1998) and endothelin ET_B (Gratton et al., 1997) receptors, respectively. Five minutes after the injection of saline (or endothelin receptor antagonists), a saline injection was followed by nicotine in doses of 0.6, 2, 6 and 20 μmol/kg, i.v. (Dominiak et al., 1985). These doses would lead to circulating levels of nicotine comparable to those achieved after smoking 1, 3, 10, and 30 cigarettes, respectively (Benowitz and Jacob, 1984). Each dose of nicotine was given when the mean arterial pressure had returned to baseline after the previous injection (usually 5–8 min). Nicotine, JKC 301, and BQ 788 were purchased from Sigma (St. Louis, MO, USA).

Table 1
Baseline mean arterial pressure (MAP) and heart rate (HR) in the control, group ET_A, and ET_B groups
Values are the mean ± S.E.M.

	Control	Group ET_A	Group ET_B
MAP (mm Hg)	120 ± 6	119 ± 5	113 ± 4
HR (bpm)	396 ± 11	408 ± 11	388 ± 14
N	10	9	9

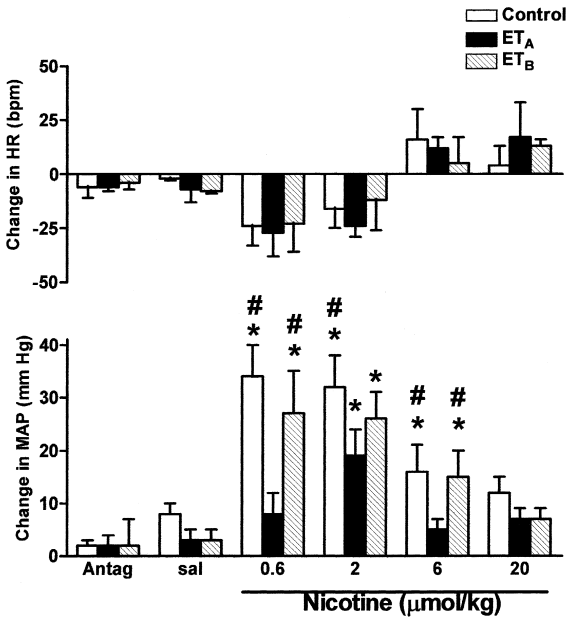

Fig. 1. Change in mean arterial pressure (MAP) and heart rate (HR) 5 min after the injection of saline (control, $n = 10$), the selective endothelin ET_A receptor antagonist JKC 301 (200 nmol/kg, ET_A, $n = 9$), or the selective endothelin ET_B receptor antagonist BQ 788 (200 nmol/kg, ET_B, $n = 9$) (Antag), and after the injection of saline (sal) or nicotine (0.6, 2, 6 and 20 μmol/kg) in anesthetized rats. Values are the mean + S.E.M. $^{*}P < 0.05$ versus saline (sal). $\#P < 0.05$ vs. the ET_A group.

2.3. Plasma thromboxane B_2 determinations

Carotid arterial blood samples were collected in tubes containing EDTA at baseline and after the administration of nicotine. The plasma was separated by centrifugation and stored at −20°C until assayed. Plasma samples were extracted using C_{18} reverse-phase cartridges (Waters, Milford, MA, USA) and the thromboxane B_2 levels were determined using a commercial enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA).

2.4. Statistical analysis

The results were expressed as means + S.E.M. The changes in mean arterial pressure and heart rate were calculated as the difference between the baseline value and those recorded at the highest values of mean arterial

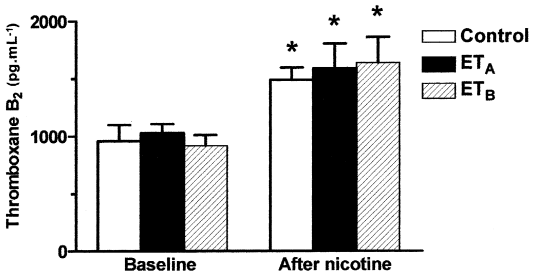

Fig. 2. Serum thromboxane B_2 concentrations at baseline and after the last nicotine administration in rats pretreated with saline (control, $n = 10$), the selective endothelin ET_A receptor antagonist JKC 301 (200 nmol/kg, ET_A, $n = 9$), or the selective endothelin ET_B receptor antagonist BQ 788 (200 nmol/kg, ET_B, $n = 9$). Values are the mean + S.E.M. $^*P < 0.05$ vs. corresponding baseline values.

pressure after each dose of nicotine. The changes from baseline values within each group were analyzed using one-way analysis of variance (ANOVA) for repeated measures followed by the Student–Newman–Keuls test. Comparisons between groups were analyzed using one-way ANOVA followed by the Student–Newman–Keuls test. A probability value < 0.05 was considered the minimum level for statistical significance.

3. Results

3.1. Mean arterial pressure and heart rate

There were no significant differences in the baseline mean arterial pressure and heart rate among the experimental groups (Table 1). Treatment with saline, JKC 301, or BQ 788 did not change the mean arterial pressure (Fig. 1). Nicotine (0.6 and 2 μmol/kg) increased the mean arterial pressure by 26% and 28%, respectively, in the control group; similar effects were observed in group ET_B. Lower pressor responses were observed after a higher dose (6 μmol/kg) of nicotine and there was no significant change in the mean arterial pressure of the control and ET_B groups with 20 μmol nicotine/kg (Fig. 1). The mean arterial pressure of rats pretreated with the selective endothelin ET_A receptor antagonist JKC 301 increased only in response to a nicotine dose of 2 μmol/kg (Fig. 1). There were no significant differences in the heart rate changes among groups after the nicotine doses.

3.2. Plasma thromboxane B_2 levels

Nicotine induced similar increases in the thromboxane B_2 levels in the three experimental groups (Fig. 2).

4. Discussion

Our results demonstrate that whereas the antagonism of endothelin ET_A receptors attenuated the increase in blood pressure after intravenous nicotine in rats, endothelin ET_B receptor antagonism had no effect on the pressor responses to nicotine. The antagonism of endothelin ET_A or ET_B receptors did not affect the thromboxane A_2 production stimulated by nicotine.

The acute pressor effects of nicotine observed here in anesthetized rats were similar to those previously reported (Dominiak et al., 1985). As part of its complex cardiovascular effects, nicotine increases heart rate and blood pressure by stimulating the central nervous system and the autonomic ganglia, and by causing the release of norepinephrine from sympathetic nerve endings and of epinephrine from the adrenal medulla (Taylor, 1990; Marano et al., 1999). The decreasing pressor effect and the lack of a significant change in heart rate after consecutive doses of nicotine strongly suggest the occurrence of tachyphylaxis, an expected finding in rats treated with pentobarbital (Cruz et al., 1994).

Although we did not measure the levels of endothelin-1 in the present study, the attenuation or blockade of the increase in mean arterial pressure after nicotine injections in the ET_A group suggests that endothelin-1, by acting on endothelin ET_A receptors, has a role in the pressor effect of nicotine in rats. In support of this hypothesis, increased blood pressure and enhanced levels of endothelin-1 were observed after the smoking of high-tar cigarettes (Haak et al., 1994; Goerre et al., 1995). In addition to its potent vasoconstrictor effect, endothelin-1 has central actions (Ouchi et al., 1989) and may also potentiate the peripheral actions of the sympathetic nervous system (Gray and Webb, 1996), thereby contributing to the pressor effects of catecholamines released by nicotine. In this regard, the cardiovascular effects of nicotine have been attributed to neurally released norepinephrine which, in turn, activates vascular α-adrenergic receptors (Marano et al., 1999). Since endothelin-1 potentiates this postjunctional action of norepinephrine (Wong-Dusting et al., 1991), we believe that the antagonism of endothelin ET_A receptors may have attenuated the contribution of endothelin-1 to the pressor actions of nicotine.

The antagonism of endothelin ET_A receptors did not affect the heart rate responses to nicotine. This finding may be explained by the probable lack of influence of endothelin-1 on heart rate, at least in rats (Beyer et al., 1994). Nicotine-induced sympathetic stimulation increases the cardiac output (Benowitz, 1988) and causes alfa-adrenergically-mediated vasoconstriction (Marano et al., 1999), thereby increasing the mean arterial pressure through effects taking place in the heart and peripheral vasculature. The lower increases in mean arterial pressure without remarkable differences in heart rate after nicotine injections in the ET_A group suggest that the antagonism of endothelin ET_A receptors in the peripheral vasculature could account for the attenuation of the nicotine-induced pressor responses. Our data, however, do not allow a detailed analysis of the mechanisms involved in the pressor effects of nicotine.

Smoking and the use of nicotine increase the formation of thromboxane A_2 (Wennmalm et al., 1991; Benowitz et al., 1993; Riutta et al., 1995), another potent vasoconstrictor substance. The mechanisms by which smoking and nicotine increase eicosanoid production are not clear, although the release of epinephrine after cigarette smoking (Cryer et al., 1976) or nicotine administration (Alanko et al., 1992) may be involved. Our results show increased thromboxane A_2 production (measured as thromboxane B_2) after nicotine confirmed previous findings in humans (Riutta et al., 1995). The antagonism of endothelin ET_A or ET_B receptors, however, did not affect the increase in thromboxane B_2 levels. Although endothelin-1 stimulates the release of thromboxane A_2 (Hyslop and de Nucci, 1992), and this eicosanoid may mediate the vasoconstrictor effects of endothelin-1 (Reynolds and Mok, 1990), our results suggest that nicotine-induced thromboxane A_2 production does not depend on endothelin-1.

In conclusion, the blockade of endothelin ET_A receptors attenuated the pressor effects of nicotine in rats, without affecting thromboxane A_2 production. This finding suggests that endothelin-1 may have a role in the acute hemodynamic effects of nicotine inhaled in cigarette smoke.

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