Sympathectomy inhibits the vasoactive effects of nicotine in conscious rats

Giuseppe Marano a,*, Agustin Ramirez b, Ileana Mori b, Alberto U. Ferrari h, c

a Laboratorio di Farmacologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy
b Centro di Fisiologia Clinica e Ipertensione, Cattedra di Cardiologia Medica, Università di Milano; Ospedale Maggiore, and CNR, Milano, Italy
c Divisione di Cardiopulmonologia, Ospedale di Seregno, Az. Osp. Civ., Vimercate (MI), Italy

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Abstract

Objective: The mechanisms underlying the pressor response to nicotine are incompletely understood. Although sympatho-adrenergic activation plays a major role, the relative contribution of adrenal vs. neurally released catecholamines and the possible role of non-adrenergic factors (e.g. vasopressin release) is not established. Methods: We examined the cardiovascular responses to graded i.v. injections of nicotine (1 to 100 μg kg−1) in conscious Wistar–Kyoto rats under control conditions and (i) after chemical sympathectomy by 6-hydroxydopamine, which destroys sympathetic endings but spares the adrenal medulla; (ii) after an α-adrenergic blockade by phenoxybenzamine; (iii) after a V_{1a} vasopressin receptor blockade by a specific antagonist. Results: In control rats, nicotine caused a dose-dependent tachycardiac and pressor response. Both responses were abolished by sympathectomy, whereas the α-blockade left the tachycardiac response unaffected but inhibited the pressor response; the V_{1a} vasopressin receptor blockade had no effect on either the tachycardiac or pressor response. Conclusions: We conclude that in the conscious rat: (1) the pressor response to nicotine mainly depends on peripheral α-adrenergically-mediated vasoconstriction; (2) the vasomotor effect is caused by neural rather than adrenomedullary catecholamine release; (3) the nicotine-induced increase in heart rate (and presumably cardiac output) is per se unable to raise blood pressure, and (4) the nicotine-induced release of vasopressin plays no significant role in the pressor response. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cigarette smoking produces a rise in blood pressure associated with an increase in plasma catecholamines and other vasoactive hormones, including vasopressin [1–3]. Although tobacco smoke is a complex mixture of biologically active agents, nicotine is generally believed to be the major factor responsible for the acute hemodynamic effects of cigarette smoking because these effects can be replicated by the intravenous administration of nicotine alone [4–7].

However, the mechanisms responsible for the nicotine-induced blood pressure rise are still incompletely understood. Sympatho-adrenergic activation is unquestionably involved [6,8,9], but the relative contribution of catecholamines released from sympathetic nerve endings vs. those released from the adrenal medulla has not been established. It is also unclear whether the blood pressure rise is mainly determined by peripheral vasoconstriction or that the tachycardiac component of the response (with the subsequent rise in cardiac output) is also essential for the full-blown pressor effect of nicotine to be observed. A further uncertainty relates to the contribution of non-adrenergically-mediated mechanisms to the pressor effects of nicotine, in particular to the possible constrictor effects of the fairly large amounts of vasopressin known to be released following nicotine administration [3–5,8].

The purpose of the present study was to address the above mentioned questions by examining the cardiovascular effects of nicotine injection in conscious unrestrained rats under the conditions of: (1) no intervention (control);
(2) chemical sympathectomy by 6-hydroxydopamine, a model in which post-ganglionic sympathetic nerve endings are destroyed but the adrenal medulla is spared [10]; (3) an acute α-adrenergic receptor blockade by phenoxybenzamine, a to nicotine after pretreatment with the alpha-receptor antagonist, phenoxybenzamine, or with the AVP receptor antagonist, [d(CH₃)₂, Tyr(Me)₂, Tyr(NH₂)₃]-Arg⁵-vasopressin, were also determined in intact animals. Ten min before nicotine administration, phenoxybenzamine or the AVP receptor antagonist were administered at the dose of 1 mg kg⁻¹ i.v. and 20 μg kg⁻¹ i.v., respectively. Effectiveness of the blockade was confirmed by abolition of the pressor response to i.v. phenylephrine, 4 μg kg⁻¹, or vasopressin, 100 ng kg⁻¹, for no less than 3 h.

2. Methods

2.1. Animal preparation and surgery

Rats were housed and taken care of in compliance with the guidelines of the Council of European Communities (86/609/EEC) for the use of laboratory animals. Male Wistar–Kyoto rats (250–300 g) were used. Sympathectomy was obtained as previously described by Ferrari et al. [10]. Briefly, 6-hydroxydopamine, 100 mg kg⁻¹, was administered three times over a 5–7 day period. The first and second doses were given intraperitoneally, whereas the third dose was administered as a slow intravenous infusion. The α-adrenergic antagonist phentolamine, 150 μg kg⁻¹ i.p., was administered with the first dose to protect the animal from the massive release of catecholamines. The effectiveness of sympathectomy was verified at the end of the experiment by showing suppression of the cardiovascular responses to tyramine, 150μg kg⁻¹ i.v.

Each animal was surgically instrumented under ketamine anaesthesia, 100 mg kg⁻¹ i.p.: the femoral artery and vein, usually on the left side, were cannulated by polyethylene catheters for systemic blood pressure measurement and drug administration, respectively. Catheter extensions were tunnelled subcutaneously, exteriorized at the dorsal neck region and kept patent by periodical flushing with heparinized saline solution.

2.2. Experimental protocol

All experiments were performed at least 24 h after the surgical procedures in the conscious unrestrained condition. Arterial blood pressure was measured by connecting the arterial catheter to a Statham pressure transducer (mod. P23) and recorded on a Grass polygraph (mod. 7P). The catheter-transducer system had a flat frequency response up to 30 Hz. Heart rate was measured beat-to-beat by tachographic conversion (mod. 7P4, Grass Instruments) of the blood pressure wave. The effects of nicotine on blood pressure and heart rate were measured in both intact and sympathectomized rats: the alkaloid was administered in separate i.v. bolus doses of 1, 3, 10, 30, and 100 μg kg⁻¹, the sequence of the different doses being randomized, and the time interval between one injection and the following being no less than 20 min. To test whether adrenal catecholamines and/or vasopressin are involved in the acute cardiovascular effects of nicotine, the responses to nicotine after pretreatment with the alpha-receptor antagonist, phenoxybenzamine, or with the AVP receptor antagonist, [d(CH₃)₂, Tyr(Me)₂, Tyr(NH₂)₃]-Arg⁵-vasopressin, were also determined in intact animals. Ten min before nicotine administration, phenoxybenzamine or the AVP receptor antagonist were administered at the dose of 1 mg kg⁻¹ i.v. and 20 μg kg⁻¹ i.v., respectively. Effectiveness of the blockade was confirmed by abolition of the pressor response to i.v. phenylephrine, 4 μg kg⁻¹, or vasopressin, 100 ng kg⁻¹, for no less than 3 h.

2.3. Drugs

The drugs employed in the experimental protocol were ketamine (Parke-Davis), 6-hydroxydopamine, Arg-vasopressin, nicotine, tyramine, phenoxybenzamine, phenotolamine (Sigma Chemical), and [d(CH₃)₂, Tyr(Me)₂, Tyr(NH₂)₃]-Arg⁵-vasopressin (Peninsula Laboratories).

2.4. Data analysis

Changes in mean arterial pressure and in heart rate following injection of the experimental drugs were calculated as the difference between the peak post-injection value and the average value observed in the 2 min preceding the injection. The responses in the different experimental conditions were compared by using Student’s t-test or two-way analysis of variance for repeated measures (groups and treatments). If a significant F value was obtained, the Fisher’s test was used to assess specific differences between groups (STATVIEW 4.02 statistical package). The level of statistical significance was set at 0.05. Data are expressed as mean±standard error.

3. Results

As shown in Table 1, 6-hydroxydopamine was highly effective in producing sympathectomy inasmuch that the pressor and tachycardiac responses to tyramine were reduced by about 90% in the rats pretreated with the

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Pressor and tachycardiac responses to tyramine in 6-hydroxydopamine-treated (symx) and vehicle-treated (intact) rats</td>
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<tr>
<td></td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
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<tr>
<td>Heart rate (beats per min)</td>
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Mean values±S.E. are shown. Data in parentheses refer to the percent reductions in the response observed in Symnx vs. Intact rats. Symnx=sympathectomized rats; Intact=intact rats. * p<0.01 vs. intact rats.
Table 2
Baseline mean arterial pressure and heart rate in intact and sympathectomized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP (mmHg)</th>
<th>HR (beats per min)</th>
</tr>
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<tbody>
<tr>
<td>Intact rats (n=12)</td>
<td>93.0±2.0</td>
<td>335±10</td>
</tr>
<tr>
<td>Sympathectomized rats (n=14)</td>
<td>82.7±1.2*</td>
<td>350±9.0</td>
</tr>
<tr>
<td>Intact rats (phenoxybenzamine pretreatment)</td>
<td>92.6±0.6</td>
<td>340±8.9</td>
</tr>
<tr>
<td>Intact rats (AVP antagonist pretreatment) (n=8)</td>
<td>93.0±1.5</td>
<td>345±11.5</td>
</tr>
</tbody>
</table>

Mean values±S.E. are shown. MAP, mean arterial pressure; HR, heart rate. *p<0.01 vs. intact rats.

neurotoxin compared to those pretreated with vehicle alone, in accordance with Ferrari et al. [11].

Sympathectomy induced a moderate but significant reduction in baseline values of mean arterial pressure, whereas it did not significantly affect heart rate (Table 2). As represented in Fig. 1, left panel, nicotine produced a clear-cut but short-lasting pressor and tachycardiac response. As shown in the average data of Fig. 2, this effect was dose-dependent. The effect plateaued at the dose of 100 μg kg⁻¹, as higher doses were unable to induce larger pressor and tachycardiac responses (data not shown). The cardiovascular effects of nicotine were completely abolished by 6-hydroxydopamine-induced sympathectomy: this is again represented in the right panel of Fig. 1 and in the average group data of Fig. 2. The nicotine-induced increase in arterial blood pressure was also completely abolished by pretreatment with the α-adrenergic receptor blocking agent phenoxybenzamine which on the contrary failed to affect the increase in heart rate (Fig. 2). At variance with the interventions that interfered with adrenergic mechanisms, pretreatment with a V₁ vasopressin receptor antagonist did not modify the responses to nicotine in terms of either the pressor or the tachycardiac effect (Fig. 2). Neither receptor antagonist significantly

Fig. 1. Original recordings showing the effects of nicotine (30 μg kg⁻¹ i.v.) on arterial blood pressure (AP) and heart rate (HR) in one intact (left panel) and one sympathectomized (right panel) conscious rat.

Fig. 2. Dose–response curves for the cardiovascular effects of nicotine obtained in conscious unrestrained rats examined in the control condition (open circles), after sympathectomy (solid triangles) or after pretreatment with either 1 mg kg⁻¹ phenoxybenzamine (solid circles), or 20 μg kg⁻¹ of a vasopressin antagonist (solid squares) on mean arterial pressure (top panel) and heart rate (bottom panel).
affected the baseline values of mean arterial pressure or heart rate (Table 2).

4. Discussion

The major findings in our study are that in unanesthetized, spontaneously-behaving rats (i) chemical sympathectomy completely abolishes the pressor and tachycardiac effects of nicotine, (ii) α-adrenergic receptor blockade abolishes the pressor effect of nicotine without affecting the concomitant tachycardiac effect, and (iii) the blockade of V₁ vasopressin receptors has no effect on either the pressor or the tachycardiac response to nicotine.

Although the concept of sympatho-excitation as the basis of the pressor effect of nicotine is obviously not a new one, we believe the above listed findings represent evidence that was previously lacking and significantly expands our insight into the mechanisms by which nicotine (and presumably cigarette smoking) stimulates the cardiovascular system.

Firstly, the obligatory role of the sympathetic system in the pressor effect of nicotine is documented by a ‘subtractive’ approach. In particular, demonstrating a suppressed response to nicotine in a model in which the influences of sympathetic nerve endings but not of the adrenal medulla are removed, indicates that the former but not the latter, are mostly responsible for the response. Secondly, abolition of the pressor response to nicotine by an α-blocker, which not unexpectedly leaves the tachycardiac response unaffected, suggests that the crucial hemodynamic component of the pressor response to nicotine is an α-adrenergically-mediated vasoconstriction, whereas the tachycardia is not essential and hemodynamically redundant to the vasomotor effect; although this was already suggested by previous experiments in which propranolol was employed to block the cardiac component of the response [12], the latter drug may not be devoid of peripheral constrictor effects due to blockade of vascular β₂-adrenergic receptors, thus potentially confounding the interpretation of the results. Therefore our phenoxybenzamine data, indicating that nicotine-induced tachycardia alone is unable to raise blood pressure, usefully complements the information so far available, and strengthens the notion that the tachycardiac effect of nicotine is not obligatory for the occurrence of the blood pressure rise.

Thirdly, the unmodified cardiovascular response to nicotine after V₁ vasopressin receptor antagonism indicates that also the vasopressin release elicited by nicotine plays no essential role in the hemodynamic effects of the alkaloid.

It is important to set the above mentioned concepts in the context of previously reported data concerning the cardiovascular actions of nicotine.

A first consideration is that our study extends to unanesthetized rats, the findings by Ea-Kim et al. based on their observations in anaesthetized guinea pigs, in which adrenalectomy failed to affect the pressor effect of nicotine [12]. On the other hand, our demonstration that the sympathetic system is essential for the pressor effect of nicotine to occur may also help interpret the intriguing observation by Grassi et al. [13] that cigarette smoking is followed by a reduction, rather than an increase, in muscle sympathetic nerve activity: we believe that a reasonable, although admittedly speculative explanation, is that nicotine (smoking) does act via a sympatho-excitatory effect in humans as well as in rats, and that this is the basis of its pressor and pressor action. However, the net change in sympathetic activity results from the opposite direct (central?) and reflex effects (via the arterial baroreceptors) and may be different in different territories – the reflex inhibition predominating in the muscle sympathetic outflow while the direct excitation predominating elsewhere, e.g. in the splanchic area.

Finally, the observation that a specific V₁ receptor antagonist had no effect whatsoever on the pressor and tachycardiac response to nicotine may be at variance with previous reports [14]. The reason for the discrepancy is not clear, although it may be that the restraint to which the animals were subjected in the above mentioned experiments [14] may have imposed a certain degree of stress on the animals and interfered with their baseline neurohumoral state. It must also be considered, that in a study of the digital artery responses to nicotine, Wigoda et al. [8] found no relationship between vasoconstriction to nicotine and changes in vasopressin serum levels, which supports, albeit limited to a single regional vascular bed, the concept of the redundancy of vasopressin release in mediating the blood pressure raising effect of nicotine in man.

The mechanisms of the nicotine-induced sympatho-excitation were not addressed by our study and remain to be elucidated. One should remember, however, that previous studies provided evidence for centrally, as well as peripherally-mediated effects of nicotine: for example, Brezenov found that the central cholinergic pathways leading to sympatho-excitation are mediated by muscarinic rather than nicotinic receptors [15]. This would imply that peripheral (ganglionic) activation of nicotinic receptors may be the major factor involved in the cardiovascular effects of nicotine. In contrast, Buccafusco [16] and Kahn [17] reported evidence for the existence of central nicotinic receptors capable of stimulating sympathetic cardiovascular neurons. The matter is made even more complicated by the observation that nicotine may also have direct, non neurally-mediated excitatory effects on the cardiovascular system, as suggested by, for example, the persisting tachycardiac response to cigarette smoking in subjects receiving beta-blocking drugs [18].

In conclusion, our findings in unanesthetized, spontaneously behaving rats support the concept that, at least in the species studied, the cardiovascular effects of nicotine are largely mediated via sympathetic activation, and in
particular via activation of vascular α-adrenergic receptors by neurally released norepinephrine. On the other hand, no essential contribution to the pressor response to nicotine is provided by other known actions of this alkaloid such as the release of catecholamines from the adrenal medulla, cardiac sympathetic stimulation or secretion of vasopressin.

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References