Acute Nicotine Pretreatment Augments Dopaminergic Pulmonary Vasodilation (44318)

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Abstract. **Nicotine use has been associated with augmented dopaminergic neurotransmission within the central nervous system by a number of researchers. We wished to determine if acute nicotine treatment would augment the pulmonary vasodilatory response to dopamine. Male Sprague-Dawley rats were pretreated with either subcutaneous nicotine or equivolume saline and a dose-response curve for dopaminergic pulmonary vasodilation was constructed *ex vivo* in isolated, salt-perfused rat lungs preconstricted with the synthetic thromboxane analogue U-46619. Nicotine pretreatment augmented the vasodilatory response to dopamine at doses falling within the mid range of the dopamine dose-response relationship, and this augmentation was blocked by the nicotinic ganglionic receptor antagonist mecamylamine. In contrast, nicotine did not augment the vasodilatory response produced by either the preferential DA_1-dopaminergic receptor agonist SKF-38393 or the preferential DA_2-dopaminergic receptor agonist quinpirole. Acute nicotine pretreatment did not affect the maximal pulmonary vasodilatory response produced by dopamine. Similarly, nicotine pretreatment failed to augment the vasodilatory response to the β-adrenergic receptor agonists isoproterenol or terbutaline, but significantly diminished the response to dobutamine. Even though acute nicotine pretreatment did not augment β-adrenergic receptor-mediated pulmonary vasodilation, the augmentation of dopaminergic responsiveness was inhibited by prior treatment with propranolol, suggesting β-adrenergic receptor involvement. Finally, nicotine pretreatment did not alter the vasodilation produced by the nitric oxide dependent agents arginine vasopressin or sodium nitroprusside. These data demonstrate that nicotine alters dopaminergic vasodilation in the pulmonary vascular bed.** [P.S.E.B.M. 1998, Vol 219]

Dopamine (DA), depending upon the contractile state of the pulmonary vasculature, may act either as a pulmonary vasoconstrictor or a pulmonary vasodilator. At basal levels of pulmonary vascular tone, dopamine produces a dose-dependent increase in pulmonary arterial pressure (P_{PA}: 1–3), presumably *via* stimulation of pulmonary vascular α-adrenergic receptors (4). In contrast, dopamine administration to the preconstricted pulmonary vasculature is associated with decrements in P_{PA} (5–8), which may be mediated *via* stimulation of vascular β-adrenergic receptors (4) or DA_1-dopaminergic receptors (8).

Although few studies have considered the systemic actions of the combined administration of the two compounds, considerable research has examined the complex relationships between nicotine and dopamine in the mammalian central nervous system (CNS). Nisell *et al.* (9) demonstrated that small subcutaneous doses of nicotine produced an approximate 50% increase in dopamine levels measured within the CNS. This was subsequently confirmed by El-Bizri and Clarke (10) who also demonstrated the specificity of the effect by blocking it with the nicotinic ganglionic receptor antagonist chlorisondamine. Outside the CNS, dopaminergic neurons innervate multiple organs, including the lungs (11). We are aware of no studies examining nicotinic/dopaminergic interactions outside of the CNS. However, it is logical to assume that acute nicotine exposure would augment dopaminergic responses in these organs in a manner similar to that demonstrated within the CNS.

Thus, the goals of the present study were to: 1) examine the vasodilatory response to dopamine in the preconstricted rat pulmonary vasculature; 2) characterize any effects of acute nicotine administration on the vasodilatory response

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to dopamine in the preconstricted rat pulmonary vasculature; and 3) examine the effects of acute nicotine administration on the vasodilatory response to agents unrelated to dopamine in the preconstricted rat pulmonary vasculature.

Methods

All surgical procedures and protocols employed in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the Mercer University School of Medicine. All rats were purchased from Harlan Sprague-Dawley and allowed to acclimate in the animal facility for at least 1 week prior to the beginning of the study.

Isolated Lung Preparation. The surgical procedure for lung isolation has been described previously (12–14). Briefly, animals were anesthetized with pentobarbital sodium (100 mg/kg, i.p.). The trachea was isolated and cannulated with a 17-gauge needle stub. A midsternal incision was made to expose the heart, and heparin (100 U) was injected directly into the right ventricle. The pulmonary artery was cannulated with a 17-gauge needle stub *via* an incision in the right ventricle, and the preparation was quickly connected to a perfusion system containing a physiological saline solution (126 m*M* NaCl, 5.4 m*M* KCl, 0.83 m*M* MgSO, 19 m*M* $NaHCO_3$, 1.8 m*M* $CaCl_2$, 5.5 m*M* glucose, and 4% albumin; w/v). The left ventricle was then cannulated with a 4-mm plastic tube, and the heart and lungs were removed *en bloc*. After removal, the heart and lungs were suspended in a humidified chamber maintained at 38°C and perfused initially at 1.0 ml/min/kg body weight. The perfusion rate was gradually increased to 40 ml/min/kg body weight. Because heparin has considerable vasoactive properties that could confound data interpretation, all blood was carefully washed out of the lung prior to the initiation of perfusate recirculation. Flow rate was normalized to body mass because cardiac output and body weight have been shown to be closely correlated irrespective of the age of the animal (15). The lungs were ventilated at 55 breaths/min (tidal volume approximately 2.5 ml) with a gas mixture containing 5% CO_2 in room air for the duration of the experiment. The gas mixture was humidified prior to entering the trachea. Inspiratory pressure was set at 9 cm H_2O and the end-expiratory pressure at 2 cm H_2O. This ventilatory pattern was sufficient to insure normal oxygenation and acid-base balance of the perfusate, which did not differ between groups (Po_2 = 158.16 ± 4.01 mmHg; Pco_2 = 23.64 ± 0.36 mmHg; pH = 7.37 ± 0.00). All experiments were conducted with the lungs under Zone 2 conditions (arterial pressure > alveolar pressure > venous pressure). Pulmonary arterial and left atrial pressures were measured using a Gould Statham P23ID pressure transducer connected to a Grass model 7D chart recorder (Grass Instrument Co., Quincy, MA). In addition, data were collected digitally and recorded online using a microcomputer-based data acquisition and analysis system (Codas, Dataq Instruments Inc., Columbus, OH) for later analysis of pressure wave forms. After maximum flow was established, lungs were allowed to equilibrate for 20 min prior to any experimental manipulation. Following the 20-min equilibration period, the pulmonary vasculature was preconstricted by adding the synthetic thromboxane analog U-46619 (9,11, dideoxy-9α, 11α-methanoepoxy Prostaglandin $F_{2\alpha}$) in 0.5-μg increments to achieve a maximal stable pressor response between 10 and 15 mmHg that did not differ significantly between groups. U-46619 was chosen as the pressor agent because, unlike the more physiological pressor stimulus hypoxia, it results in consistently stable and long-lasting pressor responses in the isolated perfused rat lung. Following stabilization of the final pressor response, lungs were tested using one of the protocols outlined below. Because of the possibility of tachyphylaxis (16), each lung was studied using only one protocol.

Isolated Lung Protocols. *Dopamine dose-response analysis.* Animals were anesthetized, and the lungs were prepared for study as outlined above. To construct the dopamine dose-response curve, once P_{PA} had increased 10–15 mmHg above baseline and was stable, dopamine (10.0, 25.0, 50.0, 100.0, or 200.0 μg) was given as a single bolus dose into the pulmonary artery to elicit a vasodilatory response. Because of the instability of dopamine solutions, each dose was prepared immediately prior to injection. Each lung received a single dose of dopamine. Percent vasodilation was then calculated as described below.

Effects of acute nicotine pretreatment on dopaminergic pulmonary vasodilation. Thirty minutes prior to sacrifice, each animal was given a single intraperitoneal injection of either nicotine (50.0, 75.0, 100.0, 300.0, or 500.0 μg/kg) or equivolume saline (vehicle control). Thirty minutes later, animals were anesthetized and prepared for study as outlined above. Once a stable pressor response (10–15 mmHg) was achieved in the isolated lung, dopamine (50 μg bolus) was administered *via* the pulmonary artery to elicit vasodilation. Based upon these studies, we used the 100 μg/kg dose of nicotine for all future protocols. Following establishment of an acceptable dose of nicotine, the dopamine dose response curve (*vida supra*) was then repeated in a separate group of animals pretreated with 100 μg/kg nicotine. Because the 50-μg bolus dose of dopamine fell along the midpoint of the dose-response relationship and demonstrated the greatest degree of augmentation following nicotinic pretreatment, this dose was used for all future protocols.

Specificity of nicotinic augmentation of dopaminergic pulmonary vasodilation. To assess the specificity of any augmentation of dopaminergic pulmonary vasodilation seen with acute nicotine pretreatment, a separate group of animals was given a single injection of the nicotinic ganglionic receptor antagonist mecamylamine (3.0 mg/kg) or equivolume saline vehicle intravenously *via* the tail vein 10 min prior to either nicotine (100 μg/kg) or saline pretreatment. Thirty minutes later, the animals were sacrificed, and

the lungs were prepared for study, dopaminergic (50 μg bolus) vasodilation was measured as outlined above.

Dopamine receptor involvement in dopaminergic pulmonary vasodilation. To examine the actions of acute nicotine pretreatment on the vasodilatory responses to DA_1- and DA_2-dopaminergic receptor stimulation, another set of animals was pretreated with either acute nicotine (100 μg/kg) or equivolume saline. Thirty minutes later, animals were anesthetized and prepared for study as described above. Once a stable pressor response (10–15 mmHg) had been achieved, a bolus dose of either DA (50 μg), the DA_1-dopaminergic receptor agonist SKF-38393 (3 m*M*), or the DA_2-dopaminergic receptor agonist quinpirole (6 m*M*) was administered *via* the pulmonary artery. These doses were chosen because previous experiments in our laboratory (data not shown) determined them to produce submaximal vasodilatory responses in isolated lungs. Thus, use of these doses allowed us to detect either increased or decreased responsiveness as a result of nicotine pretreatment.

β-Adrenergic receptor involvement in dopaminergic pulmonary vasodilation. To examine the actions of acute nicotine pretreatment on the vasodilatory responses to agents interacting with pulmonary β-adrenergic receptors, another set of animals was pretreated with either acute nicotine (100 μg/kg) or equivolume saline. Thirty minutes later, animals were anesthetized and prepared for study as described above. Once a stable pressor response (10–15 mmHg) had been achieved, a bolus dose of either isoproterenol (25 ng), the β_1-adrenergic receptor agonist dobutamine (25 μg), or the β_2-adrenergic receptor agonist terbutaline (250 μg) was administered *via* the pulmonary artery. These doses were chosen because previous experiments in our laboratory (data not shown) determined them to produce submaximal vasodilatory responses in isolated lungs. Thus, use of these doses allowed us to detect either increased or decreased responsiveness as a result of nicotine pretreatment. To examine the effect of β-adrenergic receptor antagonism on dopaminergic pulmonary vasodilation, another set of animals was pretreated and prepared for study as outlined above, with the following modification. After lungs had been isolated and prepared for study (but 10 min prior to preconstriction with U-46619) either propranolol (2.0 μg/ml final concentration) or equivolume saline vehicle was added to the perfusate reservoir to block β-adrenergic receptors. Lungs were then preconstricted and, after a stable pressor response (10–15 mmHg) was achieved, a bolus dose of either DA (50 μg), quinpirole (6 m*M*), or isoproterenol (25 ng) was given *via* the pulmonary artery. The resulting vasodilatory response was measured and percent vasodilation calculated as described below.

Endothelial involvement in dopaminergic pulmonary vasodilation. To examine the actions of acute nicotine pretreatment on the vasodilatory responses mediated by the vascular endothelium, a final set of animals was pretreated with either acute nicotine (100 μg/kg) or equivolume saline. Thirty minutes later, animals were anesthetized and prepared for study as described above. Once a stable pressor response (10–15 mmHg) had been achieved, a bolus dose of either the endothelium-dependent vasodilator arginine vasopressin (10 ng) or the endothelium-independent vasodilator sodium nitroprusside (2.5 μg) was administered *via* the pulmonary artery. These doses were chosen because previous experiments in our laboratory (data not shown) determined them to produce submaximal vasodilatory responses in isolated lungs. Thus, use of these doses allowed us to detect either increased or decreased responsiveness as a result of nicotine pretreatment.

Calculations and Statistics. For each treatment protocol, the percentage vasodilation (percentage reversal of U-46619 induced vasoconstriction) was determined by dividing the vasodilatory response by the initial amount of vasoconstriction and multiplying by 100 (17). Data were analyzed by two-way analysis of variance using a *t* test with Bonferroni correction as the *post hoc* analysis for individual comparisons (18). A value of $P < 0.05$ was accepted as statistically significant for all comparisons.

Results

Baseline Hemodynamic Data. Baseline perfusion pressures (P_{PA} following achievement of maximal flow rate but prior to administration of U-46619) ranged from 6.9–9.9 mmHg and did not differ between any of the groups studied (Tables I and II). Any preparation exhibiting a baseline perfusion pressure greater than 10 mmHg was considered

Table I. Baseline Pressures and Maximal Pressor Responses in Lungs Used for Dopamine Dose-Response Curve

Dopamine dose (μg)	Treatment group			
	Saline		Nicotine	
	Baseline pressure (mmHg)	Pressor response (mmHg)	Baseline pressure (mmHg)	Pressor response (mmHg)
10	8.50 ± 0.35	12.63 ± 0.17	8.20 ± 0.37	10.30 ± 0.61
25	8.00 ± 0.25	11.00 ± 0.25	8.50 ± 0.43	11.00 ± 0.78
50	8.42 ± 0.36	10.75 ± 0.22	8.30 ± 0.45	11.90 ± 0.57
100	8.10 ± 0.63	11.70 ± 0.57	8.20 ± 0.32	10.20 ± 0.32
200	8.40 ± 0.36	11.60 ± 0.52	8.10 ± 0.24	10.70 ± 0.23

Note. Values are mean ± SEM of six to eight determinations per group. There are no significant differences.

Table II. Baseline Pressures and Maximal Pressor Responses in Lungs Comparing Different Vasodilators

Pretreatment	Treatment group			
	Saline		Nicotine	
	Baseline pressure (mmHg)	Pressor response (mmHg)	Baseline pressure (mmHg)	Pressor response (mmHg)
No pretreatment				
Dopamine	8.42 ± 0.36	10.75 ± 0.22	8.30 ± 0.45	11.09 ± 0.57
SKF-38393	8.08 ± 0.26	11.67 ± 11.92	7.25 ± 0.49	11.92 ± 0.50
Quinpirole	7.75 ± 0.20	12.42 ± 0.69	7.25 ± 0.28	11.50 ± 0.44
Isoproterenol	7.25 ± 0.47	11.13 ± 0.39	7.10 ± 0.44	10.30 ± 0.47
Terbutaline	9.17 ± 0.22	10.33 ± 0.24	9.67 ± 0.37	11.50 ± 0.66
Dobutamine	7.92 ± 0.19	12.00 ± 0.40	8.08 ± 0.37	10.92 ± 0.37
Sodium Nitroprusside	9.83 ± 0.07	13.08 ± 0.58	9.92 ± 0.06	12.42 ± 0.78
Arginine Vasopressin	8.08 ± 0.47	11.17 ± 0.57	9.42 ± 0.41	11.67 ± 0.48
Propranolol Pretreatment				
Dopamine	7.75 ± 0.27	11.67 ± 0.51	7.92 ± 0.37	11.42 ± 0.31
Quinpirole	7.33 ± 0.30	10.83 ± 0.38	8.17 ± 0.27	11.42 ± 0.54

Note. Values are mean ± SEM of six to eight determinations per group. There are no significant differences.

unreliable and was excluded from further study. Similar to baseline pressure, the maximum pressor response attained following administration of U-46619 was between 10 and 13 mmHg and did not differ between groups (Tables I and II).

Nicotine Dose-Response Relationship. Figure 1 depicts the effects of acute nicotine pretreatment on the pulmonary vasodilatory response to a 50-μg bolus of dopamine. Acute nicotine pretreatment was associated with significantly ($P < 0.05$) augmented responses to dopamine at all nicotine doses tested above 50 μg/kg when compared with saline pretreatment (100%). This effect appeared to plateau with the 300 and 500 μg/kg doses. The specificity of this effect for nicotinic receptor stimulation was demonstrated by administering mecamylamine to animals prior to nicotine pretreatment. Administration of mecamylamine completely abolished the augmented dopaminergic pulmonary vasodilation associated with nicotine pretreatment (Fig. 1).

Dopamine Dose-Response Relationship. Dopamine elicited vasodilatory responses in the pulmonary vasculature in a dose-related manner (Fig. 2). This reached maximal effect at 200 μg. Acute nicotine pretreatment (100 μg/kg) augmented the vasodilatory response to dopamine at doses along the midpoint of the dose-response curve (25 and 50 μg) but did not increase the maximal effect attained with the agent (Fig. 2).

Dopamine Receptor Involvement in Dopaminergic Pulmonary Vasodilation. Figure 3 depicts the effects of either nicotine or saline pretreatment on the va-

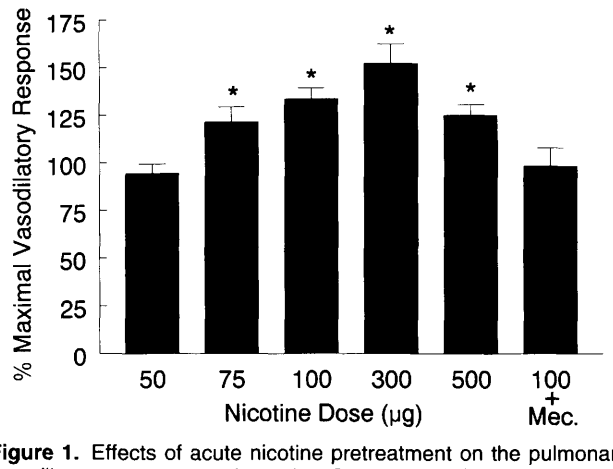

Figure 1. Effects of acute nicotine pretreatment on the pulmonary vasodilatory response to dopamine. Percentage of maximal vasodilatory response (reversal of U-46619-induced vasoconstriction) in isolated-perfused lungs. One hundred percent vasodilation is defined as that produced by dopamine with saline pretreatment. Data are mean ± SEM of six to eight determinations for each bar. * indicates $P < 0.05$ vs. 100%. Mecamylamine is abbreviated as Mec.

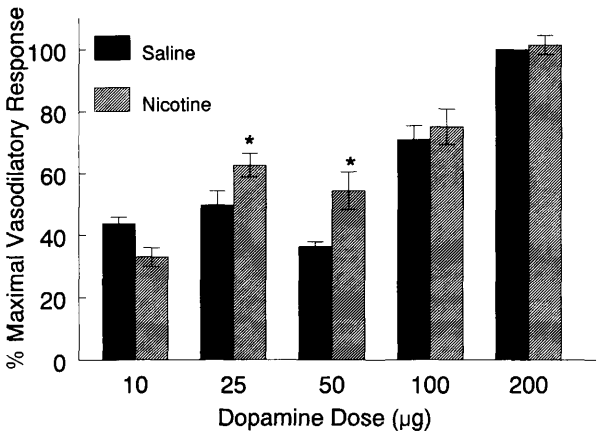

Figure 2. Effects of acute nicotine pretreatment on the dopaminergic pulmonary vasodilation dose-response relationship. Percentage of maximal vasodilatory response (reversal of U-46619-induced vasoconstriction) in isolated-perfused lungs following pretreatment with saline (black bars) or nicotine (100 μg/kg; hatched bars). One hundred percent vasodilation is defined as that produced by 200 μg of dopamine following saline pretreatment. Data are mean ± SEM of six to eight determinations for each bar. * indicates $P < 0.05$ vs. 100%.

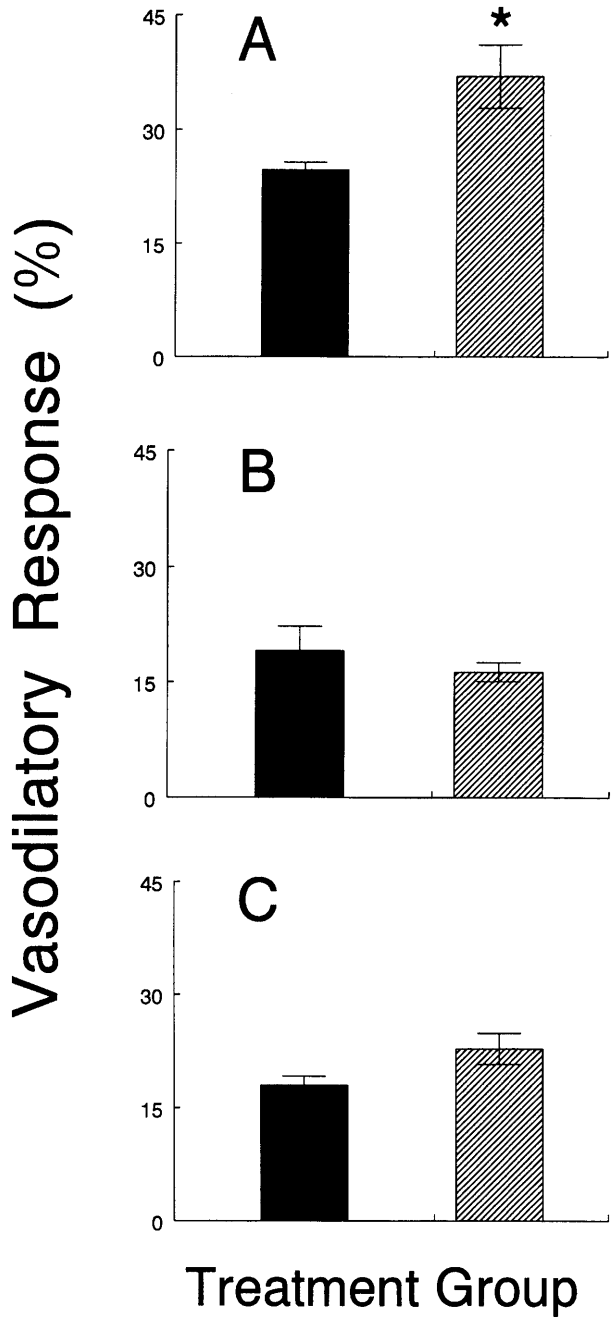

Figure 3. Effects of acute nicotine pretreatment on the pulmonary vasodilatory response (% reversal of U-46619-induced vasoconstriction) to dopamine receptor agonists. Solid bars represent saline pretreatment. Nicotine pretreatment is depicted by hatched bars. Dopamine receptor agonists tested were dopamine (3A), SKF-38393 (3B), and quinpirole (3C). Doses of agonists are explained in the text. Data represent mean ± SEM of six to eight determinations for each bar. * indicates $P < 0.05$ vs. saline controls.

sodilatory responses to dopamine, SKF-38393 and quinpirole. Nicotine pretreatment significantly augmented the vasodilatory response to dopamine (Fig. 3A). In contrast, vasodilatory responses to the preferential DA_1-dopaminergic receptor agonist SKF-38393 (Fig. 3B) and the DA_2-dopaminergic receptor agonist quinpirole (Fig. 3C) were unaffected by prior nicotine administration.

Beta-Adrenergic Receptor Involvement in Dopaminergic Pulmonary Vasodilation. Figure 4 depicts the effects of either acute nicotine or saline pretreatment on the pulmonary vasodilatory responses to the combined β_1 and β_2 receptor agonist isoproterenol, the β_1 preferential receptor agonist dobutamine, and the β_2 preferential receptor agonist terbutaline. Nicotine pretreatment did not affect the vasodilatory response to ether isoproter-

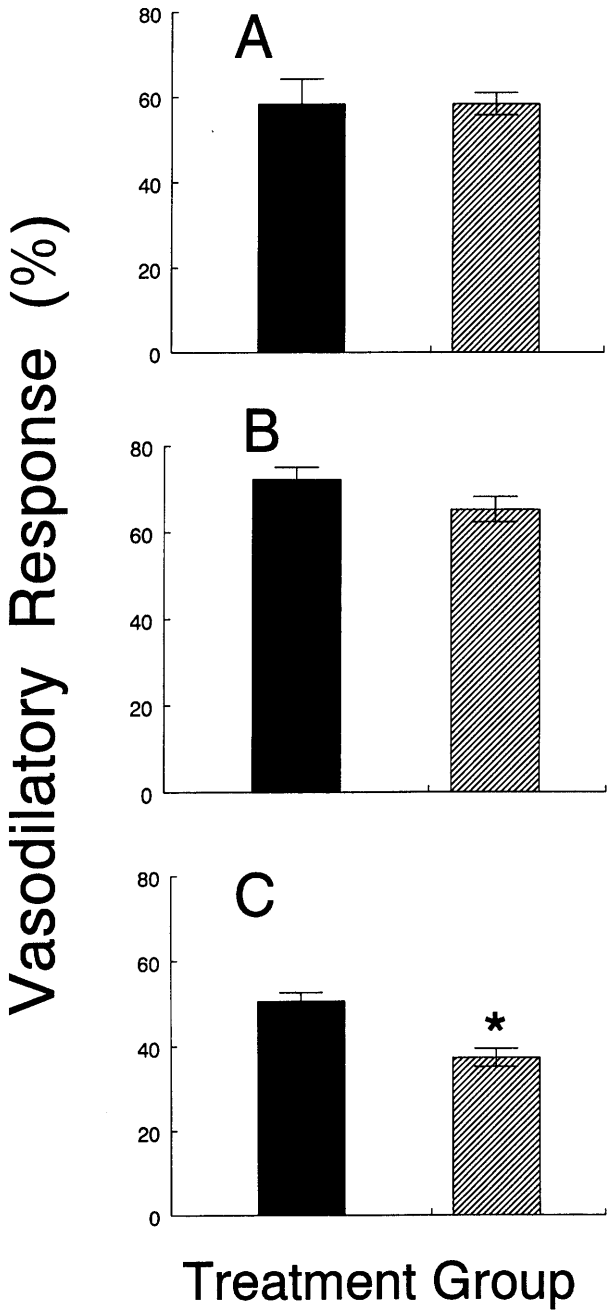

Figure 4. Effects of acute nicotine pretreatment on the pulmonary vasodilatory response (% reversal of U-46619-induced vasoconstriction) to β-adrenergic receptor agonists. Solid bars represent saline pretreatment. Nicotine pretreatment is depicted by hatched bars. β-Adrenergic receptor agonists tested were isoproterenol (4A), terbutaline (4B), and dobutamine (4C). Doses of agonists are explained in the text. Data represent mean ± SEM of six to eight determinations for each bar. * indicates $P < 0.05$ vs. saline controls.

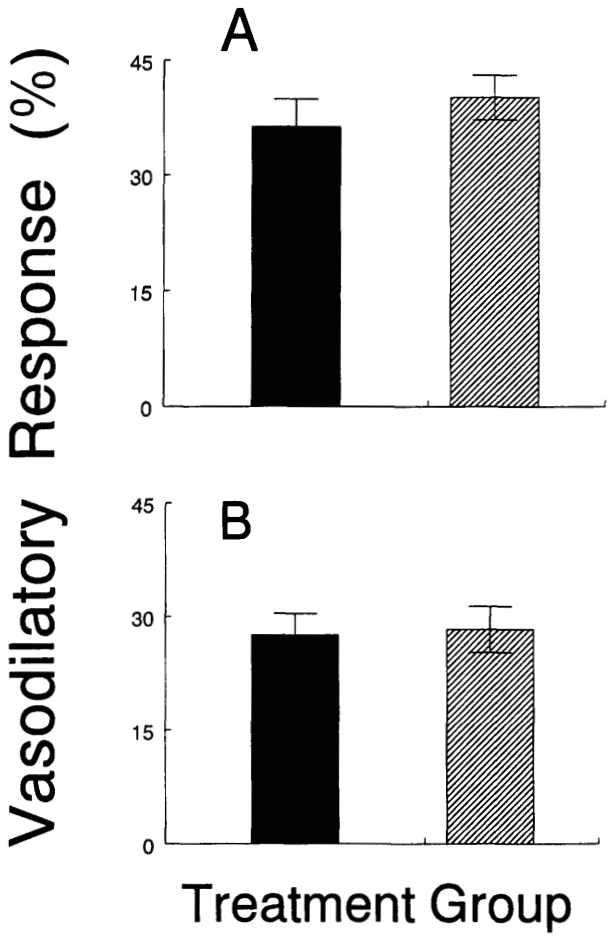

Figure 5. Effects of acute nicotine pretreatment on the pulmonary vasodilatory response (% reversal of U-46619-induced vasoconstriction) to nitric oxide–dependent vasodilators. Solid bars represent saline pretreatment. Nicotine pretreatment is depicted by hatched bars. Vasodilators tested were sodium nitroprusside (5A) and arginine vasopressin (5C). Doses of vasodilators are explained in the text. Data represent mean ± SEM of six to eight determinations for each bar. There are no significant differences.

enol (Fig. 4A) or terbutaline (Fig. 4B). In contrast, acute nicotine pretreatment significantly diminished vasodilatory responses to dobutamine (Fig. 4C).

Endothelial Involvement in Dopaminergic Pulmonary Vasodilation. To assess any contribution of the vascular endothelium in dopaminergic pulmonary vasodilation, vasodilatory responses to the endothelium-dependent vasodilator arginine vasopressin and the nitric oxide donor sodium nitroprusside were measured in lungs obtained from either nicotine- or saline-pretreated animals (Fig. 5). Sodium nitroprusside administration resulted in a 36% reversal of U-46619-induced vasoconstriction, which was not altered by nicotine pretreatment (Fig. 5A). Similarly, arginine vasopressin caused a 28% vasodilatory response, which also was not altered by nicotine pretreatment (Fig. 5B).

Effects of Propranolol on Dopaminergic Pulmonary Vasodilation. Because some evidence suggests that the pulmonary vasodilatory response to dopamine is mediated *via* β-adrenergic receptors, the effects of propranolol on dopaminergic pulmonary vasodilation were assessed. These data are presented in Figure 6. Propranolol pretreatment did not significantly affect the pulmonary vasodilatory response to dopamine when compared to animals receiving saline controls (Fig. 6A). Propranolol pretreatment did, however, abolish any stimulatory effect of nicotine on this vasodilatory response (Fig. 6A vs. Fig. 2, 50-μg dose). In contrast, propranolol did not affect the vasodilatory response to the DA2-dopaminergic agonist quinpirole (Fig. 6B). Finally, propranolol treatment was sufficient to eliminate the pulmonary vasodilatory response to isoproterenol completely (data not shown).

Conclusions

The major findings of this study are: 1) nicotine pretreatment augments dopaminergic pulmonary vasodilation in a dose-dependent manner; 2) this augmentation is present only in the mid-portions of the dose-response curve and does not increase the maximal pulmonary vasodilatory response to dopamine; 3) although the response to dopamine

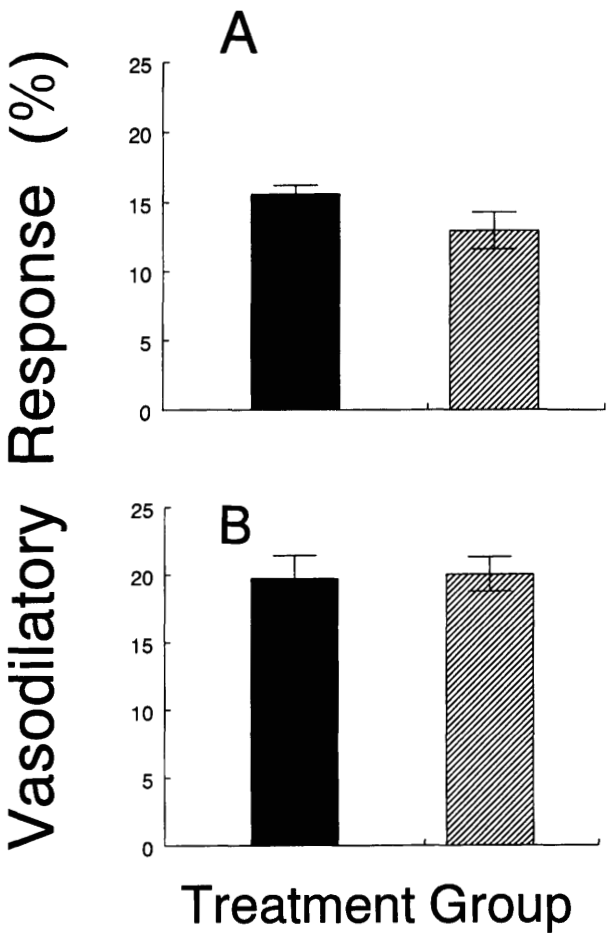

Figure 6. Effects of propranolol and acute nicotine pretreatment on the pulmonary vasodilatory response (% reversal of U-46619-induced vasoconstriction) to dopamine receptor agonists. Solid bars represent saline pretreatment. Nicotine pretreatment is depicted by hatched bars. All preparations were treated with propranolol prior to initiation of vasodilation (see text). Dopamine receptor agonists tested were dopamine (6A) and quinpirole (6B). Doses of agonists are explained in the text. Data represent mean ± SEM of six to eight determinations for each bar. There are no significant differences.

is augmented, we were unable to demonstrate augmented responses to either DA_1 or DA_2-preferential dopaminergic receptor agonists; 4) although this augmentation may be blocked by propranolol, we were unable to demonstrate augmented responses to agents acting primarily on β-adrenergic receptors; and 5) while we cannot rule out an involvement of nitric oxide in this augmentation, acute nicotine pretreatment failed to augment the pulmonary vasodilatory response to either the nitric oxide donor sodium nitroprusside or the endothelium-dependent vasodilator arginine vasopressin.

Numerous recent studies have documented the pulmonary vasodilatory actions of dopamine in rabbits (6), ferrets (5), humans (7, 8) and rats (19, 20). Most research has clearly pointed toward a specific action of dopamine on DA_1-dopaminergic receptors as mediating this phenomenon. Yamauchi *et al.* (7, 8) have reported pulmonary vasodilation in response to DA_1-dopaminergic receptor stimulation in both rabbits and humans. In their reports, this response was effectively blocked by the dopamine receptor antagonists haloperidol and fluphenazine. Similarly, Gorman (5) has reported dopaminergic pulmonary vasodilation in the rat pulmonary circulation, which was inhibited by SCH-23390. In contrast, Hyman *et al.* (4) report attenuation of dopaminergic pulmonary vasodilation following treatment with the β-adrenergic receptor antagonist propranolol. This is in agreement with the present study in which we have found that propranolol administration eliminated the augmentation of dopaminergic pulmonary vasodilation by nicotine and diminished, but did not eliminate, the response to dopamine alone (Fig. 6A). Thus, although DA_1-receptors appear to be responsible for mediating the pulmonary vasodilatory actions of dopamine, involvement of β-adrenergic receptors cannot be ruled out at this point. It is unlikely that our findings are secondary to nonspecific actions of nicotine as they were completely blocked by prior administration of the nicotinic receptor antagonist mecamylamine (Fig. 1).

Our finding that acute nicotine pretreatment augments dopaminergic responses (Figs. 1 and 2) is consistent with other reports in the biomedical literature. The stimulatory effect of acute nicotine administration on dopamine release and/or action has been well established within the CNS. El-Bizri and Clarke (10) have recently documented augmented release of tritiated nicotine from synaptosomal preparations prepared from rat striatum. Subsequently, Nisell *et al.* (9) reported similar actions of nicotine on dopamine release in the rat nucleus accumbens. These authors were able to demonstrate augmented dopamine release in response to nicotine administered either systemically or intracerebrally. In addition to augmenting the release of dopamine, nicotine may also increase synaptic levels of the neurotransmitter by inhibiting the primary mechanism for terminating its action. Two recent reports (21, 22) have demonstrated that nicotine administration, in admittedly high levels, inhibits the reuptake of dopamine into catecholaminergic storage vesicles in a manner similar to that seen with cocaine. Further, Izenwasser and Cox (23) report that this effect is maximal approximately 40 min after injection of nicotine into the animal, a time frame that correlates well with the current study. Although we cannot definitively accept or reject either of these mechanisms, the second seems more likely when our data regarding DA_1- and DA_2-preferential receptor agonists is considered. If acute nicotine pretreatment is augmenting the release of dopamine in the lung, then it would also be expected to augment the responses to these more selective agonists by increasing the total amount of dopamine receptor occupation. However this was not the case in the present study. Acute nicotine pretreatment had no effect on the pulmonary vasodilatory response to the DA_1-preferential agonist SKF-38393 (Fig. 3B) and caused only a slight, nonsignificant increase in the vasodilatory response to the DA_2-preferential agonist quinpirole (Fig. 3C). In contrast, we are aware of no data indicating that the dopamine reuptake mechanism is capable of removing either SKF-38393 or quinpirole from the synapse. Thus, inhibition of dopamine reuptake by nicotine would be expected to produce augmented responses to dopamine but not to either of the more selective agonists, a result that is consistent with our current findings. It is interesting to note that since both of these mechanisms would be consistent with elevated synaptic levels of dopamine without corresponding changes in dopamine receptors, they are both consistent with our findings of increased responsiveness in the mid-portion of the dose-response curve, without an increase in maximal responsiveness. In order for either of these mechanisms to be active in the pulmonary vasculature, dopaminergic innervation of this vascular bed would be required, since a pre-synaptic neuron is necessary for each action. Although we are aware of no reports documenting dopaminergic innervation of rat lungs, Bakhle *et al.* (11) have documented the presence of dopaminergic axons innervating pulmonary resistance vessels in guinea pigs. Thus, it is not unlikely that a presynaptic source of dopamine, which is available for modification by nicotine, exists within the rat lung as well. If rat lungs are innervated by dopaminergic neurons, then either of the two mechanisms outlined above, or possibly both acting in concert, could explain our findings.

Another potential site for nicotine to augment dopaminergic pulmonary vasodilation is *via* augmentation of nitric oxide release. Yamauchi *et al.* (8) have recently documented both endothelial-dependent and endothelial-independent components of dopaminergic pulmonary vasodilation in response to DA_1-dopaminergic receptor stimulation. It is feasible, therefore, that dopaminergic pulmonary vasodilation is augmented not by any receptor-mediated event but rather by augmentation of the production of and/or release of nitric oxide. This is unlikely in the present study for two reasons. First, the vasodilatory response to the preferential DA_1-dopaminergic receptor agonist SKF-38393 was not augmented by prior nicotine treatment (Fig. 3B)

even though DA_1-dopaminergic receptors are thought to be responsible for stimulating pulmonary nitric oxide release. Second, nicotine was unable to augment the responses to arginine vasopressin or sodium nitroprusside, both of which are agents that produce pulmonary vasodilation *via* nitric oxide (Fig. 5). Arginine vasopressin is an endothelium-dependent pulmonary vasodilator that triggers the production and release of nitric oxide following stimulation of pulmonary endothelial V_1-vasopressinergic receptors (13). Thus, if nicotine somehow acted by stimulating the cellular machinery responsible for production of nitric oxide, we would expect similar augmentation with this agent. Similarly, sodium nitroprusside acts as a pulmonary vasodilator by donating nitric oxide to the vascular smooth muscle cells themselves, effectively bypassing the endothelial component of the response. Our finding that nicotine did not augment the response to this agent suggests that it is not secondary to increased cellular production of cyclic guanosine monophosphate in response to nitric oxide. These data, taken in total, strongly suggest that nicotine is not augmenting dopaminergic pulmonary vasodilation *via* a nitroxidergic mechanism.

In summary, the present manuscript has documented a selective increase in dopaminergic pulmonary vasodilation following acute nicotine pretreatment. This finding is consistent with other reports of nicotine augmenting dopaminergic neurotransmission within the CNS, but is the first of which we are aware to document the phenomenon in the peripheral nervous system. Whether this phenomenon is important in regulation of pulmonary vascular tone in health or disease is unclear at this point.

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