Acrolein-induced vasomotor responses of rat aorta


Acrolein-induced vasomotor responses of rat aorta. Am J Physiol Heart Circ Physiol 285: H727–H734, 2003. First published May 1, 2003; 10.1152/ajpheart.00269.2003.—Acrolein is a highly reactive aldehyde pollutant and an endogenous product of lipid peroxidation. Increased generation of, or exposures to, acrolein incites pulmonary and vascular injury. The effects of acrolein on the vasomotor responses of rat aortic rings were studied to understand its mechanism of action. Incubation with acrolein (10–100 µM) alone did not affect the resting tone of aortic vessels; however, a dose-dependent relaxation of phenylephrine-precontracted aortic rings was observed. Acrolein-induced relaxation was slow and time dependent and the extent of relaxation after 100 min of application was 44.7 ± 4.1% (10 µM), 56.0 ± 5.6% (20 µM), 61.0 ± 7.9% (40 µM), and 96.1 ± 2.1 (80 µM), respectively, versus 14.2 ± 3.3% relaxation in the absence of acrolein. Acrolein-induced vasorelaxation was prevented by endothelial denudation and was abolished on pretreatment with the nitric oxide synthase inhibitor Nω-nitro-l-arginine methyl ester, the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-d]quinoxaline-1-one, or the cyclooxygenase inhibitor indomethacin. Inhibition of K⁺ channels (by tetrabutylammonium) or Na⁺-K⁺-ATPase (by ouabain) did not significantly prevent acrolein-mediated vasorelaxation. Exposure to acrolein in the presence or absence of other compounds elicited slow wave vasomotor effects in 77% of aortic vessels versus 1.4% in control. Vasomotor responses were also studied on aortic rings prepared from rats fed 2 mg·kg⁻¹·day⁻¹ acrolein for 3 alternate days by oral gavage. These vessels developed a significantly lower contractile response to phenylephrine compared with controls. Together, these results indicate that acute acrolein exposure evokes delayed vasorelaxation due to a nitric oxide-and prostacyclin-dependent mechanism, whereas in vivo acrolein exposure compromises vessel contractility.

Acrolein, a highly reactive α,β-unsaturated aldehyde, is an environmental and industrial pollutant and toxin present in automobile exhaust, wood smoke, and overheated fat-containing foods. Acrolein is used in the production of acrylic acid and polymers and is widely applied as herbicide against aqueous organisms. Acrolein is also present in cigarette smoke in amounts up to 230 µg per cigarette (21). In addition, acrolein is also a product of lipid peroxidation (18, 45) and is therefore continuously generated in biological systems under oxidative stress. Increased oxidative stress and lipid peroxidation are associated with several degenerative diseases, including atherosclerosis, Alzheimer’s and Parkinson’s disease, stroke, diabetes, and autoimmune diseases. Although several oxidants, such as peroxides and free radicals, could contribute to oxidative injury, accumulating evidence suggests that lipid peroxidation-derived aldehydes such as acrolein, malonaldehyde, and 4-hydroxynonenal (HNE) act as toxic second messengers that further propagate oxidative injury due to increased availability of reactive oxygen species (18).

The acute effects of acrolein have been extensively investigated in several experimental models (4). These studies show that acrolein is a potent respiratory tract and eye irritant and that it instigates asthma-like symptoms during inhalation exposure leading to pulmonary edema and respiratory distress. Cytotoxic effects of acrolein have been demonstrated in pulmonary artery endothelial cells (27, 32), bronchiolar epithelial cells (22), and cardiac fibroblasts (44). The toxic effects of acrolein are mainly attributed to its alkylating properties and high reactivity, resulting from the presence of a conjugated double-bond system (CH₂=CH–CHO). Acrolein is a strong electrophile and shows highest reactivity among other unsaturated aldehydes toward thiol and amino groups (49). Unlike free radicals, which are very short lived, acrolein and related aldehydes are capable of diffusing and interacting with biomolecules at sites distant from the initial event (1). The formation of protein-bound acrolein has been demonstrated in several different pathological tissues, including atherosclerotic plaques (45, 46), neurofibrillary tangles in Alzheimer’s disease (8), and in the diabetic kidney (43). Metabolic studies show that conjugation with glutathione represents an important pathway for the detoxification and elimination of acrolein (10, 24). Low-dose toxicity of acrolein has a distinct effect, although it is also, at least in part, related to the capacity of acrolein to deplete glutathione (5, 29). At high doses acrolein induces cell death by oncrosis; at low doses it inhibits cell proliferation without affecting cell viability (25, 38).

Address for reprint requests and other correspondence: S. E. D’Souza, Dept. of Physiology and Biophysics, Health Sciences Center A-1101, Univ. of Louisville, Louisville, KY 40292 (E-mail: sedsou010@wwise.louisville.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpheart.org 0363-6135/03 $5.00 Copyright © 2003 the American Physiological Society H727
Several studies have implicated acrolein in vascular injury. Acrolein produces cytotoxic effect on pulmonary artery endothelial cells (27, 32) and induces platelet aggregation (40). Alteration of vascular reactivity is considered to be earliest event in vascular injury (37). Lipid peroxidation products, including unsaturated aldehydes, modulate vascular reactivity (28, 30, 35). The present study evaluates the effects of acrolein on vascular tone and reactivity in a rat thoracic aorta preparation. Our results demonstrate that in vitro acrolein induces vasorelaxation of the precontracted vessels and that this effect is mediated via nitric oxide (NO) and prostacyclin production. Vasoconstriction was highly compromised in animals fed with acrolein.

MATERIALS AND METHODS

Reagents and Chemicals

All components of physiological salt solution (PSS), phenylephrine, acetylated albumin, N\(^\text{6-}\)nitro-L-arginine methyl ester (L-NAME), and N\(^\text{6-}\)nitro-D-arginine methyl ester (D-NAME) as hydrochloride salts, L-arginine, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ), indomethacin, tetrabutylammonium iodide (TBA), and ouabain were purchased from Sigma (St. Louis, MO). L-NAME, D-NAME, TBA, and ouabain were dissolved in the distilled water; ODQ and indomethacin were dissolved in dimethylsulfoxide. Aqueous acrolein was prepared fresh from the redistilled reagent acrolein (Sigma-Aldrich). In some experiments, free acrolein was prepared by the acid hydrolysis (pH 3.0) of the diethyl acetal for 1 h at room temperature. Acrolein solutions prepared by these methods yielded the same results. Acrolein was resuspended in distilled water at a concentration of 10 mM before use. Anti-endothelial nitric oxide (NO) synthase (eNOS) monoclonal antibody and horseradish peroxidase-conjugated anti-mouse IgG were from the Transduction Laboratories (Lexington, KY). A bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL), and electrochemiluminescence detection reagents were purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Animals

Male Sprague-Dawley rats (7 to 12 wk, 180–340 g, Harlan; Indianapolis, IN) were housed in a temperature- and humidity-controlled room of the Association for the Accreditation of Laboratory Animal Care-approved animal care facility with a 12:12-h light-dark cycle and fed with rat chow and water ad libitum. A separate group of rats was fed with acrolein (2 mg/kg) three times on alternate days over 5 days. After being anesthetized with pentobarbital (60 mg/kg) three times on alternate days over 5 days. After being anesthetized with pentobarbital (60 mg/kg) three times on alternate days over 5 days. After being anesthetized with pentobarbital (60 mg/kg) three times on alternate days over 5 days. After being

Effects of in vitro treatment with acrolein. To determine the vasorelaxant capacity of acrolein, the aldehyde was applied to the vessels precontracted with phenylephrine (1 |M|). As soon as contraction reached plateau, acrolein (10, 20, 40, or 80 |M|) was added to the bath and the tissue preparation was allowed to incubate with the aldehyde for an additional 90 min. To confirm viability, the aortic rings were washed at the end of each experiment, allowed to equilibrate for 15 min, contracted with phenylephrine, and then relaxed with acetycholine (see Animals). An identical protocol was used with vessels denuded of endothelium to establish that the responses were endothelium mediated.

To elucidate the mechanisms involved in acrolein-induced response, the rings were preincubated with inhibitors of endothelium-derived vasodilators: 1) L-NAME, a blocker of NOS (100 |M|); 2) ODQ, a selective inhibitor of soluble guanylyl cyclase (1 |M|); 3) indomethacin, an inhibitor of the cyclooxygenase (COX) pathway of arachidonic acid metabolism (6.25, 0.5, 1, 5, and 10 |M|); 4) TBA, nonselective blocker of potassium channels (3 |M|); and 5) ouabain, inhibitor of Na\(^+\)-K\(^+\)-ATPase (5 |M|). Each of the compounds was incubated with aortic rings for 10 min, after which the rings were contracted with phenylephrine (1 |M|). When the contraction reached plateau, acrolein (20 |M|) was administered and incubated for an additional 90 min. Aortic rings incubated with 20 |M| acrolein alone served as the control. In the L-NAME experiments, D-NAME (100 |M|), an isomer devoid of NOS-inhibiting activity, was used as a negative control. To ensure that the contraction that followed NOS inhibitor administration was not due to a lack of substrate availability, an additional set of experiments was conducted in the presence of L-arginine, which is a NOS substrate. Maximal contraction evoked by phenylephrine detected at 10 min was taken as 100% and responses at 20, 40, 60, 80, and 100 min of incubation were expressed as a percentage of initial tonus. Maximal relaxation (\(E_{\text{max}}\)) was determined after 100 min of incubation and expressed as a percentage of the maximal contraction evoked by phenylephrine. The time required to produce half-maximal vasoactive effect (\(T_{1/2}\)) was determined from experimental data and was considered to reflect the vasorelaxant capacity of the compound.

**Western blot analysis.** Aortic rings were precontracted with phenylephrine, incubated in PSS in the absence (control) or presence of acrolein (10, 20, and 40 |M|) for 100 min at 37°C, frozen immediately after the experiment, and kept at −86°C. Vessels were homogenized on ice in homogenization buffer containing 25 mM Tris, 1 mM EDTA, 1% SDS, and 0.5% soybean trypsin inhibitor. After centrifugation at 12,000 revolutions/min for 30 min, protein concentration was measured in supernatant using BCA protein assay kit to ensure equal protein loading on the gel. After 10% SDS-
PAGE and transfer, the membranes were probed with anti-eNOS monoclonal antibody. Horseradish peroxidase-conjugated anti-mouse IgG was used as the secondary antibody. An electrochemiluminescence detection system was used for protein visualization.

Aortic reactivity after in vivo treatment with acrolein. Aortic rings obtained from the rats treated orally with acrolein (2 mg/kg by oral gavage three times on alternate days over 5 days) were incubated in PSS and increasing concentrations of phenylephrine ($10^{-10}$ to $10^{-5}$ M) were administered at 5-min intervals. Maximal contraction elicited by 80 mM KCl was taken as 100% and the response to each concentration expressed as a percentage. Aortic rings from animals gavaged with an equal volume of water were used as controls.

Statistical Analysis

Data are presented as means ± SE. Changes in vascular tone, expressed as the percentage of maximal contraction evoked by phenylephrine, were compared separately at each time point by one-way ANOVA and Dunnett’s test when significant differences existed between groups. Differences were considered statistically significant when the $P$ value was <0.05.

RESULTS

Characterization of Vasorelaxation Induced by Acrolein

No significant change in vascular tone was observed when 10–100 μM acrolein was applied to the resting aortic rings equilibrated in PSS (data not shown). However, when applied to precontracted vascular rings, 10–80 μM acrolein induced dose-dependent relaxation during 100 min of total incubation time (Fig. 1A). The relaxation induced by 10–40 μM acrolein was apparent at 40 min and reached maximum at 100 min of incubation (total observation time). However, vasorelaxation in the presence of 80 μM acrolein was apparent at 20 min, and by 100 min it almost reached the baseline. Precontracted intact aortic rings ($n=14$) incubated in PSS in the absence of acrolein for 100 min served as controls. After 40 min of incubation, the vascular tone of aortic rings treated with 10, 20, 40, and 80 μM acrolein decreased by 4.2 ± 2.8%, 16.3 ± 4.3%, 26.6 ± 3.8%, and 56 ± 4.5%, respectively (in each case, $P < 0.05$ vs. control), whereas in control vessels vascular tone remained unchanged. The value of $E_{max}$, determined at 100 min of incubation, was 44.7 ± 4.1, 56.0 ± 5.6, 61.0 ± 7.9, and 96.1 ± 2.1% for the vessels treated with 10, 20, 40, and 80 μM acrolein, respectively, and 14.2 ± 3.3% for control vessels (in each case $P < 0.05$ vs. control). $T_{1/2}$ was significantly shorter in
acrolein-treated vessels compared with controls (Fig. 1B). Although not many differences were found among vessels exposed to 10–40 μM acrolein, T1/2 for 80 μM acrolein was considerably shorter. However, in our subsequent experiments with inhibitors, we chose to use 20 μM acrolein to avoid cytotoxicity.

The endothelium-denuded vessels did not respond to acrolein (Fig. 1C), indicating that acrolein-mediated vasorelaxation was endothelium dependent. The values of Emax were 21.0 ± 8.9, 21.3 ± 5.2, and 14.9 ± 8.9% in vessels exposed to 10, 20, and 40 μM acrolein, respectively, versus 6.3 ± 2.5% in control (vessels denuded of endothelium and precontracted with phenylephrine incubated in PSS for 100 min in the absence of acrolein). Although the isometric tension of the denuded vessels exposed to acrolein had a tendency to decrease, it did not differ significantly from control vessels.

Mechanism of Acrolein-Mediated Vasorelaxation

To examine the mechanisms of acrolein-induced vasorelaxation, the aortic ring preparations were pre-treated with inhibitors of endothelium-derived vasodilators before adding phenylephrine and acrolein (20 μM). Pretreatment with the eNOS inhibitor L-NAME (100 μM) completely prevented the vasodilator response induced by acrolein (Fig. 2A). The isometric tension of the vessels in the presence of L-NAME corresponded to 103.8 ± 11.8% after 100 min of incubation with acrolein. Pretreatment with D-NAME (100 μM), an inactive isomer of L-NAME, did not significantly affect acrolein-evoked vasodilatation and the Emax observed was 49.6 ± 6.3. These data indicate that acrolein-mediated vasorelaxation is mediated by endothelium-derived NO. Addition of L-arginine did not change the vascular tone (data not shown), indicating that vasoconstriction in this case is not caused by the lack of availability of eNOS substrate. The role of NO in acrolein-evoked vasodilation was further probed with the use of ODQ, which is a relatively specific inhibitor of NO-mediated activation of guanylyl cyclase (6). Treatment with 1 μM ODQ abolished acrolein-induced vasodilation (Fig. 2B), and the vascular tone of the rings preincubated with ODQ slightly increased to 102.0 ± 4.2% after 100 min of incubation (P < 0.05). These observations further support the view that acrolein-mediated vasorelaxation is in part NO mediated.

Pretreatment with the COX inhibitor indomethacin also abolished acrolein-induced vasodilation (Fig. 3).
Concentration of indomethacin applied in inhibitor studies in the rat aorta is 10 μM (17). However, in our experiments, 1 and 5 μM indomethacin yielded complete inhibition and 0.5 μM caused partial inhibition, whereas at 0.25 μM no inhibition was observed. The $E_{\text{max}}$ values obtained from the rings exposed to 20 μM acrolein in the presence of 0.25, 0.5, 1, 5, and 10 μM indomethacin were 35.0 ± 6.2%, 54.1 ± 4.5%, 5.0 ± 5.3%, 5.2 ± 2.1%, and 2.0 ± 0.9%, respectively, compared with an $E_{\text{max}}$ value of 56.0 ± 5.6% obtained with 20 μM acrolein alone ($P < 0.05$ in each case). These data suggest that acrolein activates the COX pathway of arachidonic acid and prostacyclin production.

Application of a nonselective blocker of potassium channels, TBA, slightly potentiated the vasodilator response to acrolein (Fig. 4). The value of $E_{\text{max}}$ obtained from vessels incubated with 3 mM TBA and 20 μM acrolein was 73.2 ± 6.1%.

Inhibition of Na$^+$-K$^+$-ATPase by ouabain did not significantly affect acrolein-mediated vasodilation. Interestingly, acrolein has been reported to mimic the effect of ouabain, causing loss of Na$^+$-K$^+$-ATPase (32), in which case it would have been expected to potentiate the effect of ouabain. However, we noted no significant changes. The $E_{\text{max}}$ value obtained from rings incubated with 5 μM ouabain and 20 μM acrolein was 41.3 ± 4.8%, which is not significantly different from that obtained from aortic rings treated with acrolein alone (data not shown).

Reversibility of Effect of Acrolein on Vessel Wall

To ensure structural and functional integrity of the vessel, after each experiment, aortic rings were washed three times, allowed to equilibrate and then contracted with 1 μM phenylephrine. The maximal contraction was recorded after 5 min of addition of phenylephrine. The aortic rings were relaxed by adding 10 μM acetylcholine to the bath. The response to phenylephrine in vessels exposed to acrolein (with or without other reagents) corresponded to 80.1 ± 4.5% of the maximal contraction, whereas in control rings it was 95.4 ± 3.5% ($P < 0.05$), indicating that vascular contractility was compromised by acrolein. Endothelium-dependent relaxation was not significantly different between these groups: 93.7 ± 3.9% (acrolein-treated vessels) versus 94.0 ± 0.63 (control).

Slow Wave Vasomotion

Slow wave vasomotion with frequency of 1–4/min and variable amplitude was present in 77% of aortic rings exposed to acrolein (in the presence or the absence of other additives) and 1.4% of the vessels incubated in PSS alone. Although preincubation with ouabain only slightly decreased the incidence of slow-wave vasomotor effect of acrolein (it was observed in 62% of the aortic rings), we noted that it attenuated the amplitude of vasomotion. Interestingly, acrolein-induced vasomotion was only present when endothelium of the vessel was preserved. None of the aortic rings denuded of endothelium display such behavior on prolonged acrolein exposures. From these observations, we infer that slow wave vasomotion observed in our experiments is a result of acrolein-mediated endothelial dysfunction (12) and indicates that acrolein primarily affects endothelial function.

Western Blot Analysis

Expression of eNOS was only slightly increased in vessels exposed to acrolein compared with control rings (by ~20% in vessels exposed to 40 μM acrolein) (data not shown). This suggests that protein is not degraded, whereas new protein synthesis of eNOS occurs only to minor extent over this relatively short incubation time and acrolein probably modifies the activity of the preformed enzyme.

Aortic Reactivity After In Vivo Treatment With Acrolein

As shown in Fig. 5, the response to phenylephrine was decreased in the aortic rings obtained from the rats exposed to acrolein in vivo. The response to $10^{-8}$ M phenylephrine was decreased by 74%, $10^{-7}$ M by 50%, and $10^{-5}$ M by 33%, respectively. This is consistent with the in vitro data and indicates that acrolein feeding compromises the contractility of thoracic aorta. Animals treated with acrolein did not exhibit any gross physical changes, such as weight or hair loss.

DISCUSSION

The main findings of the present study are that exposure to acrolein induces a dose-dependent vasorelaxation of precontracted rat aortic rings and that the response to acrolein was abolished by endothelial denudation of the aortic rings. These observations clearly indicate that acrolein-induced vasorelaxation is due to increased generation of endothelial vasodilators. Our results further show that inhibition of NOS or COX
Fig. 5. Aortic reactivity after in vivo treatment with acrolein. Aortic rings prepared from rats fed acrolein (2 mg/kg; three times on alternate days over 5 days by oral gavage) were incubated in FSS and increasing concentrations of phenylephrine were administered at 5-min intervals. Response is expressed as percentage of the maximal contraction elicited by 80 mM KCl. Aortic rings from normal animals served as control. Data are means ± SE (n = number of vessels in each data series). *P < 0.05 vs. control.

prevents the response to acrolein, indicating that increased endothelial generation or availability of NO and prostaglandins is the underlying mechanism by which acrolein induces relaxation of the in vitro preparations of aortic rings.

Impairment of vascular reactivity induced by the reactive aldehyde species has been reported. Previous studies (30, 35) show that HNE induces vasorelaxation in cerebral, coronary, and mesenteric arteries through a NO/prostacyclin-dependent mechanism. In contrast, it has been reported that acrolein and allylamine produce vasoconstriction and attenuate acetylcholine-induced relaxation in the rat thoracic aorta and coronary artery in vitro (11, 12). These findings are in accord with the hypothesis that allylamine (a dye that generates acrolein during metabolism) induces vasoospasm, leading to myocardial ischemia and necrosis. However, our results are in contradistinction with the latter findings. This variability may be due to differences in experimental design and protocol. In contrast to the study by Conklin et al. (12), in our study 1) acrolein was applied to the precontracted vessels; 2) concentrations of acrolein were in a much lower range (10–40 μM vs. 0.1–1 mM); and 3) the incubation period was longer. Thus, together with the results of our studies performed in the aortic vessels obtained from the rats fed acrolein, these results are consistent with the idea that acrolein compromises the contraction capacity of the vessel. These data suggest that acrolein may interfere with autoregulation of blood flow, which may have pathophysiological implications under different conditions.

Because reactive aldehydes readily interact with proteins resulting in modification of their structure and enzymatic activity, we hypothesize that acrolein interacts with the components of NO and/or prostacyclin synthesis pathways and thus induces vasorelaxation. Alternatively, acrolein could modify the responsiveness of vascular smooth muscle cells to the vasodilator stimuli. Interestingly, ifosfamide (which generates acrolein during in vivo metabolism) was found to induce NO production by iNOS, mediating development of the hemorrhagic cystitis in mice (34). Acrolein also stimulates eicasanoid release from the bovine airway epithelial cells (15). Several reports (31, 39, 41) indicate a cross-talk between NO and COX pathways. NO-induced COX activation has been demonstrated in several different cell types, including cardiac myocytes (42) and the ischemic brain (31). The interaction may also occur in the reverse direction. COX products prostaglandin G2 and hydroperoxyeicosatetraenoic acid stimulated NOS activity in the rat brain (19). Thus it is possible that activation of one pathway takes place first and is followed by the induction of the other. Surprisingly, we found that both L-NAME and indomethacin completely blocked vasorelaxation, raising the question of the relative contribution of each pathway to the vasodilator response in the rat aorta both under physiological conditions and after exposure to acrolein.

The releases of NO and prostacyclin are coupled at the receptor level (14), and several stimuli that release NO also release prostacyclin (47). Inhibition of iNOS was found to abrogate prostanoid synthesis, whereas inhibition of COX-2 did not affect iNOS activity (42). Our results indicate that inhibition of each pathway causes reciprocal inhibition of the other and suggests bidirectional nature of NO-COX interactions.

As one of the main components of cigarette smoke, acrolein may be partly responsible for the increased risk of vascular disease in smokers. Several reports implicate the potential role of acrolein in vascular injury and atherogenesis. Acrolein immunoreactivity was demonstrated in human atherosclerotic plaque (45), whereas HNE- and malonaldehyde-conjugated proteins were reported earlier in atherosclerotic lesions (23, 36). Allylamine, which is converted to acrolein in cardiovascular tissues, is an established cardiovascular toxin that induces acute myocardial necrosis and intimal proliferation reminiscent of the early atherosclerosis lesion in several mammalian species (6). Acrolein is produced during biotransformation of allylamine by the enzyme semicarbazide-sensitive amino oxidase. Cardiovascular toxicity of allylamine is considered to be mediated mainly through action of acrolein, because semicarbazide, an inhibitor of semicarbazide-sensitive amino oxidase, prevents or significantly reduces the cardiovascular toxicity of allylamine (11). Interestingly, although single in vitro exposure to acrolein inhibited cell growth, repeated treatment with allylamine resulted in significant enhancement of the growth capacity of the smooth muscle cells, implicating its role in chemical atherogenesis (33).

Modulation of vascular reactivity and impairment of endothelial function is one of the earliest events in the development of atherosclerosis, preceding the morphological changes of the arterial wall (37). Impairment of NO-mediated, endothelium-dependent arterial relax-
ation has been demonstrated in the earliest stage of atherosclerosis (50), whereas impairment of endothelium-independent relaxation occurs at the later stage (48). Atherosclerosis and hypercholesterolemia are associated with the hypersensitivity to the contractile stimuli and impairment of the endothelium-mediated vasorelaxation through inhibition of NO production, whereas high-density lipoprotein plays a beneficial role in normalization of endothelial function (13, 16, 24).

Cigarette smoke causes impairment of vasomotor function with a differential effect in different vascular beds: vasoconstriction in the coronary and peripheral circulation and vasodilation in pulmonary circulation (20, 51), the latter finding being in accord with our results.

In an attempt to interpret our present data in this context, we hypothesize that acrolein, although causing early vasodilation, may result in the consumption of the available pool of NO and/or prostacyclin (or their precursors and/or substrates), thereby reducing bioavailability and later compromising the relaxation capacity of the vascular wall. Further studies are needed to better characterize acrolein-mediated molecular effects, including the specific mechanisms involved in the production of NO and PGII.

**DISCLOSURES**

This study was supported in part by National Institutes of Health Grants HL-43721, ES-12062, and HL-65618, an American Heart Association (AHA) Established Investigator Award (to S. E. D’Souza), and by an AHA Ohio Valley Affiliate Postdoctoral Fellowship Award (to N. L. Tsakadze).

**REFERENCES**


