Effects of Aqueous Extracts of Cigarette Mainstream Smoke on the Norepinephrine-Induced Contraction of Rat Aortic Rings *In Vitro*

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Background

Cigarette smoking is a major cause of coronary heart disease leading to subsequent events such as myocardial infarction, heart failure, or sudden death. The main underlying pathophysiologic process of coronary heart disease, as well as of cerebrovascular disease and peripheral arterial disease, is atherosclerosis (Ambrose et al., 2004). Smoking accelerates the process of atherosclerosis (Zieske et al., 1999) by increasing the risk of thrombosis (Miller et al., 1998), and enhances the liberation of catechol-amines by activating the sympathetic nervous system (Adnot, 1998 and Cryer et al., 1976). Furthermore, smoking induces coronary artery vasoconstriction in humans (Moliterno et al., 1994) and is thereby a risk factor for coronary spasms (Caralis et al., 1992 and Sugiishi et al., 1993).

Chronic smoking has been reported to augment smooth muscle contractility of canine coronary arteries (Cox et al., 1986) and extracts of cigarette smoke have been reported to induce vasoconstriction in isolated porcine coronary arteries (Murohara et al., 1994). Raij et al. (2001) showed that incubation of rat aortic rings with cigarette smoke results in an impaired acetylcholine-induced vasorelaxation and that this effect is mediated in part by an enhanced production of cyclooxygenase-derived vasoconstricting eicosanoids. However, little mechanistic information is available on the combined effects of cigarette smoke and norepinephrine, a process that could mediate coronary vasospasm and be enhanced by the cigarette smoke-induced catecholamine release.

Objective

Investigate the effect of aqueous extracts of cigarette mainstream smoke on the norepinephrine-induced contraction of rat aortic rings *in vitro* and the possible involvement of a cyclooxygenase-dependent pathway.

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Materials and Methods

Generation of Aqueous Extracts of Cigarette Mainstream Smoke

- 2R4F (University of Kentucky Reference Cigarette)
- Mainstream smoke generated on a 20-port Borgwaldt-smoking machine according to ISO standards 3402 (1999) and 3308 (2000)
- 60 puffs (1 puff = 35 cm^3 smoke) bubbled through 20 ml phosphate-buffered saline (PBS) at room temperature
- Fresh (≤15 min) smoke-bubbled PBS (sbPBS)

Aortic Ring Assay

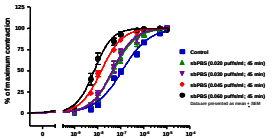
- Male Wistar Unilever rats (300 g to 450 g; Harlan Winkelmann, Germany) anesthetized and exsanguinated
- Dissection of thoracic aorta and preparation of rings (5 mm length)
- Denudation of the endothelium by incubation (3 min) with 0.3% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)
- Mounting of rings between two stainless-steel stirrups in the ADInstrument organ bath system model LEO1.046 and treated as described in test design

Test Design

- Isolation and mounting of aortic rings on transducers in the organ bath system
- Stretching 4x to 2 g tension
- Addition of 80 mM K^+ buffer (3x) to determine max. contraction
- Media change ± pre-incubation (30 min) with cyclooxygenase-inhibitor (indomethacine 10 µM, diclofenac 100 nM)
- Incubation with sbPBS (control, 0.020, 0.030, 0.045, or 0.060 puffs/ml) for different time periods (1, 15, 30, 45, or 60 min)
- Contraction with norepinephrine (1 nM to 10 µM)

Results and Discussion

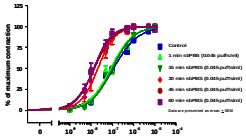
Concentration



	Control	0.020 puffs/ml	0.030 puffs/ml	0.045 puffs/ml	0.060 puffs/ml
EC_{50} [x10^{-7} M]	1.507	0.733	0.602	0.232	0.136
± SEM [x10^{-7} M]	0.393	0.280	0.210	0.088	0.059
p-value	----	p >0.05	p <0.05	p <0.001	p <0.001

ANOVA followed by Dunnett's post-Bonn test

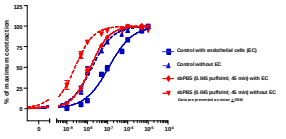
Time



	Control	5 min	15 min	30 min	45 min	60 min
EC_{50} [x10^{-7} M]	1.507	1.067	1.117	0.311	0.232	0.206
± SEM [x10^{-7} M]	0.393	0.222	0.214	0.088	0.088	0.052
p-value	----	p >0.05	p >0.05	p <0.001	p <0.001	p <0.001

ANOVA followed by Dunnett's post-test

Endothelial Cell Denudation



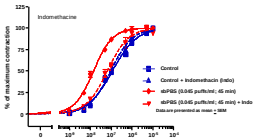
	Control + EC	Control - EC	0.045 puffs/ml + EC	0.045 puffs/ml - EC
EC_{50} [x10^{-7} M]	1.507	0.182	0.232	0.026
± SEM [x10^{-7} M]	0.393	0.026	0.088	0.005
+ EC/- EC	8.3-fold increased sensitivity to NE		8.9-fold increased sensitivity to NE	

NE: norepinephrine

Incubation with aqueous extracts of cigarette mainstream smoke (sbPBS) increases the vasoconstriction of aortic rings in response to norepinephrine in a concentration- and time-dependent manner, as indicated by a left-shift of the dose-response curves. Significant differences in the EC_{50} values were obtained at concentration ≥0.03 puffs/ml and incubation periods ≥30 min.

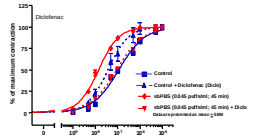
Removal of the endothelium prior to incubation with PBS or sbPBS results in a similar increase in sensitivity to norepinephrine, indicating a non-endothelial-dependent effect of sbPBS on the contractile response of rat aortic rings.

Cyclooxygenase Inhibition



	Control	Control + Indo	0.045 puffs/ml	0.045 puffs/ml + Indo
EC_{50} [x10^{-7} M]	1.507	1.130	0.232	0.921
± SEM [x10^{-7} M]	0.393	0.191	0.088	0.144
p-value	----	p >0.05	p <0.001	p <0.05

ANOVA followed by Dunnett's post test



	Control	Control + Dicl	0.045 puffs/ml	0.045 puffs/ml + Dicl
EC_{50} [x10^{-7} M]	1.507	0.697	0.232	0.984
± SEM [x10^{-7} M]	0.393	0.222	0.088	0.228
p-value	----	p <0.05	p <0.001	p >0.05

ANOVA followed by Dunnett's post test

The enhancement of the norepinephrine-induced vasoconstriction by sbPBS appeared to be a cyclooxygenase (COX)-dependent pathway because pre-incubation (30 min) with non-selective COX inhibitors (indomethacine 10 µM or diclofenac 100 nM) revealed no significant differences between PBS- and sbPBS-treated aortic rings.

Conclusion

Exposure to aqueous extracts of cigarette mainstream smoke increases the norepinephrine-induced contraction of rat aortic rings *in vitro*.

- The increase is concentration- and time-dependent.
- The increase is not endothelial cell-dependent.
- A cyclooxygenase-dependent pathway is involved.

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