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CIGARETTE SMOKE INHALATION SPECIFICALLY INHIBITS DEPRESSOR RESPONSES TO PROSTACYCLIN IN THE RAT

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Studies have been made of the effects of prior exposure to cigarette smoke on cardiovascular responses of the anaesthetized rat to arachidonic acid, prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and PGI_2. When compared with a control group, falls in systolic and diastolic blood pressure and tachycardia following intravenous PGI_2 were significantly reduced in those animals exposed to smoke 1 and 24 h previously. Responses 48 h after exposure were not significantly different. Pressor effects of $PGF_{2\alpha}$ and depressor responses to arachidonic acid and PGE_2 were not significantly affected at these times. It is suggested that the specific and long lasting attenuation of the effects of PGI_2 which occurs following cigarette smoke inhalation could contribute to both acute cardiovascular changes and the circulatory diseases associated with smoking.

Introduction Cigarette smoking causes acute circulatory effects and is known to be associated with a number of peripheral vascular diseases in man (U.S. Surgeon General, 1979). It has also been established that metabolites of arachidonic acid such as prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and more particularly PGI_2 (prostacyclin) may have importance in control of thrombus formation, vascular smooth muscle tone and blood pressure (Vane & McGiff, 1975; Dusting, Moncada, Mullane & Vane, 1978; Moncada & Vane, 1979). Recent studies have shown that prior exposure of rats to cigarette smoke decreases *in vitro* pulmonary inactivation of PGE_2 (Bakhle, Hartiala, Toivonen & Uotila, 1979). Nicotine *in vitro* inhibits conversion of arachidonic acid to prostacyclin (Wennmalm, 1978a; 1980) and formation of 6-keto $PGF_{1\alpha}$ (Wennmalm, 1978b) but facilitates synthesis of PGE_2 in rabbit hearts (Wennmalm, 1978a). To assess the relevance of these findings to the cardiovascular system *in vivo*, a study has been made of the effects of cigarette smoke on responses of the arterial blood pressure and heart rate of rats to arachidonic acid and some of its metabolic products.

Methods Male Wistar rats (300–330 g) were used. Each animal was exposed, in an inhalation chamber for 30 min, to the smoke from 1 cigarette (Rothman's Pall Mall, nicotine and tar content 1.2 mg and 20 mg respectively) by a technique identical to that of Bakhle *et al.* (1979) excepting that the air flow through the chamber was approximately 7 l/min. Control animals were placed in an identical chamber under similar conditions but the cigarette was unlit.

Cardiovascular responses were studied 1, 24 and 48 h after exposure. Anaesthesia was induced in each rat with 4% halothane in oxygen and maintained with intravenous chloralose (60 mg/kg) supplemented when necessary with 10 mg/kg. The trachea was cannulated. Drugs were administered into a cannulated right jugular vein. Recordings of arterial blood pressure were made by means of a Statham Pressure Transducer (P23A.C.) connected to the left carotid artery and of heart rate by means of a cardiotachometer triggered by the arterial pulse. Both variables were displayed on a Grass Model 7D polygraph. Rectal temperature was maintained at approximately 38°C.

Prostaglandins and arachidonic acid, as sodium salts, were stored at −20°C in ethanol or hexane (arachidonic acid). The solvent was removed under nitrogen and the required concentration obtained using 0.9% w/v NaCl solution (saline). A logarithmic series of progressively increasing doses (expressed in terms of the salt) of each of PGE_2 (10–300 μg/kg), $PGF_{2\alpha}$ (1–30 μg/kg), PGI_2 (0.3–30 μg/kg) and arachidonic acid (3–12 mg/kg) were injected intravenously, in that order. Statistical analyses of the data were carried out by Student's *t* test.

Results Mean initial basal blood pressures of the controls (n = 12) were 137 ± 3.5 mmHg systolic and 106 ± 3.1 mmHg diastolic, the mean heart rate being 420 ± 9.6 beats/min. These values were not significantly different ($P>0.05$) from those obtained using rats previously exposed to cigarette smoke (n = 10). Intravenous injection of PGE_2 or PGI_2 caused hypotension and tachycardia in a dose-dependent manner, PGI_2 being approximately 10–15 times more potent than PGE_2. In the groups exposed to cigarette smoke either 1 or 24 h previously, depressor responses and tachycardia following PGI_2 were reduced significantly when compared with controls. Figure 1 shows that 1 h after exposure there was a 3–6 fold decrease in sensitivity to the depressor effect of PGI_2. Decreased sensitivity to PGI_2 was also present in the group exposed to cigarette smoke 24 h previously. In a third group, exposed 48 h beforehand, responses were not significantly different from those of controls.

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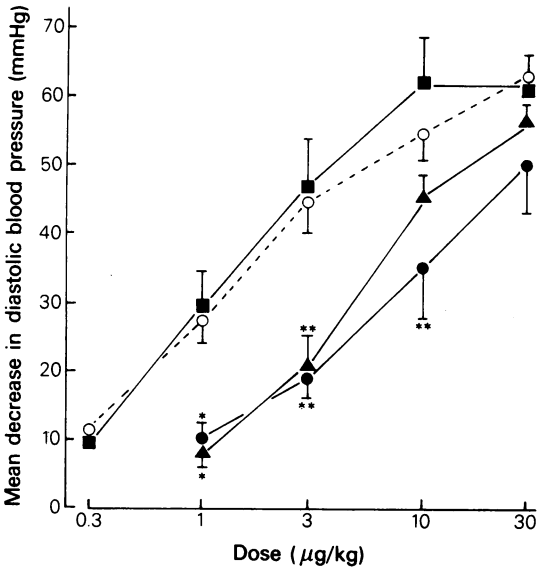

Figure 1 Mean effects of intravenous prostacyclin (PGI_2) on diastolic blood pressure in controls (○), 1 h (●), 24 h (▲) and 48 h (■) after cigarette smoke exposure. Each point is the mean response of 5–12 rats. Vertical lines represent ± s.e.mean. $^*P<0.01$; $^{**}P<0.005$ (significance of difference from controls)

Analogous findings were obtained for the effects of PGI_2 on systolic blood pressure and heart rate. In contrast, there were no significant differences between the responses of the controls and smoke-exposed animals at these times in the pressor effects of $PGF_{2\alpha}$ and the depressor effects of PGE_2 and arachidonic acid. Responses to the latter, but not PGE_2, $PGF_{2\alpha}$ or PGI_2 were inhibited in 4 control animals which received indomethacin 1 mg/kg.

Discussion The finding that cigarette smoke caused a specific and long lasting reduction in depressor responses and tachycardia following intravenous PGI_2 may have relevance to both acute circulatory changes and the cardiovascular diseases associated with smoking. There is now considerable evidence that circulating PGI_2 may make an important contribution to normal control of vascular resistance, arterial blood pressure and blood clotting (Moncada & Vane, 1979).

This preliminary study did not elucidate the mechanism underlying the inhibitory effect of cigarette smoke on responses to PGI_2. It could be due to a direct action on the cardiovascular system or to altered metabolic breakdown, perhaps in the liver, a major site of PGI_2 removal from the circulation (Wong, Malik, Desiderio, McGiff & Sun, 1980). Changes in tissue enzyme activities in rats have been detected within 1 h of exposure to cigarette smoke (Welch, Loh & Conney, 1971; Uotila, 1977; Hartiala, Uotila & Nienstedt, 1978). Depressor responses to arachidonic acid were unchanged, which was a paradoxical observation as PGI_2 in some species appears to be its major vascular metabolite (Moncada & Vane, 1979). Confirmation that the responses were indirect, as a consequence of its conversion to more active metabolites by cyclo-oxygenase, was indicated by the inhibitory effect of indomethacin. However after intravenous administration, arachidonic acid is converted into a number of depressor and pressor substances which may have masked any depression of the effects of PGI_2 being formed concomitantly. Moreover, the relative amounts of the metabolites produced appears to depend on the dose, the tissue and the species (Mullane, Moncada & Vane, 1979).

Bakhle *et al.* (1979) showed that pulmonary removal of PGE_2 was decreased *in vitro* following cigarette smoke exposure which indicated that its effects following intravenous administration might be potentiated in the smoke-exposed groups. However, no change could be detected in its action or in those of $PGF_{2\alpha}$ which is also removed by the pulmonary vasculature (Vane, 1969). A more sensitive and less indirect method of assessing circulating prostaglandin levels might have revealed altered pulmonary removal.

The finding of long lasting inhibition of cardiovascular responses to PGI_2 after cigarette smoke inhalation by rats, together with rapidly accumulating evidence of its importance in circulatory homeostasis (Moncada & Vane, 1979) indicates the necessity of confirming and extending these observations using other species. Determination is also required of the underlying mechanism and the constituent of tobacco smoke primarily responsible.

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References

BAKHLE, Y.S., HARTIALA, J., TOIVONEN, H. & UOTILA, P. (1979). Effects of cigarette smoke on the metabolism of vasoactive hormones in rat isolated lungs. *Br. J. Pharmac.*, **65**, 495–499.

DUSTING, G.J., MONCADA, S., MULLANE, K.M. & VANE, J.R. (1978). Implications of prostacyclin generation for modulation of vascular tone. *Clin. Sci. Molec. Med.*, **55**, 195–198s.

HARTIALA, J., UOTILA, P. & NIENSTEDT, W. (1978). The effects of cigarette smoke exposure on testosterone metabolsim in the isolated perfused rat lung. *J. Steroid Biochem.*, **9**, 365–368.

MONCADA, S. & VANE, J.R. (1979). Prostacyclin in perspective. In *Prostacyclin*, ed. Vane, J.R. & Bergström, S. New York: Raven Press.

MULLANE, K.M., MONCADA, S. & VANE, J.R. (1979). Formation and disappearance of prostacyclin in the circulation. In *Prostacyclin*, ed. Vane, J.R. & Bergström, S. New York: Raven Press.

UOTILA, P. (1977). Effects of single and repeated cigarette smoke-exposures on the activities of aryl hydrocarbon hydroxylase, epoxide hydratase and UDP glucuronosyltransferase in rat lung, kidney and small instestinal mucosa. *Res. Comm. Chem. Path. Pharmac.*, **17**, 101–114.

U.S. SURGEON GENERAL. (1979). *Smoking and Health.* pp. 1:13–1:15. U.S. Department of Health, Education & Welfare.

VANE, J.R. (1969). The release and fate of vaso-active hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

VANE, J.R. & McGIFF, J.C. (1975). Possible contributions of endogenous prostaglandins to the control of blood pressure. *Circulation Res. Suppl.* **36/37**, 68–75.

WELCH, R.M., LOH, A. & CONNEY, A.H. (1971). Cigarette smoke: stimulatory effect on metabolism of 3,4-benzpyrene by enzymes in rat lung. *Life Sci.*, **10**, Part I, 215–221.

WENNMALM, A. (1978a). Effects of nicotine on cardiac prostaglandin and platelet thromboxane synthesis. *Br. J. Pharmac.*, **64**, 559–563.

WENNMALM, A. (1978b). Nicotine inhibits the release of 6-ketoprostaglandin $F_{1\alpha}$ from the isolated perfused rabbit heart. *Acta. physiol. scand.*, **103**, 107–109.

WENNMALM, A. (1980). Nicotine inhibits hypoxia- and arachidonate-induced release of prostacyclin-like activity in rabbit hearts. *Br. J. Pharmac.*, **69**, 545–549.

WONG, P.Y-K., MALIK, K.U., DESIDERIO, D.M. McGIFF, J.C. & SUN, F.F. (1980). Hepatic metabolism of prostacyclin (PGI_2) in the rabbit: formation of a potent novel inhibitor of platelet aggregation. *Biochem. biophys. Res. Commun.*, **93**, 486–494.

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