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EFFECTS OF CIGARETTE SMOKE ON THE METABOLISM OF VASOACTIVE HORMONES IN RAT ISOLATED LUNGS

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1 The effects of exposure of rats to cigarette smoke have been studied on the metabolism of vasoactive hormones in isolated lungs from these animals.

2 Rats were exposed for 1 h per day to cigarette smoke for 1 day or for 10 days.

3 Angiotensin I conversion was increased after 1 day's exposure but after 10 days' exposure conversion returned to normal.

4 Inactivation of prostaglandin E_2 was decreased after 1 day's exposure. After 10 days' exposure there was a further decrease which could not be attributed to smoke alone.

5 The inactivation of 5-hydroxytryptamine and bradykinin remained unchanged after both short and longer exposures to smoke.

6 The metabolic activity of the lung towards some vasoactive hormones in the pulmonary circulation is affected by exposure of the animal to cigarette smoke and such changes may be relevant to the initiation of cardiovascular changes consequent upon cigarette smoking.

Introduction

Smokers have an increased risk of lung cancer, and cardiovascular disease (cf. U.S. Department of Health, Education & Welfare, 1975). Cigarette smoke exposure is known to increase the metabolism of polycyclic aromatic hydrocarbons, present in cigarette smoke, to carcinogenic intermediates in the lungs of experimental animals (Welch, Loh & Conney, 1971; Cohen, Uotila, Hartiala, Suolinna, Simberg & Pelkonen, 1977) and also in human alveolar macrophages (Cantrell, Warr, Busbee & Martin, 1973). Another group of substrates, the vasoactive hormones, are also metabolized by lung tissue on passage through the pulmonary circulation (Bakhle & Vane, 1974; 1977). Changes in the metabolism of such substrates induced by cigarette smoke exposure might be relevant to the cardiovascular effects of smoking. We therefore investigated the effect of exposing rats to smoke on the activation of angiotensin I to angiotensin II and on the inactivation of bradykinin, prostaglandin E_2 (PGE_2) and 5-hydroxytryptamine (5-HT) in the pulmonary circulation.

Methods

Male adult Wistar rats were exposed to cigarette smoke in an inhalation chamber as described earlier (Uotila & Marniemi, 1976). During 1 h the rats inhaled the smoke from five commercial cigarettes, each of them containing 1 mg nicotine and 16 mg tar, as quoted by the manufacturer. The rats were exposed once (1 day exposure) or daily for 10 consecutive days (10 days' exposure). Sham-exposed rats were exposed only to air in an identical chamber and control rats were kept in their cages in animal rooms.

About 20 h after the last exposure, rats were anaesthetized with sodium pentobarbitone (50 mg/kg i.p.) and the lungs removed and prepared for perfusion as described previously (Bakhle, Reynard & Vane, 1969). Oxygenated, warmed Krebs solution was pumped through the pulmonary circulation at 8 ml/min. The effluent superfused two assay tissues arranged in cascade below the lung (Vane, 1964). The contractions of the tissues were recorded on a potentiometric recorder via Harvard smooth muscle transducers and auxotonic levers. The following assay tissues were used: for angiotensin I and II, rat colon (Regoli & Vane, 1964); for bradykinin, guinea-pig terminal ileum; for prostaglandin E_2 (PGE_2), hamster stomach strip (Ubatuba, 1973). The Krebs solution also contained indomethacin (0.5 μg/ml) and methysergide (200 ng/ml; final concentration as base). Agonists were injected in a volume of 0.05 to 0.2 ml into the Krebs flow.

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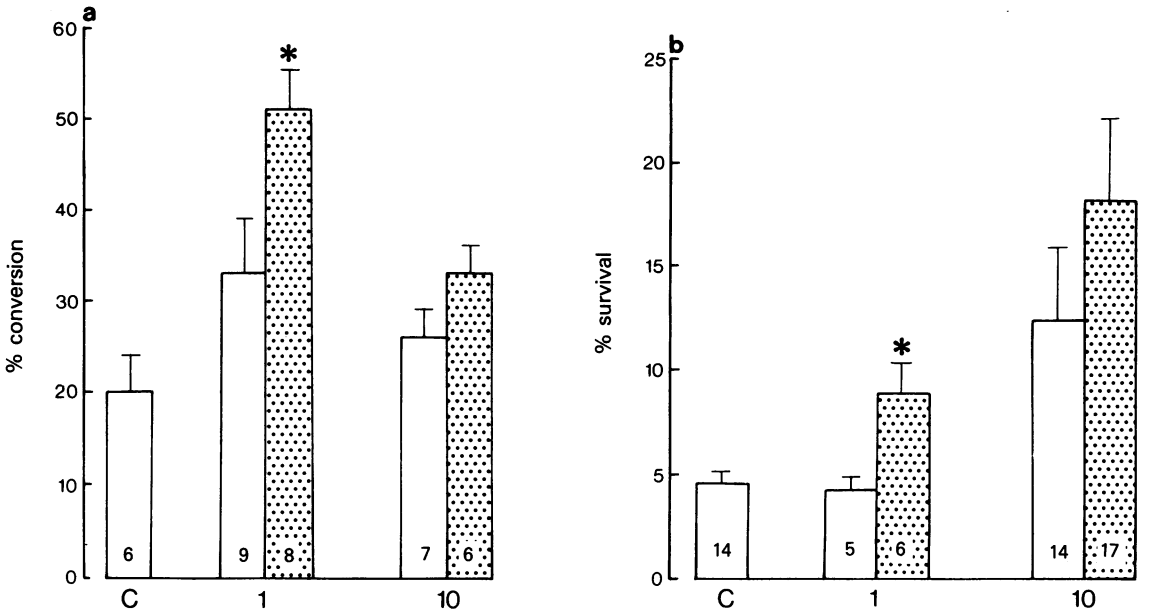

Figure 1 Effect of exposure to cigarette smoke on the metabolism of angiotensin I (a) and prostaglandin E_2 (PGE_2, b) in rat isolated lungs. The mean values are shown for the number of animals indicated within each column; vertical lines show s.e. mean. At 1 and 10 days the open columns represent sham- and the stippled columns, smoke-exposed animals; C = control rats. Metabolism of angiotensin I was measured with injections of 100 to 200 ng, and that of PGE_2 with injections of 500 to 1500 ng. For both angiotensin I conversion and PGE_2 inactivation, the value after 1 day's exposure was significantly different ($*P < 0.05$) from sham and control values. At 10 days no significant effect of smoke exposure could be demonstrated.

Metabolism of 5-HT was measured by a radiochemical method. [^{14}C]-5-hydroxytryptamine (Radiochemical Centre, Amersham, 54 mCi/mmol) was diluted with 0.9% w/v NaCl solution (saline) to give a concentration of 1 μCi/ml. This solution was infused at 0.155 ml/min over 3 min into the Krebs solution entering the pulmonary arterial cannula and the effluent from the lung collected in four fractions, 0 to 2.5 min, 2.5 to 5 min, 5 to 10 min and 10 to 20 min. By this time the radioactivity in the effluent had fallen to background levels. Aliquots (1 ml) of each effluent fraction were chromatographed on columns of ion exchange resin to separate metabolites from unchanged 5-HT (Southgate & Collins, 1969). The radioactivity in the column effluent (metabolites) was measured by liquid scintillation.

The following drugs were used: angiotensin I (Schwarz Biochemicals); angiotensin II (gift of Ciba-Geigy Ltd.); bradykinin (gift of Sandoz); methysergide bimaleate (Sandoz); indomethacin (Merck, Sharp & Dohme & Lääke Oy Turku); PGE_2 (Sigma).

Results

The effect of exposure to cigarette smoke on the pulmonary metabolism of angiotensin I is shown in Figure 1a. After 1 day's exposure, activation of angiotensin I by conversion to angiotensin II was significantly increased in smoke-exposed rats when compared with either control or sham-exposed rats. Prolonging the exposure to 10 days did not cause a greater effect. On the contrary, the increase in conversion was less than that observed after 1 day's exposure, and not significantly higher than in sham-treated animals. In contrast to these results, bradykinin metabolism was unaffected by exposure to cigarette smoke for either 1 or 10 days. In Table 1 the metabolism of bradykinin is expressed as survival, i.e. 100 − inactivation, as it is the surviving bradykinin that is measured by bioassay.

The two substrates which are metabolized by intracellular enzymes, 5-HT and PGE_2 were affected differently. Whereas the metabolism of 5-HT by monoamine oxidase was unchanged by our regime of smoke exposure (Table 1), PGE_2 metabolism was altered (Figure 1b). The effect of 1 day's exposure was to increase survival, i.e. decrease inactivation to double the control value. The animals given longer exposure to cigarette smoke showed a further increase in survival of prostaglandin E_2 compared with the control group, but as the sham-exposed rats also

Table 1 Lack of effect of cigarette smoke exposure (1 day or 10 days) on metabolism of bradykinin and 5-hydroxytryptamine (5-HT) in the pulmonary circulation of rat isolated lung

	Control animals	*Exposed animals*			
		1 day		*10 days*	
		Sham	*Smoke*	*Sham*	*Smoke*
Bradykinin	1.6 ± 0.3	2.2 ± 0.1	1.6 ± 0.3	0.9 ± 0.1	1.0 ± 0.2
% survival	(5)	(4)	(4)	(4)	(4)
5-HT	78.8 ± 1.1	74.4 ± 6.1	76.0 ± 5.9	74.0 ± 1.8	76.8 ± 3.1
% metabolism	(4)	(4)	(4)	(5)	(5)

For bradykinin metabolism, injections of 5–10 μg were made into the pulmonary arterial cannula. For 5-HT metabolism, each lung received 475 nCi [^{14}C]-5-HT infused over 3 minutes. The number of animals is given in parentheses.

showed increased survival, the difference between smoke-exposed and sham-exposed rats did not reach statistical significance.

Discussion

We have measured the metabolism of four substrates, angiotensin I, bradykinin, 5-HT and PGE_2 in the pulmonary circulation of isolated lungs taken from rats exposed to cigarette smoke. The method of smoke exposure used caused increases in the metabolism and covalent binding of benzo(a)-pyrene in rat lung (Cohen *et al.*, 1977).

The effects of smoke exposure on the metabolism of the peptides, angiotensin I and bradykinin can be considered together, as both these substrates are hydrolysed by enzymes on the luminal surface of the endothelial cells (Ryan & Ryan, 1976). The increase in angiotensin converting enzyme activity after only 1 day's exposure to smoke showed that events at the pulmonary endothelial cell membrane could be affected by a relatively distant stimulus, i.e. smoke in the airways. However, this increase was not sustained after the longer 10 day exposure. The return of altered enzyme levels to normal values during repeated exposure to smoke has already been observed by Uotila (1977) in the case of epoxide hydratase in rat lung and our results could be another example of this effect. An alternative explanation might be that here the renin-angiotensin system, of which converting enzyme is a crucial component, responded only transiently to the smoke exposure. Such a transient response to a continuing stimulus is known to occur in experimental 'one-kidney' hypertension where there is an initial increase in renin accompanying an increase in blood pressure but after some days renin levels return to normal while blood pressure remains increased (Miller, Samuels, Haber & Barger, 1975). It is also relevant to point out that pulmonary conversion of angiotensin I is lower in rats (Bakhle *et al.*, 1969; Kreye & Gross, 1971; Bakhle, 1977) than in other laboratory animals and therefore converting enzyme may, in the rat, play a greater role in controlling the overall efficacy of the renin-angiotensin system.

As bradykinin is also a substrate for converting enzyme, an increase in converting enzyme activity would be expected to cause an increase in bradykinin inactivation. However, there are other pulmonary peptidases hydrolysing bradykinin (Ryan, Roblero & Stewart, 1970) and, in rat lung, only about 10% of the bradykininase activity can be attributed to converting enzyme (Bakhle, 1977). This and the high level of normal inactivation would make it difficult to detect increases in total bradykinin inactivation due to increases in converting enzyme.

The lack of effect on 5-HT metabolism suggests that the uptake process for 5-HT is unchanged, since this is the rate-limiting step in the metabolic process in isolated lung (Gillis & Roth, 1976). Another possibility would be to suggest opposite changes in uptake and enzyme giving a net zero change.

On the other hand, metabolism of PGE_2 in rat lung is not transport-limited (Anderson & Eling, 1976) and this metabolism was unequivocally decreased after 1 day's exposure to cigarette smoke, suggesting a decreased activity of 15-hydroxyprostaglandin dehydrogenase (PGDH), the rate-limiting enzyme in PGE_2 degradation in lung. Although the change is numerically small, it does represent a doubling of the PGE_2 entering the systemic arterial circulation. The changes occurring after 10 days' exposure cannot be safely attributed to smoke exposure as survival of PGE_2 increased in both sham- and smoke-exposed animals. Although the chambers for smoke and sham exposures were in different rooms, it is possible that the sham-exposed rats could have inhaled small amounts of smoke during their movement from the exposure chamber to the animal rooms. This could partly explain the increased survival of PGE_2 in the 10 days sham group. In an earlier study (Cohen *et*

al., 1977), when similar exposure arrangements were used, we observed an increase in the metabolism of benzo(a)pyrene in perfused lungs of sham-exposed animals.

The effects of smoke exposure have been studied mostly in terms of the metabolism of exogenous substrates, often polycyclic hydrocarbons. These studies have dealt with mixed function oxidases, hydrocarbon hydroxylases and conjugating enzymes. Only recently has the fate of an endogenous substrate for some of these enzymes, testosterone, been studied after smoke exposure (Hartiala, Uotila & Nienstedt, 1978). Our experiments are the first to study a range of endogenous vasoactive substances, which are substrates for enzymes not usually considered to be affected by smoke exposure.

Although the mechanism by which the effects we have seen are produced remains unknown, it must be reasonably specific as three of the substrates (angiotensin I, bradykinin and 5-HT) are metabolized in the endothelial cell but only one substrate, angiotensin I, is affected. A non-specific 'poisoning' of endothelial cells would affect all three substrates. Furthermore, since the two enzymes affected are dissimilar, one a peptidase and one a dehydrogenase, one extracellular and one intracellular, there is no obvious mechanism that could be common to both.

The metabolic activities we have studied are physiologically important as they control the systemic arterial levels of endogenous vasoactive substances. Our results suggest, therefore, that exposure to cigarette smoke disturbs these physiological control systems and could thus produce changes in systemic blood pressure. These results may also suggest a mechanism for the initiation of cardiovascular changes associated with cigarette smoking.

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