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PULMONARY INACTIVATION OF 5-HYDROXYTRYPTAMINE IS DECREASED DURING CIGARETTE SMOKE VENTILATION OF RAT ISOLATED LUNGS

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1 The effect of cigarette smoke ventilation on the inactivation of [^{14}C]-5-hydroxytryptamine (5-HT) was studied in isolated perfused lungs of the rat.

2 [^{14}C]-5-HT 9.6 nmol was infused into the pulmonary circulation of rat lungs in 3 min. The nonrecirculating perfusion effluent was collected during the 5-HT infusion in three consecutive 1 min fractions. The amount of metabolites of 5-HT was determined from the perfusion effluent and from the perfused lungs.

3 The amount of metabolites of 5-HT in the perfusion effluent was decreased during cigarette smoke ventilation.

4 The amount of metabolites of 5-HT in the perfused lungs was also decreased by cigarette smoke ventilation, although the total amount of radioactivity in the lung tissue was not significantly changed.

5 The decreased pulmonary inactivation of 5-HT may cause increased circulating levels of 5-HT, which would explain some cardiovascular changes during smoking.

Introduction

Cigarette smoke has been shown to change the pulmonary metabolism of vasoactive substances. The metabolism of prostaglandin E_2 (PGE_2) in isolated lungs of the rat is slightly decreased after a single exposure of rats to cigarette smoke (Bakhle, Hartiala, Toivonen & Uotila, 1979) and during cigarette smoke ventilation (Männistö, Kuusisto, Matintalo & Uotila, 1981). Angiotensin I conversion in rat isolated lungs is increased after a single exposure of rats to cigarette smoke (Bakhle *et al.*, 1979).

Pre-exposure of rats to cigarette smoke for 1 h either once or daily during ten consecutive days did not affect the metabolism of 5-hydroxytryptamine (5-HT) in isolated perfused lungs (Bakhle *et al.*, 1979). However, there is evidence that cigarette smoke may change 5-HT metabolism. Perfusion of isolated lungs of the rat (Junod, 1972) and guinea-pig (Steinberg, Basset & Fisher, 1975) with anoxic solution partially inhibits both the 5-HT uptake and the oxidation of 5-HT. Cyanide, a component of cigarette smoke, also inhibits the uptake of 5-HT (Steinberg *et al.*, 1975). In mouse skin, cigarette smoke exposure inhibits 5-HT specific monoamine oxidase activity and increases 5-HT uptake (Essman, 1977).

Therefore, we have investigated whether cigarette smoke ventilation during perfusion of rat isolated lungs has any effect on the pulmonary metabolism of 5-HT.

Methods

Male adult Wistar rats weighing 260–330 g were anaesthetized with sodium pentobarbitone (Mebunat, 60 mg/kg i.p.). The lungs were removed (Bakhle, Reynard & Vane, 1969) and perfused through the main pulmonary artery at a flow rate of 8 ml/min at 37°C in a nonrecirculating system. The perfusion medium consisted of Krebs bicarbonate buffer containing glucose (5.6 mM). It was gassed continuously with 95% O_2 and 5% CO_2 to oxidize it and to maintain its pH at 7.4. The perfusion pressure remained below 20 mmHg.

The lungs were ventilated by a pressure-regulated respirator with air for 5 min before cigarette smoke ventilation was started. The main stream smoke from 2 filter cigarettes (containing 1 mg nicotine and 18 mg tar per cigarette) was added to the air flow from the respirator during a cigarette smoke ventilation period of 5 min. Two minutes after the beginning of cigarette smoke ventilation 9.6 nmol of [^{14}C]-5-HT (sp.act. 50 mCi/mmol, diluted with 0.9% w/v NaCl solution) was infused into the pulmonary circulation during 3 min at a rate of 0.155 ml/min to give a final concentration of 0.4 μM in the perfusion medium. During 5-HT infusion the lungs were ventilated with cigarette smoke and the nonrecirculating effluent from the lungs was collected in three frac-

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tions: 0–1 min, 1–2 min and 2–3 min. Control lungs were ventilated with air only. Immediately after the 5-HT infusion the perfusion was stopped and the lungs were taken for analysis.

The total radioactivity in each perfusion effluent fraction was measured by liquid scintillation counting. This represents the combined amount of unmetabolized 5-HT and its metabolites in the effluent. Samples of each effluent fraction were chromatographed on columns of ion exchange resin (Amberlite CG-50, 100–200 mesh) to separate the metabolites from unchanged 5-HT (Southgate & Collins, 1969). The amount of radioactivity in the chromatography column effluent was determined. This represents the amount of 5-HT metabolites in the effluent.

The perfused lungs were weighed, homogenized in 3 volumes of 0.5 N $HClO_4$ with an Ultra-Turrax homogenizer for 3 × 10 s and centrifuged at 2,000 *g* for 15 min; 2 ml of the supernatant was buffered with 1 ml of 0.1 M sodium phosphate buffer (pH 7.4) and neutralized with 0.5 M KOH. The neutral solution was chromatographed as above. The amount of radioactivity in the neutral solution and that in the chromatography column effluent was measured by liquid scintillation counting. All the data were analysed by Student's *t* test for unpaired data.

[^{14}C]-5-Hydroxytryptamine was from Radiochemical Centre, Amersham. The cigarettes (Marlboro) were made by Amer-Yhtymä Oy, Finland, under authority of Philip Morris Inc., U.S.A.

Results

When 9.6 nmol of [^{14}C]-5-HT was infused into the pulmonary circulation of rat isolated lungs over a period of 3 min and the effluent from the lungs was collected during that time, 72 ± 0.7% (mean ± s.e., $n = 9$) of the infused radioactivity appeared in the effluent from control lungs and 69 ± 1.2% ($n = 9$) from smoke-ventilated lungs. Although the total amount of radioactivity (i.e. the sum of unchanged 5-HT and metabolites) in the combined effluent of 0–3 min was not changed, the amount of metabolites in this effluent was decreased from 2.8 ± 0.1 nmol (29 ± 0.6% of the infused radioactivity) in the control group to 1.6 ± 0.1 nmol (16 ± 1.5%; $P < 0.001$) in the smoke-ventilated group. This decrease in the amount of 5-HT metabolites was seen in each 1 min effluent fraction of the smoke-ventilated group (Figure 1). The decrease in the metabolism of 5-HT in smoke-ventilated lungs was also seen when the radioactivity in the perfused lungs was analyzed. As calculated from the radioactivity in the lungs, the combined amount of unmetabolized 5-HT and its metabolites was 2.7 ± 0.1 nmol (28 ± 0.8% of the infused radioactivity) in control lungs and 2.9 ± 0.1 nmol (31 ± 1%) in smoke-ventilated lungs.

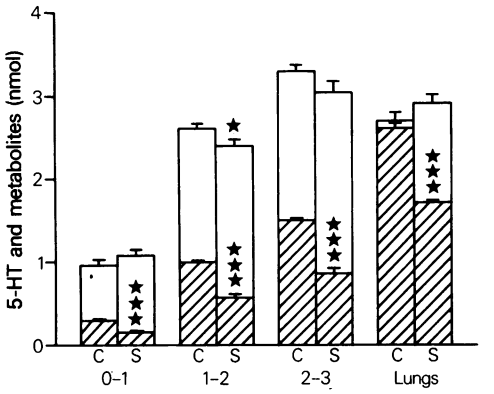

Figure 1 Effect of cigarette smoke ventilation on the metabolism of 5-hydroxytryptamine (5-HT) in isolated perfused lungs of the rat. Isolated lungs were ventilated with cigarette smoke (S) when 9.6 nmol of [^{14}C]-5-HT was infused in 3 min into the pulmonary artery. Control lungs (C) were ventilated with air only. During the 5-HT infusion the nonrecirculating perfusion effluent was collected in three consecutive 1 min fractions. The combined amount of unmetabolized 5-HT and its metabolites (the whole length of the columns) as well as the amount of 5-HT metabolites (the hatched section of the columns) were measured from each effluent fraction and from the perfused lung tissue. Cigarette smoke ventilation decreased the amount of 5-HT metabolites both in the perfusion effluent and in the perfused lung tissue. Mean values from nine separate experiments are shown; vertical lines indicate s.e.mean. Significance of difference from control: $^{*}P < 0.01$; $^{***}P < 0.001$ (Student's *t* test for unpaired data).

The amount of metabolites of 5-HT in the perfused lungs was decreased from 2.6 ± 0.06 nmol (27 ± 0.6%) in control lungs to 1.7 ± 0.3 nmol (18 ± 3%; $P < 0.001$) in smoke-ventilated lungs (Figure 1).

Discussion

The present results show that cigarette smoke ventilation inhibits the metabolism of 5-HT in isolated perfused lungs of the rat. The inhibition of 5-HT metabolism was seen in the perfusion effluent within 3 min of beginning the cigarette smoke exposure (during the first minute of 5-HT infusion) and the % inhibition remained at the same constant level during the following 2 min of exposure. After perfusion the amount of radioactivity in the cigarette smoke-ventilated lungs was at the control level. However, about one third of the radioactivity was still present as unmetabolized 5-HT, in contrast to nearly total metabolism in untreated lungs.

Pulmonary inactivation of 5-HT is a two-stage process, consisting of an energy-requiring uptake

into the pulmonary endothelial cells and subsequent oxidation by monoamine oxidase (MAO) (Alabaster & Bakhle, 1970; Bakhle & Youdim, 1979). The process is strictly uptake limited: the K_m for 5-HT uptake in rat isolated lungs is less than 10 μM (5.9 μM by Junod, 1972, and 2 μM by Bakhle & Youdim, 1979) whereas the K_m for MAO is about 300 μM (330 μM by Bakhle & Youdim, 1979, and 255 μM by Kung & Wilson, 1979). The pulmonary removal of 5-HT can be prevented by uptake inhibitors, e.g. lowered temperature, cocaine, chlorpromazine, tricyclic antidepressants or anoxia (Junod, 1972) whereas MAO inhibition by iproniazid (Alabaster & Bakhle, 1970; Junod, 1972) or mebanazide (Alabaster & Bakhle, 1970) does not significantly decrease the pulmonary 5-HT uptake in rat isolated lungs. The present findings, unaltered or slightly increased (at 1–2 min) uptake of radioactivity into the cigarette smoke-ventilated lungs during the 5-HT infusion and the increased 5-HT percentage in the lung effluent, correspond to the findings of Alabaster & Bakhle concerning MAO inhibition by mebanazide.

MAO inhibition due to cigarette smoke exposure was further supported by the analysis of the lungs after perfusion. In control lungs the 5-HT taken up was almost totally metabolized. However, in cigarette-exposed lungs, about one third of the radioactivity still appeared as unchanged 5-HT. The total radioactivity in the cigarette smoke exposed lungs was at the same level as the total radioactivity in the control lungs. As an alternative, similar results would be found if uptake of 5-HT were inhibited and 5-HT stored in the lungs outside the endothelial cells. There is an interstitial 5-HT space in rat lung estimated to be about 0.25–0.30 ml/g of wet lung (Junod, 1972). The mean weight of the lungs in our experiment was 1.3 g and cigarette smoke caused no additional gain in weight. Because the concentration of 5-HT in the perfusate inflow was 0.4 μM and passive diffusion is not able to concentrate 5-HT in the lungs, the presence of most of the unchanged 5-HT in the lungs after perfusion was the result of active uptake and not passive diffusion.

Although the present findings show that cigarette smoke strongly inhibits the metabolism of 5-HT in rat isolated lungs, they do not totally exclude the possibility of an additional inhibition of 5-HT uptake. Cyanide (a component of cigarette smoke) inhibits the uptake of 5-HT in guinea-pig lungs (Steinberg *et al.*, 1975). Anoxia is also an inhibitor of 5-HT uptake (Junod, 1972, Steinberg *et al.*, 1975). In the present study, anoxia of the lungs was unlikely because they were perfused with oxygenated buffer solution and neither was the cigarette smoke ventilation mixture totally anoxic (smoke from two cigarettes was mixed into approximately 5 litres of air). The increase of unchanged 5-HT in the lung effluent observed during the first minute of 5-HT infusion, suggests that it would have escaped the pulmonary uptake. In this situation, however, the amount of total radioactivity coming through the lungs would be expected to have increased, and this could not be detected.

The depression of 5-HT metabolism in rat isolated lungs due to cigarette smoke ventilation seems to be reversible because cigarette smoke exposure of rats before the perfusion experiments did not decrease the pulmonary inactivation of 5-HT (Bakhle *et al.*, 1979). Although the lung is thought to be the major site for 5-HT inactivation, blood platelets, liver and other tissues are also able to bind or inactivate this amine. Therefore further studies are needed in this field before the role of altered amine metabolism in the course of cigarette smoke-induced cardiovascular alterations can be evaluated.

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