Br. J. Pharmac. (1981), **73**, 461–466

EFFECT OF HYPEROXIA ON UPTAKE AND METABOLISM OF 5-HYDROXYTRYPTAMINE AND β-PHENYLETHYLAMINE IN RAT LUNG: A SEX DIFFERENCE

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1 The uptake of 5-hydroxytryptamine (5–HT) and β-phenylethylamine (PEA) and their deamination by monoamine oxidase (MAO) were studied in perfused lung from male and female rats exposed to 100% O_2 at 1 ATA for up to 60 h.

2 The uptake and metabolism of 5-HT in lungs from both male and female rats was not changed by exposure to O_2.

3 The uptake and metabolism of PEA by lungs from male rats was unchanged. Uptake of PEA by lungs from female rats was inhibited 20% and 62% after 37 h and 50 h exposure respectively.

4 MAO activity, both *in vitro* and in perfused lung, was increased towards PEA after 35 h of hyperoxia.

5 Metabolism of PEA in perfused lung, measured over 30 min, was inhibited 52% after 50 h of O_2 hyperoxia.

6 These results show that exposure to high concentrations of O_2 damages lung, resulting in inhibition of uptake of PEA and consequently in inhibition of metabolism of PEA.

7 These results also indicate that, in lung from female rats, MAO-type B is more susceptible to changes in O_2 tension than MAO type A.

Introduction

The total syndrome of oxygen poisoning is caused by multiple interacting factors (for review see Clark & Lambertsen, 1971) which include direct, toxic effects upon lung tissue itself and indirect effects related to actions of oxygen at extra-pulmonary sites. The lung is the major target organ at low pressures of O_2 (Clark & Lambertsen, 1971). Little is known about the biochemical changes that may occur in the lungs of intact animals during pulmonary O_2 poisoning.

Exposure of rats to high concentrations of O_2 causes diminished pulmonary metabolism of prostaglandin E_2 (Klein, Fisher, Soltoff & Colburn, 1978), (Bakhle, Hartiala & Toivonen, 1979), angiotensin I and bradykinin (Bakhle *et al.*, 1979) and 5-hydroxytryptamine (5-HT) (Block & Fisher, 1977). Pulmonary metabolism of 5-HT and β-phenylethylamine (PEA) consists of uptake into cells and deamination by the enzyme, monoamine oxidase (MAO). The uptake systems for both 5-HT and PEA in lung have been studied (Junod, 1972; Ben-Harari & Bakhle, 1980) but it is not known whether or not high concentrations of O_2 affect this component of the metabolic process or MAO activity. To provide information on this, the uptake and deamination of [^{14}C]-PEA and [^{14}C]-5-HT were studied in lungs from rats exposed to 100% O_2 at 1 ATA for varying periods of time. A preliminary account of some of this work has been communicated to the Federation of European Biochemical Societies (Ben-Harari, Youdim & Lanir, 1980).

Methods

Animal and exposure to oxygen

Male and female rats (Sprague-Dawley strain, 100 to 150 g) were exposed to 1 atmosphere (ATA) for varying periods of time in a perspex chamber. The chamber, containing rats (6), was continuously flushed with 100% O_2 at a flow rate of 3.0 l/min; O_2 and CO_2 concentrations in the chamber were monitored continuously, the O_2 concentration exceeding 98% and the partial pressure of CO_2 being less than 1.5%. Control rats were exposed to an equivalent air flow in an equivalent cage. Food and water were provided *ad libitum*.

Preparation of lungs

Animals were killed within 1 h of the end of the O_2 exposure. The lungs were removed and perfused via

0007-1188/81/060461-006 $01.00

the pulmonary artery as described previously (Alabaster & Bakhle, 1970) with Krebs solution at 37°C gassed with a 95% O_2 and 5% CO_2 mixture at 8 ml/min. After 10 to 15 min of perfusion, the effluent was free of blood and the perfusion continued for measurement of uptake and metabolism of 5-HT and PEA.

Measurement of uptake and metabolism in perfused lungs and deamination in lung homogenates

The methods used to measure uptake and metabolism in perfused lungs, to prepare the homogentate and to assay deamination were those described previously (Tipton & Youdim, 1976; Ben-Harari & Bakhle, 1980).

Materials

Radioactive 5-HT ([2-^{14}C]-5-HT, 58 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, and radioactive PEA ([1-^{14}C]-PEA, 48 mCi/mmol) was obtained from New England Nuclear, Frankfurt. 5-HT creatinine sulphate was obtained from B.D.H. and PEA hydrochloride from Sigma. Other chemicals used were of analytical reagent grade.

Statistical methods

The significance of differences between means was calculated by Student's t test for unpaired samples and values of $P < 0.05$ were accepted as significant.

Results

General effects of O_2 exposure

Rats exposed to 100% O_2 at 1 ATA for up to 40 h appeared well and were indistinguishable on inspection from air-exposed control rats. After 40 h exposure the rats became lethargic and suffered from slight respiratory distress. At 60 h O_2 exposure, 3 rats out of 35 (8.6%) died. Isolated lungs from rats exposed to O_2 for up to 50 h were indistinguishable to the eye to lungs from control rats; after 50 h exposure the lungs contained localized points of haemorrhage.

Uptake in isolated perfused lungs

Since MAO is an intracellular enzyme, the deaminated metabolite in the effluent emerging from the lungs during the 3 min infusion is derived from amine taken up into the lung. Therefore, uptake of amine was calculated from the total radioactivity still retained in the lung after the infusion, together with the radioactive metabolite in the effluent collected. The uptake of 5-HT and PEA was studied at a single concentration of 10 μM and 50 μM respectively since the *K*ms for 5-HT and PEA uptake in rat lung are 2 μM (Junod, 1972; Bakhle & Youdim, 1979) and 25 μM (Ben-Harari & Bakhle, 1980) respectively.

Female rats were chosen at random and were, therefore, at all stages of the oestrous cycle. In female rats uptake of 5-HT was measured after 20 h, 35 h and 50 h exposure to O_2. Although after 35 h and 50 h of O_2 exposure, uptake of 5-HT was 71% and 55% of

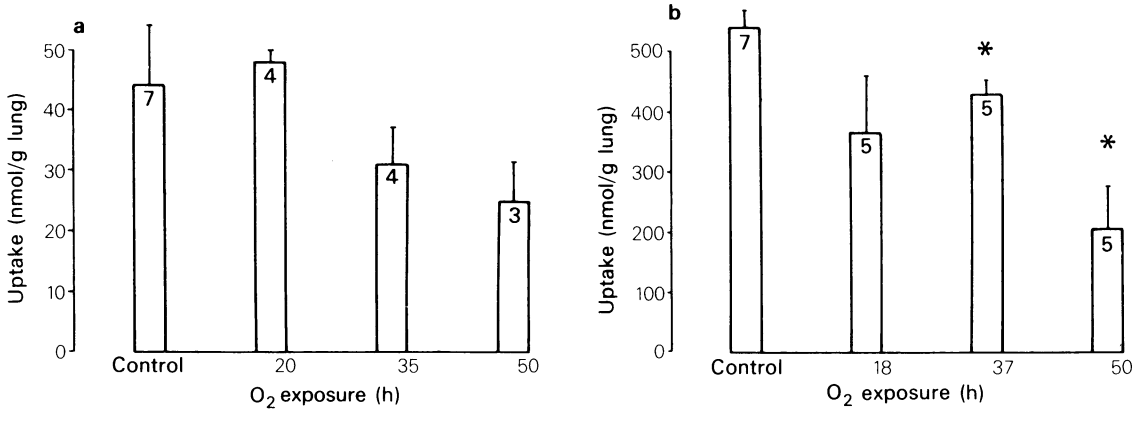

Figure 1 Effect of hyperoxia on uptake of 5-hydroxytryptamine 10 μM (a) and β-phenylethylamine 50 μM (b) in perfused lungs from female rats. The rats were at various stages of the oestrous cycle. Uptake was measured as the sum of the radioactivity retained in the lung and the radioactive metabolite in the effluent following a 3 min infusion of ^{14}C-amine and is expressed as nmol/g lung. Column height represents the mean of the number of experiments indicated; vertical lines show s.e.mean.

control values respectively (Figure 1a), these values were not significantly different compared with controls. PEA uptake was inhibited by hyperoxia and this inhibition was dependent on the duration of exposure to O_2 (Figure 1b). Exposure to 100% O_2 for 18 h, 37 h, and 50 h produced 23%, 20% and 62% inhibition and the inhibition was statistically significant at 37 h and 50 h.

For males, uptake of 5-HT and PEA in lungs from rats exposed to 100% for 60 h was unchanged.

Monoamine oxidase activity in perfused lung and in homogenates

When the metabolite in the lung is expressed as a proportion of the total radioactivity retained by the lung, these values have been shown to reflect accurately MAO activity of the lung (Ben-Harari & Bakhle, 1980). Analysis of radioactivity retained by the lung from female rats showed that during O_2 exposure, MAO activity towards 5-HT was unchanged (Table 1), but towards PEA, MAO activity appeared to increase after hyperoxia although this did not become statistically significant until after 37 h. To confirm these results obtained in perfused lung and in order to determine MAO activity directly, the deamination of [^{14}C]-5-HT (0.5 mM) and [^{14}C]-PEA (0.05 mM) was measured in homogenates of lungs from female rats. The concentrations of both amines were in excess of the *Km* of lung mitochondrial MAO for these amines (Bakhle & Youdim, 1979). Table 2 shows values for MAO activity in lung from female rats exposed to 100% O_2 for varying intervals of time. As in perfused lung, no significant changes in MAO activity occurred towards 5-HT. However, MAO activity towards PEA tended to increase after 20 h and 35 h exposure, but seemed to fall again after 50 h exposure. None of these changes were significantly different from the control values, but there was a significant difference between exposure after 50 h and exposure after 20 and 35 h.

Metabolism in isolated perfused lungs

Amine metabolism was measured after a 3 min infusion of the ^{14}C-substrate into the pulmonary artery and collecting the lung effluent for a total of 30 min in rats exposed to 100% O_2 (1 ATA) for 50 h (Table 3). As expected the metabolism of 5-HT (10 μM) in lungs from both male and female rats was unchanged after exposure to O_2 since no changes in either uptake or MAO occurred after hyperoxia. For the same reasons, pulmonary metabolism of PEA was unchanged in male rats but was significantly inhibited in lungs from female rats. Table 3 also shows that lungs from female rats have a greater capacity than those from males to metabolise both 5-HT and PEA.

Discussion

Earlier studies demonstrating an inhibition of 5-HT metabolism or clearance in lung after hyperoxia (Block & Fisher, 1977; Bakhle *et al.*, 1979) made no attempt to determine whether this was an effect on uptake or on MAO activity towards 5-HT. This study has attempted to clarify this point by measuring both uptake and MAO activity separately and to extend the studies to the amine PEA.

Hyperoxia inhibited uptake of PEA in lungs from female rats after only 37 h exposure and after 50 h exposure uptake was inhibited 62%. No significant changes occurred in the deamination of PEA by MAO from the controls. Therefore, inhibition of

Table 1 Effect of hyperoxia on the proportion of metabolite retained by isolated lungs from female rats

Treatment	*Metabolite retained (% total radioactivity retained)*	
	5-HT(10 μM)	*PEA*(50 μM)
Control (air-exposed)	87.2 ± 3.6	50.9 ± 4.9
100% O_2 (h)		
18	—	61.6 ± 3.1
20	91.8 ± 2.5	—
35	84.4 ± 7.0	—
37	—	70.5 ± 4.3*
50	78.8 ± 5.7	62.7 ± 7.6

Female rats were exposed to 100% O_2 at 1 ATA for varying periods between 18 and 50 h. The control rats were exposed to air. The rats were at all stages of the oestrous cycle. The radioactive metabolite retained in the lung after a 3 min infusion of [^{14}C]-5-hydroxytryptamine (5-HT) or [^{14}C]-β-phenylethylamine (PEA) was expressed as a percentage of the total radioactivity retained in the lung. The values shown are the mean ± s.e. from between 4–10 experiments.

*Significantly different ($P < 0.05$) from control values.

Table 2 Effect of hyperoxia on 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) deamination in homogenates of lung from female rats

Treatment	*MAO activity* (μmol mg^{-1} protein min^{-1})	
	5-HT (0.5mM)	*PEA* (0.05mM)
Control (air-exposed)	3.4 ± 0.03	1.4 ± 0.1
100% O_2 (h)		
20	3.6 ± 0.1	1.9 ± 0.2
35	3.6 ± 0.6	1.8 ± 0.2
50	3.8 ± 1.5	1.0 ± 0.2*

Female rats were exposed to 100% O_2 at 1 ATA for 20, 35 and 50 h. The control rats were exposed to air. The rats were at all stages of the oestrous cycle. Monoamine oxidase (MAO) activity was measured in lung homogenates and is expressed as nmol deaminated metabolite mg^{-1} protein min^{-1}. The procedures for preparations of the homogenates and for the incubations are described in the text. The values shown are the mean ± s.e. from at least 4 different experiments.

*Significantly different ($P < 0.05$) from 20 h and 35 h.

PEA metabolism measured over 30 min is due to changes in PEA uptake and not MAO activity. The ability to demonstrate depression of PEA uptake in female but not in male rats is consistent with sex steroid hormones hastening the onset or increasing the severity of oxygen poisoning (Clark & Lambertsen, 1971).

Uptake and deamination of 5-HT in lung from both male and female rats was not changed after hyperoxia. This result agrees with that of Bakhle *et al.* (1979), who showed that metabolism of 5-HT in lung from male rats exposed to 95% O_2 at 1 ATA was not changed until after 60 h exposure. In contrast, Block & Fisher (1977) showed a 35% inhibition of clearance of 5-HT in lung perfused in a recirculating system for 20 min after exposure of rats to 100% O_2 at 1 ATA for 48 h. This discrepancy may be due to the use by Bakhle *et al.* (1979) and in this study of a single pass 3 min infusion of 5-HT.

The site of uptake and metabolism of PEA in lung is unknown. The earliest effect of high concentrations of O_2 is on endothelial cells (Kistler, Caldwell & Weibel, 1967). Since uptake of PEA was inhibited as early as 37 h after exposure to O_2 and uptake of two other amines, 5-HT and noradrenaline, is known to occur in pulmonary endothelial cells (Strum & Junod, 1972; Nicholas, Strum, Angelo & Junod, 1974; Cross, Alabaster, Bakhle & Vane, 1974), the site of uptake and metabolism of PEA in lung may be the capillary endothelium. Uptake of PEA into lung comprises a saturable component, suggesting a specific site of uptake, and a large passive component (Ben-Harari & Bakhle, 1980). If hyperoxia is inhibiting the uptake of PEA at the specific site it would suggest that the site of uptake of PEA on the endothelium is different from that for 5-HT. However, if the passive component is being inhibited by O_2 this would still exclude an effect on 5-HT uptake since this amine has no passive component to its uptake in the lung. Non-endothelial cells may also be implicated since prostaglandin metabolism in lung, like PEA uptake, was inhibited as early as 36 h after exposure to 95% O_2 at 1 ATA (Bakhle *et al.*, 1979) and prostaglandins are not metabolized in pulmonary endothelial cells (Ryan & Ryan, 1977; Ody, Dieterle, Wand, Stalder & Junod, 1979). Mechan-

Table 3 Effect of hyperoxia on metabolism of 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) in perfused lungs from male and female rats

	Metabolite (nmol)			
	5-HT (10μM)		*PEA* (50μM)	
Treatment	*Male*	*Female*	*Male*	*Female*
Control (air-exposed)	71 ± 11	103 ± 11	494 ± 137	795 ± 146
100% O_2 at 1 ATA; 50 h	77 ± 11	104 ± 8	501 ± 143	375 ± 69*

Male and female rats were exposed to 100% O_2 at 1 ATA for 50 h. The control rats were exposed to air. The female rats were at all stages of the oestrous cycle. The values shown represent the total amount of metabolite (nmol) collected in 30 min in the effluent following a 3 min infusion of ^{14}C-labelled amine and are the mean ± s.e. from at least 4 experiments.

*Significantly different ($P < 0.05$) from control values.

isms by which O_2 might affect cells membranes and, therefore, inhibit PEA uptake have been described (Balentine, 1976).

The activity of MAO in a variety of tissues increases with high O_2 concentrations. These increases are dependent on the amine substrate used to assay for activity and the mechanisms involved have been described (Fowler & Oreland, 1979). In lung, MAO activity metabolizing 5-HT was unchanged, both *in vitro* and in perfused lung. But for PEA, both *in vitro* and in perfused lung, MAO activity tended to increase at approximately 35 h exposure to O_2 (Tables 1 and 2), although the increase was statistically significant in perfused lung only. The MAO activity in lung includes both A and B types; type A MAO deaminates 5-HT selectively and type B MAO deaminates PEA selectively (Bakhle & Youdim, 1979). It would, therefore, appear from our results that in lung, MAO-A activity is resistant to hyperoxia while MAO-B activity is more sensitive. This finding is in agreement with that of Tipton (1972), who showed that MAO-A is relatively insensitive to any fluctuations in the local concentrations of O_2 within the cell.

The lung has an enormous capacity to metabolize biogenic amines (Bakhle & Vane, 1974). Inhibition of uptake by exposure to O_2 could result in amine remaining in the arterial circulation and this could elicit unwanted effects in the lung as well as in other organs. In view of the clinical use of hyperbaric O_2 (Davis & Hunt, 1977) and the known metabolism of 5-HT in lungs of humans (Gillis, Greene, Cronau & Hammond, 1972) inhibition of pulmonary amine metabolism could produce deleterious effects in man. Conversely, the amine metabolism, particularly of PEA, in lung may provide a reliable index of O_2 poisoning in lung. Its potential for early warning of pulmonary O_2 toxicity is strengthened by the fact that depression of uptake occurred after a shorter exposure to O_2 than that reported to cause morphological and physiological changes in the lung (Kistler *et al.*, 1967).

The support of the Wellcome Trust, who provided the salary of R.R.B.H., is gratefully acknowledged. This work was supported by a grant from the Israel Ministry of Health, Jerusalem.

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(Received September 22, 1980.
Revised January 5, 1981.)