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Short communication

Specific uptake of 5-hydroxytryptamine is reduced in lungs from hypoxic pulmonary hypertensive rats

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Abstract

In this study, the aim was to determine whether 5-hydroxytryptamine (5-HT) removal by the pulmonary endothelium is reduced in 1-week hypoxic, pulmonary hypertensive rats by directly measuring [^{3}H]5-HT uptake in isolated lungs. In lungs from hypoxic rats, specific 5-HT uptake was reduced. This was due to a 50% decrease in the maximal initial rate of uptake rather than a decrease in affinity of 5-HT for its transporter. It is possible that reduced removal of 5-HT may contribute to the elevation in plasma levels of this vasoactive amine in pulmonary hypertension.

Keywords: Chronic hypoxia; 5-HT (5-hydroxytryptamine, serotonin) uptake; Pulmonary hypertension; Lung, rat

1. Introduction

One of the functions of the pulmonary vascular endothelium is the production of nitric oxide in response to endothelium-dependent vasodilators, e.g. acetylcholine. Another important function is the removal/metabolism of various vasoactive substances. For example, uptake of 5-HT by its transporter, which is located in pulmonary vascular endothelial cells (Bryan-Lluka et al., 1995), is part of the metabolic process that culminates in the degradation of 5-HT by monoamine oxidase. In rats with pulmonary hypertension induced by chronic hypoxia, the first of these functions is known to be impaired, especially in the early stages of the disease. For example, in pulmonary arteries from rats exposed to hypoxia for 1 week, relaxant responses to a number of endothelium-dependent vasodilators, including acetylcholine and adrenomedullin, are reduced (Wanstall and Crilley, 1996; Jeffery and Wanstall, 1998; Crawley et al., 1992). It is presently unknown whether removal of 5-HT by the pulmonary endothelium is similarly reduced in 1-week hypoxic rats. Therefore, the aim of the present study was to investigate this possibility by examining specific [^{3}H]5-HT uptake in isolated perfused lungs from normoxic and hypoxic rats. We directly measured specific 5-HT uptake; this is in contrast to a previous study that measured arterio-venous differences in 5-HT concentration (Hill et al., 1990). Moreover, we have obtained for the first time in isolated lungs from hypoxic rats, values for the affinity of the transporter for 5-HT (inversely related to K_m) and the maximal initial rate of 5-HT uptake (V_{max}), using Michaelis–Menten kinetic analysis.

2. Materials and methods

2.1. Rats

Male Wistar rats, aged 8 weeks on the day of the experiment, were used. Some were housed in hypoxic chambers (10% O_2) for 1 week before the experiment (Jeffery and Wanstall, 1998). Normoxic rats were housed in room air (21% O_2). Body weights of the rats were: normoxia, 269 ± 4.5 g, $n = 31$; hypoxia, 217 ± 3.6 g, $n = 30$. Rats were pretreated with pargyline (75 mg/kg i.p.), 18 and 2 h prior to the experiment to inhibit monoamine oxidase (Paczkowski et al., 1996). This investigation conforms to the ''Code of Practice for Animal Experiments'' of the National Health and Medical Research Council of Australia.

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2.2. Isolation of lungs

On the day of the experiment, rats were anaesthetized with pentobarbitone (60 mg/kg i.p.). The thoracic cavity was opened, heparin (2500 U/kg) was injected intracardially and a blood sample was collected for measurement of haematocrit. The rats were exsanguinated via the vena cava and cannulae were inserted into the trachea, pulmonary artery (via the right ventricle, RV) and left atrium (via the left ventricle). The lungs were removed, set up in a humidified chamber (37°C) and ventilated, via the tracheal cannula, under negative pressure (frequency 70 times/min, peak inspiratory pressure −2 kPa). Lungs were perfused via the pulmonary artery cannula at a constant flow rate of 10 ml/min (Desaga peristaltic pump) with physiological salt solution (PSS; composition in mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $CaCl_2$ 3.2, KH_2PO_4 1.18, $NaHCO_3$ 22.6, glucose 5.5, sucrose 50, ascorbic acid 1.02, and 4% w/v bovine serum albumin).

2.3. Experimental protocol

The method used for the measurement of 5-HT uptake in lungs has been previously described (Paczkowski et al., 1996). Lungs were equilibrated for 15 min with PSS, which was recirculated. The lungs were then perfused for 2 min with PSS containing 0.6, 2, 6, 20 or 60 μM [^{3}H]5-HT and also 100 μM [^{14}C]sorbitol. To determine non-specific 5-HT uptake, paroxetine (5-HT uptake inhibitor; 10 μM) was included in the perfusate for some experiments with 0.6 and 60 μM [^{3}H]5-HT. At the conclusion of the experiment, the perfusate was collected and the lungs were blotted and weighed. If the lung weight was greater than 0.65% of the body weight the lungs were discarded due to the development of oedema (four out of 34 lungs discarded for hypoxic rats; none of the 31 lungs discarded for normoxic rats). The lungs were then placed in 0.4 M perchloric acid containing antioxidants (2.7 mM Na_2EDTA and 10 mM Na_2SO_3) at 4°C. After at least 1 h in perchloric acid, lungs were homogenised and centrifuged at $10\,000 \times g$ for 20 min and samples of the supernatant were collected. The samples of the perfusion solution and supernatant of the lung homogenate were counted for [^{3}H] and [^{14}C] (liquid scintillation counter). The hearts were also dissected free, divided into RV and left ventricle plus septum (LV + S), blotted, weighed and the ratio of RV/(LV + S) was calculated.

2.4. Data and statistical analyses

The amount of [^{3}H]5-HT in the lung was used to calculate 5-HT uptake. The data obtained for 0.6 and 60 μM 5-HT in the presence of paroxetine was used to calculate non-specific uptake of 5-HT for each of the other experimental concentrations based on the assumption that non-specific uptake of 5-HT was linear with respect to 5-HT concentration. The initial rate of specific 5-HT uptake at each concentration was calculated by subtracting non-specific uptake from total uptake and dividing by the perfusion time of 2 min, after correction for distribution of [^{3}H]5-HT in the extracellular space (determined from [^{14}C] content of the lung). The initial rates of specific 5-HT uptake vs. 5-HT concentration were analysed by non-linear regression analysis according to a hyperbolic model to obtain the K_m (Michaelis–Menten constant) and V_{max} (maximal initial rate of uptake) for 5-HT uptake in the lungs.

Mean values are quoted together with S.E.M. and were compared by unpaired *t*-test (GraphPad Prism).

2.5. Drugs and solutions

Bovine serum albumin (Sigma, St. Louis, USA); heparin (Fisons, Sydney, Australia); 5-hydroxytryptamine (5-HT; Sigma); [^{3}H]5-HT (803 Bq/pmol; New England Nuclear Life Science Products, Boston, USA); pargyline hydrochloride (Sigma); paroxetine (gift from SmithKline Beecham, Worthing, UK); pentobarbitone sodium (Rhone Merieux, Brisbane, Australia); D-sorbitol (Sigma); D-[^{14}C]sorbitol (12 Bq/pmol; New England Nuclear Life Science Products).

Stock solutions of drugs were prepared in deionised water except pargyline hydrochloride (92.5 mg/ml), which was dissolved in saline (0.9% w/v NaCl).

3. Results

Exposure of rats to hypoxia for 1 week led to significant increases in haematocrit (normoxia, 46 ± 0.8%, n = 25; hypoxia, 65 ± 1.3%, n = 25; $P < 0.001$) and RV/(LV + S) (normoxia, 0.24 ± 0.01 g/g, n = 31; hypoxia, 0.39 ± 0.01 g/g, n = 30; $P < 0.001$). These indicate the devel-

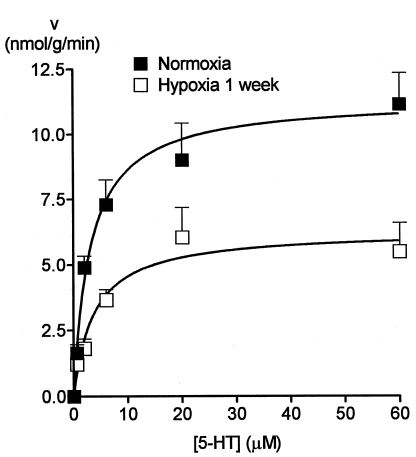

Fig. 1. Kinetic plots for 5-HT uptake in lungs from normoxic and hypoxic rats. Values are means ± S.E.M. For each concentration n = 4–7 normoxic rats, 3–5 hypoxic rats. v = rate of 5-HT uptake.

Table 1
Values for K_m and V_{max} calculated from kinetic plots in isolated lungs from normoxic and hypoxic rats
Values are means ± S.E.M.

Treatment	K_m (μM)[a]	V_{max} (nmol/g/min)[b]
Normoxia ($n = 23$)	3.16 ± 0.97	11.36 ± 0.77
Hypoxia ($n = 20$)	3.77 ± 1.92	6.28 ± 0.77[c]

[a] K_m = Michaelis–Menten constant.
[b] V_{max} = maximal initial rate of 5-HT uptake.
[c] Significantly less than the corresponding value for normoxic rats ($P < 0.001$).

opment of polycythemia and right ventricular hypertrophy, both of which are characteristic of hypoxic pulmonary hypertension.

The kinetic plots (Fig. 1) show that 5-HT uptake in lungs was saturable and in lungs from hypoxic rats was reduced when compared with lungs from normoxic rats. Values for K_m and V_{max} (Table 1) were calculated from these plots. In lungs from hypoxic rats, V_{max} was significantly reduced compared with that from normoxic rats, but there was no change in the K_m of 5-HT.

4. Discussion

In the present study, we have shown that one of the functions of the pulmonary vascular endothelium relating to metabolism, namely 5-HT uptake, is impaired in perfused lungs from 1-week hypoxic pulmonary hypertensive rats. The reduction in 5-HT uptake was due to a decrease in the maximal initial rate of uptake (V_{max}) rather than a decrease in affinity of 5-HT for its transporter (inversely related to K_m).

In only one other study has 5-HT uptake been quantified in lungs from rats exposed to chronic, in vivo hypoxia (Hill et al., 1990). However, in contrast to the current investigation, 5-HT uptake was unchanged. The disparate conclusions may reflect the fact that Hill et al. (1990) calculated the amount of 5-HT uptake from 5-HT arterio-venous differences. In situations where arterio-venous differences are large (as seen in the study of Hill et al., 1990), any change in 5-HT uptake in the hypoxic rats could be masked by the corresponding change in the 5-HT concentration gradient as it perfuses through the pulmonary circulation. In the method used in the present study this problem is circumvented. Two other differences between the two studies are the species of rat (Sprague–Dawley vs. Wistar) and duration of hypoxic exposure (2 weeks vs. 1 week). It is unknown whether these could explain the different conclusions.

Some speculations can be made as to the mechanism behind the reduction in V_{max} seen in hypoxic rats. Firstly, there could be fewer 5-HT transporters per endothelial cell due to decreases in 5-HT transporter mRNA and/or trafficking of the protein to the cell membrane or, alternatively, incorrect folding of the protein in the cell membrane. A decrease in 5-HT transporter mRNA is unlikely since in lungs from 2-week hypoxic rats, in situ hybridization studies demonstrated increased, rather than decreased, 5-HT transporter mRNA when compared with normoxic lungs (Eddahibi et al., 1999). Despite the increase in 5-HT transporter mRNA, a decrease in trafficking or incorrect folding of the protein cannot be excluded. Secondly, the endothelial cells themselves could be altered, i.e. decreased in number or damaged. It is improbable that the number of endothelial cells per vessel is reduced since there is evidence that there is hyperplasia of the pulmonary endothelial cells of rats with hypoxic pulmonary hypertension (Meyrick and Reid, 1980). If there is actual loss of pulmonary vessels in the microcirculation the total number of endothelial cells would be reduced, but the evidence for loss of vessels in hypoxic rats is conflicting (Hislop and Reid, 1976; Finlay et al., 1986). It is more likely that the reduction in 5-HT uptake reflects endothelial cell damage because pulmonary endothelial cells are abnormal and/or necrotised in hypoxic rats (Meyrick and Reid, 1980). Furthermore, in another model of pulmonary hypertension (monocrotaline treated rats), where endothelial damage occurs, 5-HT uptake is reduced (Hilliker et al., 1983).

In contrast to the present findings, when cultured pulmonary endothelial cells are exposed to hypoxia in vitro (3% O_2; 72 h), 5-HT uptake is increased (Lee and Fanburg, 1986) suggesting that the consequences of in vitro and in vivo hypoxia are different. It should be noted that, unlike chronic in vivo hypoxia (Meyrick and Reid, 1980), exposure of endothelial cells to in vitro hypoxia does not cause alterations in their structure (Lee and Fanburg, 1986) and this may explain the differing results.

In conclusion, the endothelial dysfunction that is seen in the pulmonary circulation of 1-week hypoxic rats comprises not only a decrease in endothelium-dependent vasodilatation, as previously reported (Crawley et al., 1992; Jeffery and Wanstall, 1998), but also a reduction in the pulmonary removal of 5-HT. It is known that there are increased circulating 5-HT levels in both humans and rats with pulmonary hypertension (MacLean, 1999). It is possible, though yet to be proven, that reduced pulmonary removal of 5-HT, as described in the present study, may contribute to the elevation in plasma levels of this vasoactive amine.

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