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The Effect of Fenfluramine on the Pulmonary Disposition of 5-Hydroxytryptamine in the Isolated Perfused Rat Lung: a Comparison with Chlorphentermine

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Abstract

A possible mechanism for fenfluramine-induced pulmonary hypertension has been investigated. Fenfluramine, like chlorphentermine, may inhibit the pulmonary uptake and/or metabolism of 5-hydroxytryptamine (5-HT). This allows more 5-HT to remain in the pulmonary circulation, where it may exert a greater vasoconstrictor action resulting in pulmonary hypertension. Chlorphentermine has been shown to inhibit the uptake and metabolism of 5-HT.

The effect of fenfluramine on the pulmonary disposition of [^{14}C]5-HT has been investigated, in comparison with chlorphentermine, using a recirculating isolated perfused rat lung system. The pulmonary disposition of [^{14}C]5-HT was assessed by measuring the change in [^{14}C]5-HT concentration in the perfusion medium during the experiment and at the end, and the concentration in the lung at the end of the experiment. The concentration of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, was measured in perfusate and lung samples. Mean pulmonary clearance of 5-HT for the control lung and lungs challenged with either fenfluramine (2·5 μM) or chlorphentermine (2·5 μM) was 4·514, 1·316 and 1·007 mL min^{-1}, respectively (n = 5). The concentration of 5-HT found in the lungs at the end of the experiment for the control and the lungs preloaded with fenfluramine or chlorphentermine was 695·05 ± 9·69, 638·65 ± 10·27 and 617·3 ± 14·38 ng g^{-1}, respectively.

Fenfluramine, like chlorphentermine, inhibited the pulmonary disposition of 5-HT resulting in an elevated perfusate level of 5-HT. This is a possible contributing mechanism for fenfluramine-induced pulmonary hypertension. The effect of fenfluramine was less pronounced than chlorphentermine.

Fenfluramine is an appetite-suppressant reported to induce pulmonary hypertension in man (Pouwels et al 1990; Brenot et al 1993; Abenhaim et al 1996), but the mechanism for this effect is unknown. Drugs may induce pulmonary hypertension by potentiating the effect of endogenous mediators such as 5-hydroxytryptamine (5-HT) (Hervé et al 1995) present in the pulmonary circulation. The pneumophilicity of 5-HT and its pulmonary vasoconstrictor effect makes it a possible endogenous mediator involved in the pathogenesis of pulmonary hypertension (Ozdemir et al 1972).

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Chlorphentermine inhibits the pulmonary uptake and metabolism of 5-HT producing higher levels of 5-HT in the pulmonary circulation. This hypothesis has been accepted as a possible mechanism for chlorphentermine-induced pulmonary vasoconstriction (Seiler et al 1972, 1974; Angevine & Mehendale 1982). According to this hypothesis, if fenfluramine should exert a similar effect on the pulmonary 5-HT disposition, then this may indicate a possible mechanism for fenfluramine-induced pulmonary hypertension. The objective of this study was to assess whether fenfluramine inhibited the pulmonary disposition of [^{14}C]5-HT in the isolated perfused rat lung in comparison with chlorphentermine and to discover the extent of the inhibition.

Materials and Methods

Animals and surgical procedure

Fifteen female BD9 rats (Provincial Animal Centre, Kuilsriver, South Africa) had free access to food and water and were kept on a 12-h light:dark cycle. Each rat was sedated with ether (Holpro, Cape Town, South Africa) before being anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.) (Maybaker, Port Elizabeth, South Africa). The external jugular vein was exposed and 1000 units kg^{-1} heparin sodium (Intramed, Johannesburg, South Africa) was administered via this vein to prevent blood coagulation. The trachea, heart and lungs were removed intact. A clamp on the trachea prevented blood from entering the lungs. Throughout the surgical procedure extreme care was taken not to puncture the lungs. The isolated heart–lung preparation was immersed immediately in cold saline maintained at approximately 4°C.

Isolated perfused rat lung system

The recirculating isolated perfused rat lung system was used in this study (Valodia 1989). The perfusion medium consisted of Krebs–Ringer bicarbonate buffer to which 4·5% bovine serum albumin (BDH, Poole, UK) and 5 mM glucose were added (Angevine & Mehendale 1980). The pH of the perfusion medium was adjusted to 7·4 initially and during the perfusion by a drop-wise addition of 1 M NaOH or with a direct stream of carbon dioxide. The cannula containing the flowing perfusion medium was inserted into the pulmonary trunk via the right ventricle and tied into place. The heart was trimmed away to provide an outlet for the perfusion medium. The lungs were perfused under gravitational force to wash away residual blood and the perfusate flowing out of the lungs was collected. Only lungs which were satisfactorily perfused were mounted in the artificial thorax. The final volume of the perfusion medium was 50 mL. After all the parameters were set, the lungs were equilibrated for 10 min. Visual monitoring for development of oedematous areas on the lungs was performed throughout the experiment. A drop in the flow of perfusate from the lungs or a rise in the pulmonary arterial pressure were assumed to indicate fluid accumulation in the lungs. The lungs so afflicted were discarded.

Experimental protocol

For the control experiments (n = 5), 5 μg ^{14}C-labelled 5-HT (Sigma, St Louis, MO) was added to the perfusion medium in the reservoir as a bolus dose. Perfusate samples of 0·5 mL were withdrawn from the reservoir at 3, 5, 10, 15, 20, 30, 40, 50 and 60 min after the addition of [^{14}C]5-HT to the perfusate. The samples were analysed for total radioactivity, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of 5-HT). The sample concentrations were corrected for the volume of perfusate removed from the reservoir. To monitor the effects of either fenfluramine (donated by Potchefstroom University, obtained from Servier Laboratories) (n = 5) or chlorphentermine (n = 5) on 5-HT disposition, 2·5 μM of each drug was added to the perfusate in the reservoir and this was followed by a 20-min equilibration period. This was followed by the addition of 5 μg [^{14}C]5-HT as a bolus dose and the same procedure as for the control experiment was followed. Chlorphentermine hydrochloride was synthesized according to the procedure for clortermine hydrochloride (Marshall 1979). The only difference between chlorphentermine and clortermine is that for chlorphentermine the chlorine atom is in the para- instead of the ortho-position on the benzene ring. Thus for the synthesis of chlorphentermine, *p*-α-dichlorotoluene was used as the starting material instead of *o*-α-dichlorotoluene as for clortermine.

At the end of the experiment, the lungs were removed promptly from the perfusion apparatus and cut into small pieces, blotted dry and weighed. They were homogenized in 3 vols 0·5 M perchloric acid (Angevine & Mehendale 1980) for 30 s at 4°C. Perchloric acid was used to stop the metabolism of 5-HT to 5-HIAA. The resulting homogenate was centrifuged at 10 000 *g* for 20 min at 4°C. Since 5-hydroxyindoles are unstable in perchloric acid, the resulting supernatant was rapidly adjusted to pH 6 with 0·4 M potassium bicarbonate (Angevine & Mehendale 1980). The volume of supernatant obtained after the adjustment of pH was recorded and a sample of this solution was analysed for total radioactivity, 5-HT and 5-HIAA.

5-HT is metabolized by monoamine oxidase and to determine the effect which fenfluramine and chlorphentermine might have on such metabolism the method of Angevine & Mehendale (1980) was used. The concentrations of [^{14}C]5-HT, chlorphentermine or fenfluramine used in our experiment were the same as that used in our isolated lung perfusion studies (n = 10). The metabolism of [^{14}C]5-HT in-vitro indicated the activity of the monoamine oxidase enzyme. Lung homogenates served as a source of monamine oxidase. The monoamine oxidase preparation was pre-incubated with either fenfluramine or chlorphentermine for 5 min. This was followed by the addition of [^{14}C]5-HT. The procedure as described by

Angevine & Mehendale (1980) was then followed. The percentage inhibition of metabolism of [^{14}C]5-HT by either fenfluramine or chlorphentermine was determined.

Chromatography

5-HT and 5-HIAA were separated on a chromatographic column prepared by packing Biorex 70 cation exchange resin (Bio-Rad, California) to a height of 2·5 cm in a Pasteur pipette. Each column was freshly packed and discarded after a single use. Samples (250 μL) of perfusate or lung were applied to separate chromatographic columns. This was followed by distilled water (3 mL) and the eluates containing 5-HIAA were collected in pre-weighed glass vials. The adsorbed 5-HT was subsequently eluted with 0·5 M hydrochloric acid (3 mL) into other pre-weighed glass vials (Gillis & Roth 1977). The vials were re-weighed and the volume of each eluate was determined. All of the above was performed in duplicate.

Assay

The radioactivity of the eluates and the non-separated perfusate and lung samples were quantified using a liquid scintillation method. A 0·5-mL sample of each eluate was radioassayed. Samples (250 μL) of each perfusate and lung were assayed for total radioactivity. Standard curves were constructed to quantify the total radioactivity in the perfusate or lung, 5-HT concentration in perfusate, and 5-HT concentration in the lung. Correlation coefficients were 0·999, 0·90 and 0·998, respectively. Each assay was performed in duplicate. The radiochemical purity of [^{14}C]5-HT was established as 90% by thin-layer chromatography. This was taken into account when determining the final concentration of 5-HT present in the perfusate and lung samples.

Results

The perfusate concentrations of [^{14}C]5-HT, 5-HIAA and total concentration of 5-HT and 5-HIAA (μg/50 mL) for the control lung preparations (i.e. no fenfluramine or chlorphentermine added) at specific times following the administration of 5-HT are illustrated in Figure 1. The graph obtained was characterized by an initial sharp decrease in the concentration of 5-HT in the perfusate indicating a rapid uptake of 5-HT into the lung. The concentration of 5-HT declined for 20 min to a plateau and thereafter maintained a steady state for the rest of the duration of the experiment. The 5-HIAA appeared rapidly in the perfusate as evidence of rapid metabolism of lung accumulated 5-HT. The percent of 5-HT inactivated during the perfusion was calculated as follows:

$$\text{percent of removal} = \left(\frac{C_0 - C_t}{C_0}\right) \times 100 \qquad (1)$$

where C_0 and C_t are the concentrations of 5-HT in the reservoir at times 0 and t, respectively (Cook & Brandom 1982; Cook et al 1982; Olson & Rankin 1983). In the control lung preparations 88·86% of the 5-HT was inactivated in 60 min. Figure 1 illustrates that the concentrations for the total radioactivity decreased rapidly at first, then increased. This increase in total radioactivity was paralleled by the rapid appearance of 5-HIAA in the perfusate. An overlap of the curve for the total radioactivity and for the concentration of 5-HIAA in the perfusate, although not possible theoretically, was observed. This discrepancy and the large standard deviations sometimes found for the individual points were due probably to experimental errors such as differences in lung volumes or surface areas and the volume of the perfusate in the 'circulation'. This discrepancy was not significant, as the trends obtained in these experiments were clear.

For the lungs preloaded with fenfluramine, the concentrations of 5-HT and 5-HIAA obtained in the perfusate at specific times during the perfusion are illustrated in Figures 2 and 3, respectively. These graphs are compared with those obtained for the control lungs and for lungs preloaded with chlorphentermine. The mean concentrations of 5-HT in the perfusate at specific times during the perfusion for lungs preloaded with fenfluramine were higher than those obtained for the respective control

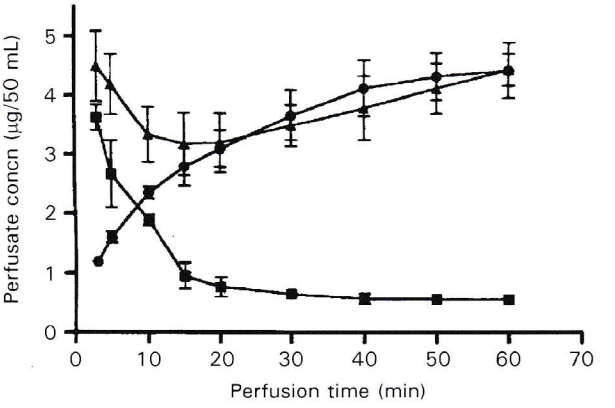

Figure 1. Perfusate concentration of 5-HT (■), 5-HIAA (●) and total ^{14}C (▲) as a function of perfusion time for the control lung preparations. Each data point represents the mean ± s.d. of five experiments.

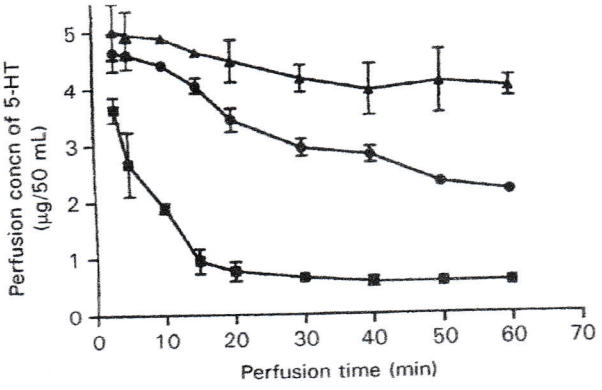

Figure 2. Perfusate concentration of 5-HT as a function of perfusion time for the control lung (■) and lungs preloaded with fenfluramine (●) or chlorphentermine (▲). Each data point represents the mean ± s.d. of five experiments.

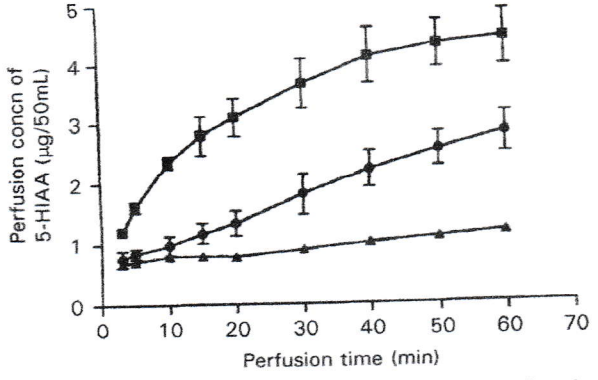

Figure 3. Perfusate concentrations of 5-HIAA as a function of time for the control lungs (■) and lungs preloaded with fenfluramine (●) or chlorphentermine (▲). Each data point represents the mean ± s.d. of five experiments.

values. A further increase in the mean concentrations of 5-HT for the lungs preloaded with chlorphentermine was found. At the end of the experiment, the percentage of 5-HT inactivated by the lungs challenged with either fenfluramine or chlorphentermine was 56·88 and 20·32%, respectively.

The percent inhibition of 5-HT removal due to the effect of either fenfluramine or chlorphentermine was calculated as follows:

$$\text{percent inhibition} = \left(\frac{R_c - R_t}{R_c}\right) \times 100 \quad (2)$$

where R_c and R_t are the percentages of 5-HT removal for the control and test drugs, respectively (Cook & Brandom 1982). The percent inhibition of 5-HT removal for lungs challenged with either fenfluramine or chlorphentermine was 35·99 and 77·13%, respectively. The mean concentration of 5-HT in the perfusate at the end of the experiment for the control lungs and for the lungs challenged with either fenfluramine or chlorphentermine was 11·15, 32·42 and 77·86% respectively of the total amount of 5-HT added to the perfusate, i.e. 5 μg. The results indicated that at the end of the experiment less 5-HT was present in the perfusate for the lungs pretreated with fenfluramine than with chlorphentermine.

The metabolism of 5-HT and the subsequent release of 5-HIAA into the perfusate occurred very rapidly for the control lungs. The exposure of the lungs to either fenfluramine or chlorphentermine for 60 min reduced the production of 5-HIAA. The extent of 5-HT metabolism was greater for the fenfluramine-challenged lungs when compared with the chlorphentermine challenged lungs (Figure 3). The mean concentration of 5-HIAA in the perfusate was higher for the lungs preloaded with fenfluramine than for chlorphentermine throughout the experiment (Figure 3). At the end of the experiment, the mean concentration of 5-HIAA in the perfusate was 59·11 and 24·62% of the control value for fenfluramine and chlorphentermine, respectively.

A semilogarithmic plot of [^{14}C]5-HT concentration in the perfusate vs perfusion time (Figure 4) showed a linear decrease over the entire period for the lungs preloaded with the test drugs. The graph suggests that first-order kinetics were applicable over the period studied (Cook & Brandom 1982). The control lungs did not show this relationship because the 5-HT concentration in the perfusate decreased linearly only over the initial 15 min after the addition of [^{14}C]5-HT to the perfusate. For the control, the initial decline in the concentration (distributive phase) indicated that 5-HT was rapidly distributed into the lungs and the latter part of the graph illustrated that metabolism of 5-HT became saturated. In lungs preloaded with fenfluramine or

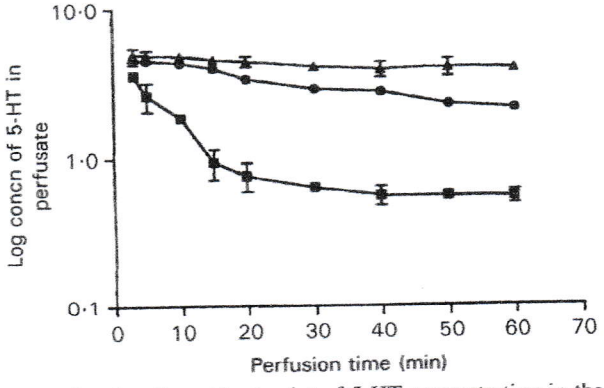

Figure 4. Semilogarithmic plot of 5-HT concentration in the perfusate as a function of time for the control (■) and lungs preloaded with fenfluramine (●) or chlorphentermine (▲). Each data point represents the mean ± s.d. of five experiments.

chlorphentermine, the 5-HT is shown to distribute less into the lungs, thus indicating that both drugs inhibited the uptake of 5-HT into the lungs. The metabolism of 5-HT in the presence of fenfluramine or chlorphentermine was not saturated (Figure 4).

The graphs showing the total radioactivity present in the perfusate (Figure 5) illustrated that the total radioactivity was lower for the fenfluramine-preloaded lungs than for chlorphentermine throughout the experiment. The initial rapid decrease in the total radioactivity in the perfusate as seen for the control was not seen for the test drugs, thus indicating that the test drugs inhibited the uptake of 5-HT into the lungs or that 5-HIAA was rapidly effluxed from the lung.

The concentration of 5-HT in the lungs ($ng\,g^{-1}$) at the end of the experiment for the control and the lungs preloaded with either fenfluramine or chlorphentermine, and the pulmonary clearance ($mL\,min^{-1}$) of 5-HT in the presence or absence of the test drugs are presented in Table 1. More 5-HT was found in the lungs in the presence of fenfluramine compared with chlorphentermine (Table 1). This implies that fenfluramine inhibited the pulmonary disposition of 5-HT to a lesser extent than chlorphentermine. The pulmonary clearance of 5-HT was calculated from the graphs in Figure 2 using the following equation (Shargel & Yu 1985):

$$\text{Clearance} = \frac{\text{Dose of 5-HT (resevoir)}}{\text{Area under the curve}} \qquad (3)$$

The pulmonary clearance of 5-HT in the presence of either fenfluramine or chlorphentermine was 29·15 and 22·31%, respectively, of the control value. The data conclusively show that these drugs inhibited the pulmonary clearance of 5-HT resulting in a higher perfusate concentration of 5-HT.

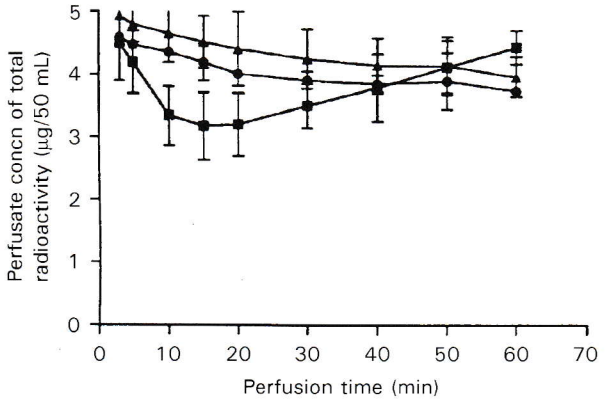

Figure 5. Perfusate concentrations of total radioactivity as a function of time for the control lungs (■) and lungs preloaded with fenfluramine (●) or chlorphentermine (▲). Each data point represents the mean ± s.d. of five experiments.

Table 1. The pulmonary clearance ($mL\,min^{-1}$) and concentration in the lung ($ng\,g^{-1}$) of 5-HT for the control lungs and lungs challenged with fenfluramine and chlorphentermine.

Treatment	Amount of 5-HT in the lung ($ng\,g^{-1}$)	Clearance ($mL\,min^{-1}$)
Control	695·05 ± 9·69	4·514
Fenfluramine	638·65 ± 10·27	1·316
Chlorphentermine	617·3 ± 14·38	1·007

The results indicated that fenfluramine was a weaker monoamine oxidase inhibitor compared with chlorphentermine. In the presence of fenfluramine, 48·37% of the 5-HT was metabolized compared with 41·6% in the presence of chlorphentermine ($n = 10$) ($P < 0·05$, Student's t-test).

Discussion

5-HT is a pulmonary vasoconstrictor (MacLean et al 1996) and an inhibition of its uptake and/or metabolism may have major consequences on vascular function in the pulmonary vessels. Our study demonstrated that fenfluramine caused an elevated level of 5-HT in the perfusate. A mechanism for fenfluramine-induced pulmonary hypertension may be that it inhibits the pulmonary disposition of 5-HT. As the effect of fenfluramine was less than chlorphentermine it may have a lower potential for inducing pulmonary hypertension.

Our investigation demonstrated that 5-HT was extensively metabolized by the rat lung, a finding consistent with that reported by Angevine & Mehendale (1980). Those authors reported that 82% of [^{14}C]5-HT added to perfusate was metabolized during 60 min of perfusion in their isolated rabbit lung preparation. The rat lungs in our study metabolized 88·86% of [^{14}C]5-HT for the same time period.

In our study, fenfluramine and chlorphentermine increased the concentration of 5-HT in the perfusate with a concomitant decrease in the concentration of 5-HIAA. Similarly Angevine & Mehendale (1980) noted this with chlorphentermine when using the isolated lung perfusion system. A similar effect with chlorphentermine was noted in rabbits in-vivo during a single pass circulation through the lungs (Mehendale et al 1983). The effect of chlorphentermine on 5-HT clearance is mediated via two mechanisms. Firstly, chlorphentermine inhibits the pulmonary uptake of 5-HT. If the uptake of 5-HT is hindered by chlorphentermine, less 5-HT will reach the sites in the lungs for metabolism. Secondly, chlorphentermine decreases 5-HT clearance by inhibition of its

metabolism. This effect was illustrated by incubating 5-HT in-vitro with chlorphentermine and lung homogenates, the latter serving as a source of monoamine oxidase. Chlorphentermine effectively inhibited monoamine oxidase thus inhibiting the metabolism of 5-HT (Angevine & Mehendale 1980). Our study demonstrated that chlorphentermine and fenfluramine both inhibited lung monoamine oxidase activity in-vitro but not to the same extent. The results indicated that fenfluramine was a weaker monoamine oxidase inhibitor compared with chlorphentermine ($P < 0.05$).

The extent to which fenfluramine and chlorphentermine affect the concentration of 5-HT in the perfusate is of significance. It is the concentration of 5-HT in the pulmonary circulation that will determine its potential to induce adverse effects such as pulmonary hypertension. This study shows that a possible mechanism for fenfluramine-induced pulmonary hypertension is that it inhibits the pulmonary disposition of 5-HT resulting in an elevated perfusate level of 5-HT.

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