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# 5-Hydroxytryptamine and platelets: uptake and aggregation in hypoxic pulmonary hypertensive rats

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**Abstract**

Pulmonary hypertension is associated with various alterations in 5-hydroxytryptamine (5-HT) physiology. In this study in platelets from hypoxic pulmonary hypertensive rats (10% $O_2$; 1 week) and normoxic rats (room air), (i) initial rates of specific [$^3$H]5-HT uptake were measured and (ii) potentiation of collagen- and ADP-induced aggregation by 5-HT was quantified. The platelet count was almost halved in hypoxic rats. In uptake experiments, there was a decrease in 5-HT uptake in platelets from hypoxic compared with normoxic rats, due to a 36% reduction in the maximal initial rate of uptake. The aggregation experiments showed that 5-HT (1–100 μM) increased the magnitude of responses to collagen and the duration of responses to ADP, but there was no difference between hypoxic and normoxic rats. Abnormalities in platelet function may conceivably lead to increases in plasma 5-HT levels in hypoxic pulmonary hypertension, but are unlikely to aggravate pulmonary thromboembolism.


*Keywords:* Platelet; 5-HT (5-hydroxytryptamine, serotonin); SERT (serotonin transporter); Hypoxic pulmonary hypertension; Aggregation

## 1. Introduction

5-Hydroxytryptamine (5-HT) is a pulmonary vasoconstrictor and vascular smooth muscle mitogen, and has been implicated in the pathophysiology of pulmonary hypertension (Egermayer et al., 1999; MacLean et al., 2000). There are several lines of evidence suggesting a role for 5-HT in humans with pulmonary hypertension. For example, in patients with primary pulmonary hypertension, it has been shown that plasma levels of 5-HT are elevated (Herve et al., 1995) and that their pulmonary arteries have increased sensitivity to the vasoconstrictor effects of 5-HT (Brink et al., 1988). In addition, pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension, when compared with those from normal individuals, show increased expression of the 5-HT transporter (SERT), greater uptake of 5-HT and more rapid proliferation in response to 5-HT (Eddahibi et al., 2001). Furthermore, the SERT gene L variant, which is associated with a greater mitogenic response to 5-HT in vascular smooth muscle than the S variant, occurs more frequently in these patients than in the general population (Eddahibi et al., 2001). Finally, an increased incidence of primary pulmonary hypertension is associated with administration of the anorectic agents, aminorex (Gurtner, 1985) and fenfluramine (Douglas et al., 1981), both of which are known to be substrates for SERT (Rothman et al., 1999).

Further support for the role of 5-HT in pulmonary hypertension has been obtained in animal models of the disease. In rats with pulmonary hypertension, induced by either hypoxia or monocrotaline, vasoconstrictor responsiveness to 5-HT was increased in pulmonary arteries (Doggrell et al., 1999; Jeffery and Wanstall, 2001; Kanai et al., 1993; MacLean et al., 1996; Wanstall and O'Donnell, 1990), but not in systemic vessels (Doggrell et al., 1999; Wanstall and O'Donnell, 1990). Increased plasma levels of 5-HT have been demonstrated in rats with monocrotaline-induced pulmonary hypertension (Kanai et al., 1993). Lungs and pulmonary arteries from chronically hypoxic rats showed increased levels of SERT mRNA (Eddahibi et al., 1999), and the development of hypoxic pulmonary hypertension was attenuated in mice genetically deficient in SERT (Eddahibi et al., 2000a). Paradoxically, uptake of 5-HT by

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SERT in pulmonary vascular endothelium was reduced in rats with hypoxic pulmonary hypertension (Jeffery et al., 2000).

Platelets represent an important cell type in the physiology of 5-HT. Circulating levels of 5-HT are regulated by active transport by SERT into platelets where the amine is stored in dense granules (Born et al., 1972). Furthermore, 5-HT is released from aggregating platelets and is itself proaggregatory (Poole, 1996). There is evidence in humans that alterations in platelet function may occur in association with primary pulmonary hypertension. For example, (i) in a patient with a rare hereditary platelet storage disease, decreased platelet 5-HT storage was associated with the development of primary pulmonary hypertension (Herve et al., 1990) and (ii) increased uptake of 5-HT and increased binding of the SERT inhibitor, citalopram, were reported in platelets from patients with primary pulmonary hypertension (Eddahibi et al., 2001).

Currently, it is not known whether alterations in platelet function occur in hypoxic pulmonary hypertension. Hence, the purpose of this study was to examine two aspects of platelet function in hypoxic rats, viz. (i) uptake of 5-HT by the SERT and (ii) the proaggregatory effects of 5-HT.

Preliminary data from this study have been presented to meetings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Dunedin, NZ (Awabdy et al., 2001) and the European Society for Microcirculation, Devon, UK (Wanstall et al., 2002).

## 2. Materials and methods

### 2.1. Induction and assessment of hypoxic pulmonary hypertension

The experiments in this study conform to the "Code of Practice for Animal Experiments" of the National Health and Medical Research Council of Australia and have been approved by the University of Queensland Animal Ethics Committee. Male Wistar rats, aged 7 weeks, were placed in a normobaric, hypoxic chamber containing 10% oxygen for a period of 1 week. This regime has been shown previously to induce pulmonary hypertension and right ventricular hypertrophy in rats (Jeffery and Wanstall, 2001). Control rats were housed in room air (21% oxygen; normoxic rats). The weights of the rats on the day of the experiment were: normoxic $353 \pm 14$ g ($n = 19$); hypoxic $293 \pm 6$ g ($n = 44$). After obtaining platelet preparations (Section 2.2), a blood sample was obtained for measurement of haematocrit. Polycythemia was taken as evidence of chronic hypoxia. The heart was then removed, separated into right ventricle (RV) and [left ventricle plus septum] ([LV + S]), blotted and weighed. Using these heart weights, ratios of RV/[LV + S], RV/body weight, and [LV + S]/body weight were calculated. Right ventricular hypertrophy was interpreted as evidence of pulmonary hypertension (Jeffery and Wanstall, 2001).

### 2.2. Isolation of rat platelets

On the day of the experiment, rats were anaesthetised with sodium pentobarbitone (90 mg/kg i.p.). Blood (7 ml) was collected from the abdominal aorta using a syringe containing heparin (105 IU; final heparin concentration 15 IU/ml) and then transferred into a 10 ml polypropylene centrifuge tube. Platelet-rich plasma was obtained by centrifuging the blood sample at $200 \times g$ for 10 min. In some experiments, the volumes of platelet-rich plasma were recorded and found to be significantly lower in hypoxic rats ($0.59 \pm 0.05$ ml, $n = 12$) than in normoxic rats ($1.44 \pm 0.14$ ml, $n = 5$; $P < 0.001$, Student's $t$-test). The number of platelets per microliter of platelet-rich plasma was determined microscopically using a haemocytometer. For uptake experiments (Section 2.3), the number of platelets was counted in platelet-rich plasma samples from individual rats, but in platelet aggregation experiments (Section 2.4), the platelet count was determined from pooled platelet-rich plasma samples. The remaining blood was centrifuged for a further 15 min at $4000 \times g$ to obtain platelet-poor plasma.

### 2.3. Uptake experiments

For each experiment on platelets from normoxic rats, the amount of platelet-rich plasma obtained from one rat was adequate. In the hypoxic rats, there were decreases in both the volume of platelet-rich plasma (see Section 2.2) and the platelet count (see Section 3.1), so the platelet-rich plasma from two to three hypoxic rats was pooled for each experiment. The platelet-rich plasma sample from normoxic or hypoxic rats was centrifuged for 8 min at $1000 \times g$ and the supernatant was removed. The resulting pellet was resuspended in platelet incubation buffer (NaCl 140 mM, KCl 5 mM, $MgSO_4$ 1.2 mM, HEPES 10 mM, glucose 10 mM; pH adjusted to 7.4 with 1 M Tris; supplemented with 50 μl/ml platelet-poor plasma) containing 100 μM pargyline (to inhibit monoamine oxidase) and incubated for 30 min at 37 °C. The platelets were centrifuged for a further 8 min at $1000 \times g$, and the resulting pellet was resuspended in platelet incubation buffer to give a platelet-rich suspension containing $0.65–1.0 \times 10^6$ platelets/μl.

Samples (100 μl) of the platelet-rich suspension were incubated with or without 10 μM paroxetine for 15 min at 37 °C and [$^3$H]5-HT (10–1000 nM) was then added for 20 s. Uptake was terminated by the addition of ice-cold platelet incubation buffer supplemented with 100 μM dopamine at 0 °C followed by filtration (Whatman GF/F filters pre-soaked with 0.3% polyethylenimine) under a constant vacuum of 10–12 mm Hg. Each filter paper was then placed in a counting vial with 2.5 ml Starscint scintillation medium (Packard, Melbourne, Victoria, Australia), vigorously vortexed for 1 min and shaken overnight. [$^3$H]5-HT content was then determined in a 2500 TR Packard liquid scintillation counter. Samples of 10 nM [$^3$H]5-HT (50 μl) were also counted to determine specific activity.

Uptake of [$^3$H]5-HT was expressed in pmol per $10^8$ platelets using the results of liquid scintillation counting and the number of platelets present. Data obtained from platelets exposed to 10–1000 nM [$^3$H]5-HT in the presence of 10 μM paroxetine were used to calculate non-specific uptake for each corresponding experimental concentration. The rates of specific [$^3$H]5-HT uptake were calculated by subtracting non-specific [$^3$H]5-HT uptake from total uptake, and dividing by the number of platelets and the incubation time. The initial rates of specific [$^3$H]5-HT uptake versus [$^3$H]5-HT concentration were analysed by non-linear regression analysis according to a hyperbolic model to determine the $K_m$ (Michaelis–Menten constant) and $V_{max}$ (maximal initial rate of uptake) of [$^3$H]5-HT uptake into the platelets.

### 2.4. Platelet aggregation experiments

For the aggregation experiments, platelet-rich plasma from two normoxic rats or four hypoxic rats was pooled for each experiment. The pooled platelet-rich plasma samples were then diluted with platelet-poor plasma, to achieve a platelet count of $6 \times 10^5$ platelets per μl, and then 1:1 with normal saline [0.9% NaCl].

Platelet aggregation was determined in platelet-rich plasma using a turbidimetric aggregometer (Chrono-log, Havertown, PA, USA). The contents of the cuvettes (total volume 500 μl, including drugs) were stirred constantly at 1200 rpm at 37 °C and changes in light transmission were recorded. Light transmission through platelet-poor plasma (diluted 1:1 with normal saline) and diluted platelet-rich plasma was used to calibrate maximum (100%) and minimum (0%) light transmission, respectively. The proaggregatory effects of 5-HT (0.1–100 μM) on aggregation induced by collagen (1.5–4.5 μg/ml) or ADP (0.3 μM) were determined. The concentrations of aggregating agent were selected so that control responses (no 5-HT) were not greater than 50% aggregation. Platelet-rich plasma was incubated with 5-HT for 30 s prior to the addition of the aggregating agent. In some experiments, platelet-rich plasma samples were pre-incubated with paroxetine (10 μM) for 15 min at 37 °C before the addition of 5-HT.

The response to the aggregating agent (collagen or ADP), recorded at the peak of aggregation, was measured as the difference in light transmission through diluted platelet-rich plasma before and after the addition of aggregating agent, and expressed as a percentage of the difference between minimal and maximal light transmission, as defined above. Unlike responses to collagen, responses to ADP were not sustained. Hence, in experiments with ADP, the duration of the response was also measured and is defined as the time from peak response to 50% recovery (minutes). The effect of 5-HT on aggregation responses (magnitude or duration of response) was expressed as a 'potentiation factor' defined as: response with 5-HT present/response with 5-HT absent.

### 2.5. Drugs and solutions

ADP (Helena Laboratories, Melbourne, Victoria, Australia); collagen (Helena Laboratories); dopamine hydrochloride (Sigma-Aldrich, Sydney, NSW, Australia); 5-[1,2-$^3$H(N)]-HT creatinine sulphate (specific activity: 888 MBq/μmol; NEN Life Science Products, Boston, MA, USA); 5-hydroxytryptamine creatinine sulphate (Sigma-Aldrich); pargyline hydrochloride (Sigma-Aldrich); paroxetine hydrochloride (donated by GlaxoSmithKline, Uxbridge, Middlesex, UK); sodium pentobarbitone (Rhone Merieux, Brisbane, Queensland, Australia).

For uptake experiments, stock solutions were prepared in deionised water, except 5-HT and dopamine which were prepared in 10 mM HCl; all dilutions were made in platelet incubation buffer. For aggregation experiments, collagen was obtained in aqueous solution; stock solutions of 5-HT and ADP and all dilutions were prepared in normal saline.

### 2.6. Statistical analysis

Data are presented as either arithmetic means ± S.E.M. or geometric means and 95% confidence limits as indicated in Section 3. The significance of differences between mean values was determined by either Student's *t*-test, repeated measures one-factor analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test, two-factor ANOVA followed by Bonferroni post test or Mann–Whitney test, as indicated in Section 3. All data were analysed using Prism 3 (GraphPad Software, San Diego, CA, USA), Instat (GraphPad Software) or Microsoft Office Excel (Microsoft, Seattle, WA, USA).

## 3. Results

### 3.1. Haematocrit, heart weights and platelet count

Rats exposed to chronic hypoxia for 1 week had significantly higher haematocrits than normoxic rats (Table 1), indicative of polycythemia. The ratio of RV to [LV + S] was significantly higher in hypoxic than in normoxic rats (Table 1). The difference reflected an increase in RV/body weight rather than a decrease in [LV + S]/body weight (Table 1). This confirmed the development of right ventricular hypertrophy, which is indicative of pulmonary hypertension (Jeffery and Wanstall, 2001). Hypoxic rats also had lower platelet counts than normoxic rats. The values from the individual platelet-rich plasma samples used in the uptake experiments were: normoxic rats $2.2 \pm 0.25 \times 10^6$ platelets/μl ($n = 5$); hypoxic rats $1.3 \pm 0.14 \times 10^6$ platelets/μl ($n = 12$) ($0.01 > P > 0.001$; Student's *t*-test). The values from the pooled platelet-rich plasma samples used in the aggregation experiments were: normoxic rats $2.0 \pm 0.19 \times 10^6$ platelets/μl ($n = 13$), hypoxic rats $1.2 \pm 0.11 \times 10^6$ platelets/μl ($n = 8$) ($0.05 > P > 0.01$, Student's *t*-test). Hence, hypoxic rats had thrombocytopenia.

Table 1
Haematocrit and heart weights in normoxic and hypoxic rats

| | Normoxic rats ($n=19$) | Hypoxic rats ($n=44$) |
|---|---|---|
| Haematocrit (%) | 41 ± 0.5 | 57 ± 0.6[a,b] |
| RV/[LV + S][c] (mg/mg) | 0.29 ± 0.01 | 0.43 ± 0.01[b] |
| RV/body weight[c] (mg/g) | 0.68 ± 0.03 | 1.02 ± 0.03[b] |
| [LV + S]/body weight[c] (mg/g) | 2.32 ± 0.04 | 2.38 ± 0.03 |

Values are means ± S.E.M. from $n$ rats.
[a] $n=43$.
[b] Significantly greater than the corresponding value for normoxic rats ($P<0.001$, Student's $t$-test).
[c] Each heart was separated into right ventricle (RV) and left ventricle plus septum ([LV + S]), blotted and weighed. From the heart weights and rat body weights, the values of RV/[LV + S], RV/body weight and [LV + S]/body weight were calculated.

### 3.2. Uptake of 5-HT into platelets

The kinetic plots (Fig. 1) show that uptake of [$^3$H]5-HT into platelets from hypoxic and normoxic rats was saturable. Specific [$^3$H]5-HT uptake was lower in platelets from hypoxic rats than in platelets from normoxic rats (Fig. 1). Values for $K_m$ and $V_{max}$ (Table 2) were calculated from the kinetic plots. $K_m$ values for specific [$^3$H]5-HT uptake into platelets from normoxic and hypoxic rats were not significantly different (Table 2). However, the $V_{max}$ for specific [$^3$H]5-HT uptake into platelets from hypoxic rats was 36% lower than the value in platelets from normoxic rats (Table 2).

### 3.3. Proaggregatory effects of 5-HT in platelets

5-HT did not cause any aggregation in platelets from either normoxic or hypoxic rats when added alone, but it potentiated responses to collagen and ADP (Figs. 2 and 3).

In platelets from normoxic rats, the effects of 0.1 or 1 μM 5-HT on collagen-induced aggregation were examined in the absence and presence of paroxetine (10 μM; Fig. 2). In the absence of paroxetine, collagen-induced aggregation was potentiated by 1 μM, but not by 0.1 μM, 5-HT. However, in the presence of paroxetine, collagen-induced aggregation was potentiated by both 0.1 and 1 μM 5-HT (Fig. 2), i.e. paroxetine caused an increase in the sensitivity to 5-HT.

Concentration–response (potentiation) curves for 5-HT in platelets from normoxic and hypoxic rats are shown in Fig. 3. In these graphs, the ordinate represents potentiation of either the magnitude of collagen-induced aggregation (Fig. 3A) or the duration of ADP-induced aggregation (Fig. 3B), expressed as a potentiation factor (see Section 2). It should be noted that, in the absence of 5-HT, neither the magnitude of the collagen response nor the duration of the ADP response differed between the normoxic and hypoxic rats (see data in legend to Fig. 3). 5-HT caused a concentration-dependent increase in the magnitude of the collagen response in platelets from both normoxic and hypoxic rats, but there was no significant difference between the potentiation factors in the two groups of rats at any of the 5-HT concentrations ($P>0.05$; Mann–Whitney test; Fig. 3A). In platelets aggregated with ADP, 5-HT had minimal effects on the magnitude of the aggregation response. The potentiation factor for 10 μM 5-HT (the maximally effective concentration) was only 1.2 ± 0.1 ($n=4$) in platelets from normoxic rats and was no different in hypoxic rats (1.4 ± 0.1,

Table 2
Kinetic parameters for [$^3$H]5-HT uptake by SERT in platelets from normoxic and hypoxic rats

| | Normoxic rats ($n=5$) | Hypoxic rats ($n=5$) |
|---|---|---|
| $K_m$ (nM) | 139 (77,252) | 103 (59,180) |
| $V_{max}$ (pmol/10$^8$ platelets/min) | 5.80 ± 0.23 | 3.72 ± 0.40[a] |

Values are from $n$ experiments. $K_m$ values are geometric means and 95% confidence limits and $V_{max}$ values are means ± S.E.M.
[a] Significantly less than the corresponding value for normoxic rats ($0.01>P>0.001$, Student's $t$-test).

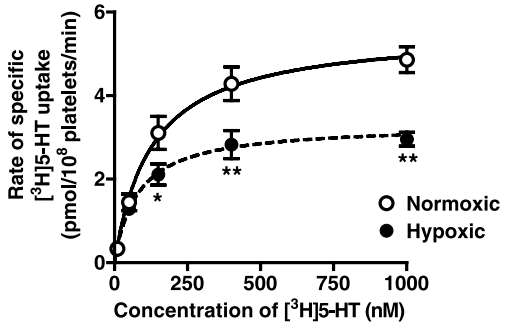


Fig. 1. Kinetic plots for [$^3$H]5-HT uptake by platelets from normoxic and hypoxic rats from five experiments. Specific [$^3$H]5-HT uptake was calculated as the difference between uptake in the absence and presence of 10 μM paroxetine. Rate of specific [$^3$H]5-HT uptake was expressed in pmol/10$^8$ platelets/min. Data from normoxic and hypoxic rats for each concentration of [$^3$H]5-HT were compared by two-way ANOVA followed by Bonferroni post tests (*$0.05>P>0.01$, **$0.01>P>0.001$).

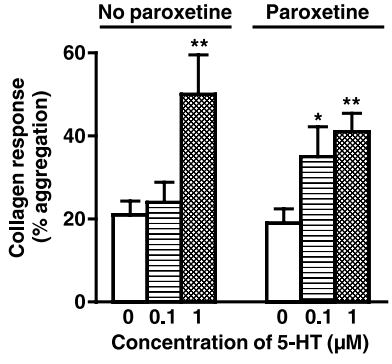


Fig. 2. The effects of 5-HT on the magnitude of aggregation responses induced by collagen in platelets from normoxic rats. Data in the absence ($n=7$) and presence ($n=5$) of paroxetine (10 μM) are shown. Values represent means ± S.E.M. Data in the presence of 5-HT were compared with data in the absence of 5-HT in the same platelet-rich plasma sample by repeated measures one-factor ANOVA followed by Dunnett's multiple comparisons test (*$0.05>P>0.01$, **$0.01>P>0.001$).

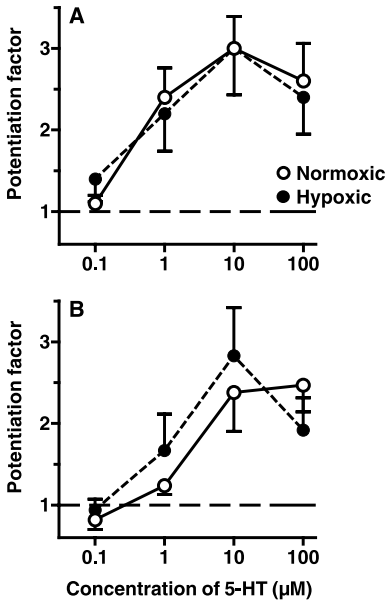


Fig. 3. Potentiating effects of 5-HT on aggregation of platelets from normoxic and hypoxic rats. (A) Effects of 5-HT on the magnitude of aggregatory responses to collagen (normoxic rats $n=7$; hypoxic rats $n=4$). (B) Effects of 5-HT on the duration of aggregatory responses to ADP (normoxic rats $n=4$; hypoxic rats $n=4$). Data are expressed as potentiation factors (see Section 2) where a value of 1 (indicated by the horizontal broken line) represents no potentiation by 5-HT. Values represent means ± S.E.M. Magnitudes of collagen responses in the absence of 5-HT were 21 ± 3.3% and 21 ± 4.5% aggregation in normoxic and hypoxic rats, respectively. Durations of ADP responses in the absence of 5-HT were 0.74 ± 0.11 min and 0.70 ± 0.11 min in normoxic and hypoxic rats, respectively. In both (A) and (B), effects of 5-HT for hypoxic rats did not differ from those for normoxic rats at any of the concentrations of 5-HT ($P>0.05$, Mann–Whitney test).

$n=4$; $P>0.05$, Mann–Whitney test). 5-HT had a more marked effect on the duration of the aggregation response to ADP, but again there was no significant difference between normoxic and hypoxic rats (Fig. 3B; $P>0.05$).

## 4. Discussion

The first objective of this study was to determine whether uptake of 5-HT into platelets by SERT is altered in an animal model of hypoxic pulmonary hypertension. The data showed a decrease in 5-HT uptake in platelets from hypoxic pulmonary hypertensive rats compared with normoxic rats. This decrease could be attributed to a decrease of 36% in the maximal initial rate of uptake, $V_{max}$; there was no change in the affinity (inversely related to $K_m$) of 5-HT for SERT. In the experiments, uptake was expressed as pmol/$10^8$ platelets, so the decrease in $V_{max}$ indicates that the capacity of individual platelets for 5-HT uptake was impaired in the hypoxic rats. This effect could reflect decreased SERT expression in the platelets, decreased trafficking of the protein to the cell membrane of the platelets and/or incorrect SERT assembly in the cell membrane. However, the current experiments were not designed to distinguish between these possibilities. The reduction in $V_{max}$ is consistent with previous findings in perfused lungs from hypoxic pulmonary hypertensive rats, where $V_{max}$ for 5-HT uptake was reduced by 45% with no change in $K_m$ (Jeffery et al., 2000). In contrast, another study showed that there was an increase in SERT mRNA in lungs from hypoxic rats (Eddahibi et al., 1999), but it was not possible to distinguish whether this increase occurred in endothelial cells or vascular smooth muscle cells. In light of the reduced uptake in endothelial cells of perfused lungs of hypoxic rats (Jeffery et al., 2000), it is likely that the increased SERT expression, as indicated by increased mRNA (Eddahibi et al., 1999), occurred in vascular smooth muscle cells. Interestingly, platelets from rats with systemic, rather than pulmonary, hypertension also showed a decrease in the $V_{max}$ of 5-HT uptake (Prina et al., 1981).

5-HT uptake in platelets has been evaluated in the context of pulmonary hypertension in only one other study in which 5-HT uptake was greater in platelets from patients with primary pulmonary hypertension than in those from healthy individuals (Eddahibi et al., 2001). Increased citalopram binding was also found in platelets from the pulmonary hypertensive patients, indicating that elevation of SERT protein levels was responsible for the increase in 5-HT uptake (Eddahibi et al., 2001). Hence, the findings in hypoxic pulmonary hypertensive rats (present study) are in direct contrast to those in human primary pulmonary hypertension (Eddahibi et al., 2001). It is not currently known whether these contrasting findings are due to species differences (human versus rat) or differences between the types of pulmonary hypertension (primary pulmonary hypertension in the human patients and hypoxic pulmonary hypertension in the rats in this study). There is no rat model of primary pulmonary hypertension, but studies on humans with hypoxic pulmonary hypertension would help to resolve this question.

Increased 5-HT plasma levels have been implicated in the pathophysiology of pulmonary hypertension (MacLean et al., 2000). In the present study, not only was there a decrease in 5-HT uptake per platelet (see above), but also the platelet count was almost halved. Since uptake and storage of 5-HT by platelets control plasma levels of 5-HT, these two changes found in the hypoxic pulmonary hypertensive rats could be expected to increase plasma 5-HT levels. In view of this, the absence of any differences in plasma 5-HT levels between hypoxic and normoxic rats (Eddahibi et al., 1997) is surprising. However, in that study, when rats were infused with 5-HT, plasma levels were increased to a greater extent in hypoxic rats than in normoxic rats (Eddahibi et al., 1997). The authors speculated that this might reflect a decrease in uptake by platelets in hypoxic rats (Eddahibi et al., 1997) and the results from our study provide evidence to support this speculation. It has also been speculated that the increased plasma 5-HT levels found in primary pulmonary hypertensive patients are

due to reduced 5-HT uptake into platelets (Herve et al., 1995). Reduced 5-HT uptake per platelet cannot be the explanation in humans, since in the one study where this was investigated, there was an increase rather than a decrease in 5-HT uptake in platelets from primary pulmonary hypertensive patients (Eddahibi et al., 2001). Nevertheless, one cannot exclude a reduction in overall 5-HT uptake into the entire population of platelets, since the total number of platelets is reduced in primary pulmonary hypertensive patients (Eddahibi et al., 2000b).

It was confirmed in the present study that 5-HT alone cannot induce aggregation of rat platelets, but does potentiate the aggregatory response induced by aggregating agents as previously reported (Poole, 1996). Hence, in the platelet aggregation experiments in this study, the effects of 5-HT on aggregation induced by collagen and ADP were examined. The first question we investigated was whether uptake of 5-HT by platelets might influence the potency of 5-HT as a proaggregatory agent. Data showed that paroxetine, a selective SERT inhibitor, did increase 5-HT potency in platelets from normoxic rats, in that the threshold 5-HT concentration was decreased by a factor of 10. Therefore, removal of 5-HT by SERT is sufficient to reduce the concentration of 5-HT in the immediate vicinity of the 5-HT receptors responsible for the proaggregatory response. Hence, it was speculated that the reduction in 5-HT uptake seen in platelets from hypoxic rats might likewise increase the proaggregatory effects of 5-HT. However, there was no difference in the potency of 5-HT in platelets from hypoxic and normoxic rats, as evidenced by the location of the 5-HT concentration–response curves, when either collagen or ADP was used as aggregating agent. This suggests that the 36% reduction in the $V_{max}$ of 5-HT uptake in platelets from hypoxic rats, in contrast to the complete inhibition achieved with paroxetine, is insufficient to have any influence on the proaggregatory potency of 5-HT.

The absence of any change in the sensitivity of platelets to the effect of 5-HT in hypoxic rats contrasts to findings in pulmonary arteries where the vasoconstrictive response to 5-HT is more pronounced in hypoxic pulmonary hypertensive rats (Jeffery and Wanstall, 2001; MacLean et al., 1996). It is, however, in agreement with the lack of change in the sensitivity of systemic vessels, i.e. aorta and mesenteric artery, to 5-HT in hypoxic rats (Doggrell et al., 1999). Taken together, these findings suggest that the sensitisation to 5-HT in animal models of pulmonary hypertension may be a phenomenon confined to the pulmonary vasculature. Selective sensitisation to 5-HT in pulmonary blood vessels is consistent with the concept that 5-HT could contribute to the increase in pulmonary vasoconstriction characteristic of pulmonary hypertension.

In conclusion, it is interesting to note that SERT activity is altered in hypoxic pulmonary hypertension in different ways in different tissues. In endothelial cells (Jeffery et al., 2000) and platelets (present study) 5-HT uptake is reduced. Because these tissues are the main sites of removal of 5-HT from the plasma, this abnormality in 5-HT physiology has the potential to contribute to the pathology of pulmonary hypertension by raising plasma levels of 5-HT. In contrast, in pulmonary vascular smooth muscle, SERT mRNA is increased in hypoxic rats, and, furthermore, 5-HT uptake is increased in cultured pulmonary vascular smooth muscle cells under hypoxic conditions (Eddahibi et al., 1999). This, too, can contribute to the pathology of the disease because uptake by SERT is reported to be a prerequisite for the mitogenic effect of 5-HT in pulmonary vascular smooth muscle (Eddahibi et al., 1999; Lee et al., 1991). Hence, augmented uptake in this tissue could play a role in pulmonary vascular remodelling, a characteristic feature of pulmonary hypertension. Thus, each of the changes in 5-HT uptake by SERT so far reported in hypoxic pulmonary hypertension could have pathological consequences. On the other hand, our finding that the proaggregatory effects of 5-HT are not increased in platelets from hypoxic rats suggests that 5-HT is not likely to contribute to the predisposition to pulmonary thromboembolism that is a feature of most types of pulmonary hypertension (Chaouat et al., 1996).

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