Dynamics of platelet mobilisation into lungs in response to 5-hydroxytryptamine (serotonin) in mice

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Summary

In experimental animals, the lung rapidly removes intravenously injected 5-hydroxytryptamine (5HT), but the mechanism underlying this pulmonary SHT removal (P-SHT-R) and the responsible cells remains unclear. SHT reportedly induces rapid pulmonary platelet accumulation (P-PLT-A). Here, we examined the relationship between P-SHT-R and P-PLT-A in mice by comparing the platelet count in the blood with the endogenous 5HT in the tissues (a marker for platelets because the 5HT is largely contained within platelets), 5HT levels in murine blood and tissues were also examined after intravenous injection of 5HT. The data revealed that: (i) 5HT injection (at ≥ 0.04 μmol/kg) induced a transient P-PLT-A (occurring within 6 seconds), (ii) platelets rapidly took up injected 5HT, (iii) the P-SHT-R was saturated following injection of 5HT at 1 μmol/kg, (iv) ketanserin (5HT2-receptor antagonist) strongly inhibited P-PLT-A, (v) under fluoxetine (5HT-uptake inhibitor), 5HT levels at 6 seconds after 5HT injection were markedly higher in blood, but significantly lower in lung (versus fluoxetine-untreated mice), (vi) P-SHT-R was barely detectable in mutant mice with platelets lacking dense bodies, and was much reduced in platelet-depleted mice, (vii) 5HT injected intravenously at 10 μmol/kg had a half-life in the lung of < 20 seconds, and (viii) unlike 5HT, injected histamine was largely excreted by the kidney. These results demonstrate that platelets rapidly translocate into the lung upon stimulation of 5HT2 receptors, take up 5HT (and possibly swiftly metabolise it), and then return to the circulation. Hence, pulmonary platelet accumulation plays an important role in pulmonary SHT removal in mice.

Keywords
Platelet, lung, 5-hydroxytryptamine, animal model

Introduction

As long ago as 1925, Starling and Verney noted that the vasoconstrictor activity of shed blood could be removed by perfusing the blood through the lungs of dogs (1). Later, this activity was shown to be due to 5-hydroxytryptamine (SHT or serotonin) (2). Actually, the lung can remove SHT very rapidly (3, 4), and surprisingly the lung of the anaesthetised dog can remove more than 90% of intravenously (i.v.) injected SHT in a “single circulation” time (5). Capillary endothelial cells and/or mast cells have been suggested to be involved in such rapid pulmonary SHT removal (P-SHT-R) (6–8). However, platelets are known actively to take up SHT in vitro. In fact, it has been proposed that the blood SHT, having originated from intestinal enterochromaffin cells, is largely stored within dense granules inside platelets (9–12). The observation that in patients with carcinoid tumors (which produce SHT at a high rate), platelets contain a large amount of SHT supports platelets taking up SHT in vivo (although there have been few in vivo studies of SHT uptake by platelets). Interestingly, Oyekan and Botting (14) found that SHT induced pulmonary platelet accumulation (P-PLT-A) in anaesthetised rats in vivo, even though SHT did not cause aggregation of rat platelets in vitro (the underlying mechanism, including the responsible receptors, was not clarified). These observations led us to hypothesise that pulmonary platelet accumulation might be causally related to pulmonary SHT removal.

In view of the above, it seemed important to examine how platelets behave and how (or indeed whether) they take up SHT in vivo. It should be noted that anaesthesia itself interferes with the properties of platelets (15, 16). Hence, in the present study

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we used non-anaesthetised animals to address the following questions: (i) how platelets contribute to the rapid P-5HT-R, (ii) how much 5HT is taken up by platelets, (iii) how rapidly 5HT is metabolised within platelets, and (iv) what types of 5HT receptors are involved in the platelet responses to 5HT. Like 5HT, histamine (H) is a typical vasoactive inflammatory amine, and H is included in human platelets and has been suggested to play important roles in the circulatory system, as well as in inflammation (17). In the present study, therefore, the effects of H on platelets and the changes in the levels of H in the blood and lung were also examined, and the data compared with those obtained for 5HT.

Materials and methods

Animals and materials

BALB/c male mice (6–7 weeks old) were provided by the animal facility of our university. Mutant mice defective in the cappuccino gene (cappuccino mice) (18) were provided by Masao Ono (an author of this study). 5HT hydrochloride, adenosine 5'-diphosphate (ADP) sodium salt, histamine hydrochloride, and ketanserin tartrate (Sigma, St. Louis, MO, USA) were dissolved in saline and injected either intravenously (i.v., via a tail vein) or intraperitoneally (i.p.) (0.1 ml/10 g body weight). ± Fluoxetine hydrochloride (Wako Pure Chemical Ind. Ltd., Tokyo, Japan) was dispersed in saline by sonication and injected i.p. Experiments were carried out at 24 ± 1°C. Hybridoma cells producing a monoclonal anti-mouse platelet antibody, Pm-1, were provided by Dr. T. Nagasawa (Division of Hematology, University of Tsukuba, Japan) (19). This antibody was produced in the peritoneal cavity of BALB/c nude mice inoculated with the hybridoma cells, and a preparation of the antibody (IgG fraction) was obtained by precipitation with ammonium sulfate and dialysis of the precipitant. Control IgG was prepared by precipitation with ammonium sulfate. Pm-1 selectively depletes platelets in mice (19, 20). All experiments complied with the Guidelines for the Care and Use of Laboratory Animals in Tohoku University. Experimental protocols and doses of these reagents are described in the text or in the legends to the figures relating to each experiment.

Platelet count

Since we aimed to analyse the very rapid in vivo responses of platelets (occurring within a few seconds), blood flowing from the neck blood vessels was collected after instant decapitation of non-anaesthetised mice. Two or three drops of blood were directly collected into a pre-weighed test tube containing 1.0 ml of 4 mM EDTA in 0.01 M phosphate-buffered saline (pH 7.0). The amount of collected blood was estimated by reweighing the tube. Incidentally, we could not detect any significant difference in the specific gravity of blood between saline-injected mice and 5HT-injected mice. After a certain amount of practice, we could collect blood at 6 ± 1 seconds (s) after an i.v. injection. The number of platelets was ascertained using a cell counter (Sysmex SF-3000; Toa Medical Electronics Co. Ltd., Kobe, Japan). The utility of this method for the measurement of the platelet count was confirmed in our previous studies (21–23).

Determination of 5HT and H

In the present study we evaluated 5HT-induced P-PLT-A by measuring 5HT as a marker for platelets because the 5HT present in the blood in mice is largely contained within platelets (21, 24, 25). After collecting the blood needed for the measurement of the platelet count (see above), the next two or three drops of blood from the same mouse were collected into another pre-weighed tube containing 3 ml of 0.4 M HClO₄, 0.2 % N-acetyl-cysteine-HCl, and 4 mM EDTA-2Na. After reweighing, the platelets were destroyed by sonication, and each tube was cooled in an ice bath. In addition, lung, liver, and kidney were rapidly removed and stored at –80°C until needed. 5HT and H were determined fluorometrically after their separation by chromatography, as described previously (24, 26).

Electron microscopy

Electron microscopic analysis was performed as described previously (27).

Statistical analysis

Experimental values for 5HT, H, and platelet count are given as mean ± standard deviation (SD). The statistical significance of differences was assessed by a Student’s unpaired t-test (for comparing two means) or by a Bonferroni multiple-comparison test after ANOVA (using InStat software). P-values less than 0.05 were considered to indicate significance.

Results

Platelet and 5HT levels after ADP injection

In the present study we evaluated 5HT-induced P-PLT-A by measuring 5HT as a marker for platelets. To confirm the utility of this method, we compared the changes in platelet count in the blood with the changes in 5HT levels in the blood and lung following an i.v. injection of ADP, because ADP induces P-PLT-A in rats (14). ADP at 1 μmol/kg induced no clear signs of illness, but did induce very rapid, mirror-image changes in the platelet count in the blood and the 5HT level in the lung (Fig. 1A and B). The change in 5HT in the blood paralleled the change in the blood platelet count. These results indicate that (a) ADP induces a very rapid and transient (i.e. reversible) P-PLT-A in mice without any
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detectable loss of 5HT or signs of illness, and (b) the increase in 5HT in the lung does indeed reflect P-PLT-A.

Platelet and 5HT levels after 5HT injection

As the heart rate of the mouse is very high (several hundred beats/min) (28), i.v. injected 5HT may be distributed throughout the entire body within a few seconds, if it is not cleared. It should also be noted that if platelets take up the administered 5HT and accumulate in the lung in response to 5HT, the lung-accumulated platelets might be expected to contain both the 5HT derived from the platelets themselves and the 5HT that was exogenously administered. In the present experiment, the 5HT level in the blood was markedly reduced (vs. “time 0 values”) at 6 s after an i.v. injection of 5HT at 0.2 μmol/kg (Fig. 2A), and the changes in the blood platelet count and in the 5HT level in the lung (Fig. 2B) were very similar to those induced by ADP (Fig. 1). In mice, the weight of the total blood is around 7% of the body weight (29). The body weight of the mice used in the present study was around 25 g, suggesting that the total weight of blood in these mice was around 1.7 g. Thus, injection of 0.2 μmol/kg of 5HT (5 nmol per mouse) would result in an increase in 5HT of approx. 3 nmol per 1 g of blood, if the 5HT is not eliminated from the plasma. In the liver and kidney, increases in 5HT, if present, were very small (less than 1 nmol/g) following 5HT injection (Fig. 2C and D). A large number of platelets was detected by electron microscopy within the capillaries of the lung at 6 s after the injection of 5HT (Fig. 3). It should be noted that these platelets retained many granules (i.e. loss of granules was not observed). These results suggest that (i) 5HT, like ADP, induces a rapid P-PLT-A, (ii) the lung-accumulated platelets soon return to the circulation, and (iii) the administered 5HT is rapidly eliminated from the blood within 6 s of its injection. However, the results shown in Figure 2 do not reveal where the administered 5HT had gone.

Platelet and 5HT levels at 6 s after injection of various doses of 5HT

Within 6 s of an i.v. 5HT injection at 0.2 μmol/kg or more, platelets had almost completely disappeared from the blood (Fig. 4A), whereas H, even at 10 μmol/kg i.v., did not reduce the platelet count (data not shown). In spite of a 5HT injection at 0.2 or 1 μmol/kg, the 5HT level in the blood was significantly reduced (vs. saline) at this time (6 s), but it was increased by 5HT injection at 5 or 10 μmol/kg (Fig. 4A). Hence, in the following sections, we consider these results separately.

(1) Effects of low doses of 5HT (1 μmol/kg or less)

It should be noted that injection of 1 μmol/kg of 5HT would be expected to result in an increase in 5HT of approx. 15 nmol per 1 g of blood, if the 5HT is not eliminated from the blood. Nevertheless, we could not detect any increase in 5HT in the blood...
after administration of 5HT at this dose (actually, the level of 5HT in the blood decreased) (Fig. 4A). In contrast, in the lung, liver, and kidney, 5HT increased or tended to increase (Fig. 4B-D) after 5HT injection at 0.04–1 μmol/kg. It should be noted that the 5HT increase in the lung (in terms of nmol/g) was much greater than those in the liver and kidney. It should also be noted that the 5HT level in the lung increased markedly when 5HT was injected at 1 μmol/kg (Fig. 4B). At this dose, platelets had disappeared almost completely from the blood (Fig. 4A). These results suggest that during the response to 5HT, the platelets that accumulated in the lung had taken up the bulk of the administered 5HT from the blood.

(2) Effects of high doses of 5HT (more than 1 μmol/kg)
Injection of 5HT at these doses increased 5HT in the blood in a dose-related manner (Fig. 4A). This indicates that 5HT increased in the plasma and/or in cells other than platelets, because platelets were completely absent from the blood (Fig. 4A). The elevated 5HT levels observed in the lung after injection of 5HT at 5 and 10 μmol/kg were similar to that observed after injection of 5HT at 1 μmol/kg (Fig. 4B). In the liver, too, the elevated 5HT levels were similar among 1, 5, and 10 μmol/kg (Fig. 4C), but this increase was very small (in terms of nmol/g). In contrast, the 5HT level in the kidney was markedly increased at both 5 and 10 μmol/kg (Fig. 4D). In the liver, the increase in 5HT was very small. These results suggest that (i) 5HT-uptake by platelets is saturated following injection of 5HT at 1 μmol/kg, (ii) the excess 5HT is largely excreted by the kidney, and (iii) the increased 5HT levels in the liver and kidney are largely due to the 5HT present in the blood within them (although we cannot exclude the possibility that a small amount of 5HT may be taken up by endothelial cells). In the subsequent experiments, we focused on the changes in the blood platelet count and in the 5HT levels in the blood and lung.

**Effects of ketanserin, a 5HT2-receptor antagonist**
*In vitro.* 5HT acts via 5HT2 receptors to enhance the aggregation of human platelets induced by other stimulators (30). We tested the effects of ketanserin, an antagonist of the 5HT2 receptor, on the P-PLT-A observed at 6 s after an injection of 5HT at 1 μmol/kg (i.v.) [which induces an almost complete disappearance of both platelets and 5HT from the blood (see Fig. 4A)]. Ketanserin itself slightly, but significantly, reduced the 5HT in the blood (Fig. 5B), an effect possibly due to its ability to release 5HT from platelets (31, 32). Ketanserin largely prevented both the 5HT-induced disappearance of platelets from the blood (Fig. 5A) and the 5HT-increase in the lung (Fig. 5C). The blood 5HT level in mice given ketanserin plus 5HT was significantly higher than that of control mice (Fig. 5B), suggesting a summation of the pre-existing 5HT in platelets with the administered 5HT. These results suggest that the 5HT-induced P-PLT-A is mediated by 5HT2 receptors. Incidentally, ketanserin did not inhibit the ADP (1 μmol/kg)-induced P-PLT-A (data not shown).

**Effects of fluoxetine, a 5HT-uptake inhibitor**
To determine whether 5HT is indeed taken up by platelets, we tested the effects of a 5HT-uptake inhibitor, fluoxetine (33). In this experiment, too, blood and lungs were taken at 6 s after 5HT injection (1 μmol/kg, i.v.). However, unlike ketanserin (Fig. 5), the fluoxetine was injected i.p., because i.v. fluoxetine was highly toxic. Fluoxetine (50 mg/kg, i.p.) alone had no detectable effects on the levels of platelets, blood 5HT, or lung, and fluoxetine did not affect the 5HT-induced decline in platelet count (or 5HT-induced P-PLT-A) (Fig. 6). However, the blood 5HT level was markedly higher in mice given fluoxetine + 5HT than in those given saline + 5HT, whereas the lung 5HT level was significantly lower in the former group than in the latter. A lower dose of fluoxetine (10 mg/kg, i.p.) tended to have similar effects, although significance was not demonstrated (data not shown). These results support the ideas that (i) administered 5HT is indeed largely taken up by platelets, and (ii) platelets, after being stimulated by 5HT and taking up 5HT, translocate into the lung.

**Effects of 5HT in cappuccino mice**
Recently, Yoshida et al. established a unique strain (called “cappuccino mice”) from EOD mice (18). EOD mice are a strain established by brother-sister mating of F1 animals derived from the mating of female lpr x male BXSB (34). Cappuccino mice have a frame-shift mutation due to a single nucleotide deletion in the *cappuccino* gene. The *cappuccino* protein in normal mice is a component of BLOC-1 (biogenesis of lysosome-related orga-
nelles complex-1) (35), and BLOC-1 is involved in the biosynthesis of melanosomes and platelet dense granules (36). Thus, cappuccino mice exhibit diluted colours in the skin and eye, and they also have a profound defect in platelet dense bodies (inability to store 5HT and thus lacking 5HT) and reduced functions of platelets (18). Since both lpr and BXSB mice have a common C57BL/6 genetic background, we used C57BL/6J mice as normal control mice.

As shown in Figure 7, platelet levels were similar between cappuccino and control mice. In cappuccino mice, however, 5HT was below detectable levels in the blood and tissues. In control C57BL/6 mice, as in BALB/c mice, 5HT injection at 1 μmol/kg markedly reduced both the platelet count and the 5HT level in the blood, and markedly increased 5HT in the lung. In cappuccino mice injected with 5HT at this dose: (a) the 5HT level in the blood was increased (to a much higher level than in control mice), but (b) the 5HT level in the lung, although increased, was much lower than in control mice. The above results might possibly be explained if the 5HT transporter in the plasma membrane is, in some unknown way, functionally coupled with the platelet’s dense granules. If so, platelets that lack dense granules (as in cappuccino mice) would have an impaired ability to take up 5HT. Although this hypothesis remains to be clarified, the above data also support the idea that in normal mice (in this case, C57BL/6 mice), platelets do indeed translocate into the lung in response to 5HT, and that they take up the injected 5HT.

Changes in 5HT levels following 5HT injection into platelet-depleted mice

I.v. injection of Pm-1 induced powerful depletion of platelets (or 5HT) from blood and lung within 3 hours of its injection (Fig. 8A). There was no detectable level of 5HT in the kidney or lung of Pm-1-treated mice (data not shown). We therefore examined the effect of platelet-depletion on the changes in 5HT levels that occurred following 5HT injection. In this experiment, we injected a high dose of 5HT (10 μmol/kg, i.v.) (see Fig. 4A and B). In control mice (injected with control IgG), the recovery of the platelet count was partial even at 30 s (Fig. 8B, left panel), although in the experiment illustrated by Figure 2A it was nearly complete within 18 s when 5HT was injected at 0.2 μmol/kg. In platelet-depleted mice, the total 5HT increase (i.e. “area under the curve”) in the blood was greater than in control mice (Fig. 8B, middle panel), but the 5HT increase in the lung was markedly smaller than in control mice (Fig. 8B, right panel). These results support the idea that platelets take up the bulk of the injected 5HT. Although there was still a significant 5HT accumulation (or uptake) in the lung in the platelet-depleted mice, it should be noted that the 5HT increase in the blood was more marked in these mice. Thus, it is reasonable to consider that the increased 5HT level in the lung in platelet-depleted mice is largely due to the 5HT present in the blood, although we cannot exclude the possibility of a minor uptake of 5HT by cells other than platelets (such as endothelial cells or mast cells) in the lung. It should also be noted that in control mice, the lung 5HT level at 30 s was about 40% of that at 10 s (Fig. 8B, right panel), indicating that the 5HT level within platelets decreased rapidly after reaching peak in this experiment. Incidentally, in mice injected with H (10 μmol/kg i.v.) the levels of H in blood and tissues did not differ between platelet-depleted and non-depleted mice in ways described above for 5HT (data not shown).

Contrasting changes in 5HT and H following their injections

Finally, we compared the changes in 5HT and H levels following their i.v. injections at 10 μmol/kg. At this dose, 5HT induced signs of shock (staggering, crawling, and prostration, but no convulsions or death) within 30 s of its injection, while H induced...
only staggering (5HT at 1 and 5 μmol/kg induced only weak staggering). In the blood, 5HT and H levels reached maximum in around 10 s, and these increases were similar to each other in magnitude (Fig. 9A). In the lung, the increase in 5HT was greater than that in H, maximum values being recorded at 10 s (Fig. 9B). In the liver, the changes in both 5HT and H were very small (Fig. 9C). In contrast, in the kidney the level of H was markedly higher than that in 5HT (Fig. 9D). These results suggest that H, unlike 5HT, is largely excreted by the kidney.

Discussion

In the present study, we evaluated 5HT-induced pulmonary platelet accumulation (P-PLT-A) by measuring 5HT as a marker for platelets. The levels of 5HT in the blood of both cappuccino mice (Fig. 7) and platelet-depleted mice (Fig. 8) were very low. Moreover, there is no difference between mast cell-deficient mice and their control mice in the amounts of 5HT in blood, lung, and liver (24). These observations clearly indicate that at least in the mice used in these studies, 5HT is not present in detectable amounts in cells other than platelets in the blood and tested tissues (although we do not deny that a very small amount of 5HT may be present in cells other than platelets [such as endothelial cells and mast cells]). The above results therefore support the contention that changes in 5HT levels can be used as an index of P-PLT-A, and evaluation of such changes allowed us to address the issues on which this study focused (see Introduction and Results).

Platelets accumulated in the lung within 6 s of an injection of 5HT or ADP (Figs. 1 and 2), whereas in anaesthetised rats (in which 111In-labeled platelets were monitored), it took 50 s or more for the maximal accumulation in the lung (14, 15). Thus, in mice translocation of platelets into the lungs and the uptake of 5HT by such platelets may occur almost immediately after 5HT injection. In addition, the rapid decrease in the 5HT in the lung (i.e., within the platelets accumulated in the lung) that occurred following its uptake (Fig. 8B) suggests that 5HT may be swiftly destroyed or metabolised within the platelets. Although monoamine oxidase (MAO) is known to metabolise 5HT, Thomas and Vane (5) doubted such a rapid metabolism by MAO (in the dog). Thus, it remains to be clarified whether the 5HT present in the platelets accumulated in the lung can be metabolised swiftly enough by MAO to account for the above short half-life. 5HT is released from platelets following their activation (37). Although the in vitro platelet-aggregation response to 5HT is weak, when combined with a low (by itself ineffective) concentration of another agonist, 5HT triggers an irreversible platelet aggregation (32). This in vitro amplification is inhibited by ketanserin (30). Our data suggest that in vivo, stimulation of the 5HT receptor (the major 5HT receptor in platelets) (38) induces translocation of platelets to the lung in response to 5HT. Interestingly, a significant number of platelets may already be present in the lung since the lungs of our mice contained a high level of 5HT (compare the 5HT level in the lung with those in the liver and kidney at time 0 in Fig. 2), and this 5HT was largely lost following Pm-1 administration (Fig. 8A, right panel). Actually, it has been reported that 20 ~ 50% of the mature megakaryocyte population ultimately reaches the lung in humans, and that 7 ~ 17% of the platelets are released there (39, 40). It is in the lung that the i.v. injected 5HT first encounters capillaries. Thus, it is likely that this 5HT activates the platelets already present in the lung, as well as the circulating platelets, leading to P-PLT-A. However, we need to do further research to test this hypothesis.

5HT is present only in a limited number of cell-types (platelets, nerve cells, and enterochromaffin cells in the digestive tract) (24, 41), but upon its release 5HT stimulates various cells throughout the body, including vascular cells (41). Thus, if 5HT were to be delivered to the entire body, it could induce profound systemic effects. In addition to 5HT, various other substances are reportedly taken up and inactivated by the lung (42 -46). Blood that has passed through the systemic circulation passes first to the lungs before being re-circulated. Thus, a P-PLT-A-mediated uptake of 5HT by platelets would seem to be an excellent mechanism for the prevention of a systemic delivery of 5HT. More-

What is known about this topic?

- The lung rapidly removes intravenously injected 5-hydroxytryptamine (5HT), although neither the underlying mechanism nor the underlying cell-type has been identified.
- 5HT reportedly induces a rapid pulmonary accumulation of platelets, despite its poor ability to aggregate platelets.

What does this paper add?

- In anaesthetised mice, a rapid translocation of platelets into the lung is induced by stimulation of 5HT2 receptors (location unknown). During the translocation and/or in the lung, the platelets take up 5HT (and possibly swiftly metabolise it) before returning to the circulation.
- Hence, pulmonary platelet accumulation plays an important role in pulmonary 5HT removal, at least in mice, and may represent one of the body’s defense mechanisms against 5HT.

Figure 9: Changes in blood and tissue levels of H and 5HT following their injection. H or 5HT was injected (i.v.) into mice, each at a dose of 10 μmol/kg, and the mice were killed at the times indicated. The "time 0" values were obtained from mice not injected with H or 5HT. Each value is the mean ± SD from four mice. For 5HT, *P < 0.05 and **P < 0.01 vs. time 0. For H, #P < 0.05 and ##P < 0.01 vs. time 0.
over, recent studies suggest that platelets may play an important role in immune responses (10–12, 47). Platelets even release bactericidal products, as well as capturing bacteria and ingesting them (48). I.v. injection of bacterial components (such as lipopolysaccharide) (22, 27, 49), bacteria themselves (50), or allergens (23) also induces a rapid P-PLT-A in mice. Collectively, the above evidence implies that the lung is an important front in the battle against pathogens and that platelets may be considered “front-line soldiers”.

The present findings are consistent with the following sequence: platelets rapidly translocate into the lung upon stimulation of 5HT2 receptors (possibly on platelets, but location not yet identified), take up 5HT (and possibly metabolise it), and then rapidly return to the circulation. As mentioned above, one inference that could be drawn is that pulmonary platelet accumulation, and uptake of 5HT by the accumulated platelets, may be an example of the body’s defense mechanisms against dangerous substances derived from either the external or the internal environment (45, 46). However, it remains uncertain whether platelets take up 5HT (a) in the circulation or (b) after their accumulation within the lung [or indeed both (a) and (b)].

**Limitations of the study**

To analyse platelet translocation from blood to tissues, a method utilising 111indium-labelled platelets has been employed by others (14, 15). Unfortunately, however, this method can be applied only to anaesthetised animals (16), and the anesthesia itself might interfere with the properties of platelets (15, 16). To evaluate P-5HT-R, it would be convenient to use 5HT labelled with an isotope such as 3H or 14C. However, such in vivo experiments using radio-isotopes require equipment not possessed by our institute. As mentioned at the beginning of the *Introduction*, the method used in the present study (and in our previous studies: [21, 24, 25]) cannot distinguish endogenous 5HT from injected 5HT. Consequently, we carefully evaluated both P-5HT-R and P-PLT-A by considering 5HT changes in blood and tissues, the platelet count in the blood, electron microscopy observations, platelet depletion, and data obtained from specific “cappuccino mice”.

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