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# Reflex circulatory collapse following intrapulmonary entrapment of activated platelets: mediation via 5-$HT_3$ receptor stimulation

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1 The role of 5-$HT_2$ and 5-$HT_3$ receptors in the mediation of direct and reflex vascular responses to intrapulmonary platelet activation was investigated.

2 Anaesthetized rabbits were challenged intravenously with an emulsion of autologous bone marrow that produced a sharp increase in pulmonary blood pressure, a fall in systemic blood pressure, platelet consumption and death.

3 Platelet depletion before the challenge nearly abolished all the cardiovascular effects and prevented death. Bilateral vagotomy prevented the fall in systemic blood pressure and death but did not prevent the increase in pulmonary pressure. The intravenous administration of the 5-$HT_3$ antagonist, MDL-72222, resembled the protection afforded by vagotomy whereas the 5-$HT_2$ antagonist, ketanserin, only reduced the increase in pulmonary pressure without affecting the systemic response or mortality.

4 The effects of intravenous 5-HT and of electrical stimulation of the cephalic ends of the cut vagi nerves were also explored. 5-HT injection increased the pulmonary vascular pressure but its effects on systemic blood pressure were variable. These responses were modified by the 5-HT antagonists in a manner that resembles their effects on bone marrow embolism. Afferent vagal stimulation produced a fall in systemic blood pressure that was not prevented by MDL-72222.

5 This study indicates that a centrally mediated reduction of peripheral vascular tone is the cause of the potentially lethal circulatory collapse that follows the intrapulmonary entrapment of activated platelets. This reflex is initiated by the action of 5-HT on 5-$HT_3$ receptors in the lung.

**Keywords:** Platelet; 5-hydroxytryptamine; 5-$HT_3$ receptors; circulatory shock; pulmonary embolism; fat embolism

## Introduction

The haemodynamic changes that follow different types of pulmonary embolism are not merely the result of the mechanical obstruction created by the plug but involve direct and indirect responses to mediators liberated either by the blood or the vessel wall in a cascade of events the ultimate consequence of which is a state of peripheral circulatory collapse (Robb, 1963). In fact, an inert material embolizing the lung requires a relatively massive size to produce a significant rise in the pulmonary vascular resistance and to reduce the systemic blood pressure by decreasing the pulmonary venous return to the left atrium (Gurewich *et al.*, 1968; Vaage, 1982).

On the other hand, platelets play an outstanding role in the pathophysiology of fat embolism (Peltier, 1984). This was suspected early since petechiae and thrombocytopenia are common clinical findings in patients with fat embolism (Evarts, 1965; Moylan *et al.*, 1976). Bone marrow embolism in man is characterized by aggregates of fat, platelets, fibrin and leukocytes that are lodged in the microvasculature of the lung (Gossling, 1982).

A variety of models have been used to explore the role of mediators released by platelets in pulmonary embolism. In this study we produced bone marrow embolism to create a situation in which marked platelet activation and release reaction occurred with the minimal possible mechanical obstruction (Saldeen, 1976).

Among the substances liberated by activated platelets, 5-Hydroxytryptamine (5-HT) and thromboxane $A_2$ ($TXA_2$) have prominent direct vascular effects. However, they are both evanescent in the lung (Vane, 1969; Svensson & Fredholm, 1977) and therefore it is unlikely that the systemic vascular responses observed after platelet entrapment in the lung can be due to direct effects of these substances. A reflex mechanism is more likely (Comroe *et al.*, 1953) and, in fact, the existence of vagal nerve terminals sensitive to 5-HT in the lung is well documented (McQueen, 1990).

The elucidation of different types of 5-HT receptors and their conspicuous locations and roles (Bradley *et al.*, 1986), suggest the possibility that 5-$HT_2$ and 5-$HT_3$ receptors play a differential role in the complex events following pulmonary embolism.

In view of this we have now assessed the overall role of 5-$HT_2$ and 5-$HT_3$ receptors in the mediation of direct and reflex vascular responses elicited by 5-HT released from activated platelets after autologous bone marrow embolism in anaesthetized rabbits.

## Methods

### *General procedure*

Male New Zealand rabbits (2.5-3.5 kg) were tranquilized with xylacine (3 mg $kg^{-1}$, i.m.) and propiomacine (2 mg $kg^{-1}$, i.m.), and further anaesthetized with sodium pentobarbitone (15-25 mg $kg^{-1}$, i.v. initially and then as needed to maintain anaesthesia). The trachea was intubated for mechanical ventilation (Palmer ventilator, 25 ml per stroke, 30 strokes $min^{-1}$).

Indwelling cannulae were placed in the left jugular vein for treatment administration, in the left carotid artery for blood sampling and recording of systemic blood pressure (SBP). The right ventricle was reached through the right jugular vein. SBP, heart rate (HR) and pressure of the right ventricle (RVP) were continuously monitored by means of Statham transducers and displayed on a Grass polygraph model 79E (Grass Instrument Co., Quincy, MA. U.S.A. The respirator pump was disconnected at the end of the surgical procedures. Ten min later

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pretreatments were administered, except the platelet antibody (see below) and after a further stabilization period of 20 min the challenge was infused.

### *Experimental series*

*Bone marrow embolism* The tissues of external side of one thigh were infiltrated with 1 ml of lignocaine diluted in saline (0.5%) the femur of one side was exposed and the diaphysis fractured with a costotome. Two to four ml of bone marrow were aspirated through a 16 gauge needle attached to a plastic syringe. Afterwards the fractured ends of the bone were sealed with wax.

Bone marrow was diluted 1:1 in isotonic saline prior to homogenization in a Tekmar homogenizer (Tissumizer Mark II) for 10 min at 20,500 r.p.m. The resultant emulsion was administered i.v. to the rabbit over a period of 5 min by means of an infusion pump (1 ml kg$^{-1}$, pump Sage 355).

Arterial blood samples were collected prior to the bone marrow challenge and 5 min after the end of the infusion for platelet count in a Coulter Counter model T-540. The cardiovascular effects after bone marrow challenge were measured 5 min after the end of the challenge, which coincided with the peak of the changes in most surviving animals. When a biphasic effect occurred the second wave was measured 5 min after its initiation. The survival rate at 6 h was recorded.

Eight groups of at least 6 animals each received one of the following pretreatments: (1) saline 1 ml kg$^{-1}$; (2) a selective platelet antibody (see below) given 24 h before the experiment, which produced a pronounced and sustained fall of platelet count of at least 90%; (3) bilateral vagotomy performed at the neck level; (4) ketanserin 300 $\mu$g kg$^{-1}$, i.v. (Vandeplassche *et al.*, 1993); (5) MDL-72222 300 $\mu$g kg$^{-1}$, i.v. (Fozard, 1984); (6) a mixture of ketanserin and MDL-72222 at the same doses; (7) aspirin 10 mg kg$^{-1}$, i.v. and (8) a combination of aspirin plus ketanserin at the doses stated above.

*Intravenous 5-HT* In order to assess the ability of exogenous 5-HT to reproduce some of the cardiovascular effects of fat embolism and its interaction with the studied antagonists, 5-HT (500 $\mu$g kg$^{-1}$, i.v.), was infused over a period of 2 min to 3 groups of six animals each, pretreated with: (1) saline; (2) ketanserin 300 $\mu$g kg$^{-1}$, i.v. or (3) MDL-72222 300 $\mu$g kg$^{-1}$, i.v.

*Bilateral vagotomy plus electrical stimulation of the cephalic trunks of the nerves* To explore whether an increase in the afferent flow of the vagal nerves was capable of emulating the peripheral vascular responses observed during pulmonary embolism, two groups of six rabbits each were submitted to bilateral vagotomy at cervical level and platinum electrodes were placed on the cephalic ends of both sides for electrical stimulation (Grass SD9 electrical stimulator, trains of 1 min at 40V, 10 Hz, 0.5 ms) and two consecutive trains of stimuli were applied with a 15 min interval.

In one group, the second train was preceded by an i.v. injection of saline (1 ml kg$^{-1}$); the other group was included to exclude a central effect of MDL-72222 in our model, and in this case the antagonist was administered (300 $\mu$g kg$^{-1}$, i.v.) between the two trains of stimuli.

### *Statistics*

Results are presented as the means ± s.e.mean of $n$ experiments. A two way analysis of variance for repeated measures followed by a Newman-Keuls test was performed to assess the significance of the differences among the groups except for the survival time, where a chi-square test was applied. A difference was considered significant at a level of $P<0.05$.

### *Chemicals*

Chemicals were obtained as follows: xylacine hydrochloride (Rompun, Bayer, Mexico), propiomacine (Combelen, Bayer, Mexico), sodium pentobarbitone (Anestesal, SmithKline, Mexico), lignocaine hydrochloride (Astra, Mexico), ketanserin (Janssen Pharmaceutica, Mexico), citric acid (Allied, Chemical, Morristown, N.J., U.S.A.), disodium EDTA (Baker, Mexico), MDL-72222 (3-tropanyl-3,5-dichlorobenzoate: Research Biochemicals Inc., Natick, MA., U.S.A.), 5-hydroxytryptamine hydrochloride (Sigma Chemical Co., St. Louis MO., U.S.A.), wax (Ethycon, Johnson & Johnson).

All drug were dissolved in saline. Ketanserin and MDL-72222 solutions were acidified (pH = 3) with citric acid.

### *Platelet antibody*

Washed rabbit platelets were prepared according to the method of Van Loghem (1959). Briefly, a priming dose of platelet antigen (3 ml of washed platelets, 500,000 platelets $\mu$l$^{-1}$ plus 3 ml of Freund's adjuvant) was given subcutaneously to a 25 kg ram. Boosting doses of 3 ml were injected intraperitoneally once a week over 5 weeks, and then every month until the end of the experiments. After the fifth challenge, blood samples were taken every month and the gamma globulin fraction was precipitated from serum with a saturated solution of ammonium sulphate and thrice dialysed against a borate buffer pH 7.6. One g pellets of rabbit washed red blood cells were added to 5 ml aliquots of the dialysate, mixed and incubated at 37°C for 0.5 h and then at 4°C for 12 h. Red blood cells were precipitated by centrifugation, and the supernatant, containing the platelet antibodies, filtered through a Metricel membrane (0.45 $\mu$m) and freeze-dried. The specificity of the antibodies was tested by injection of 15 mg of protein i.v. to conscious rabbits. In all cases this resulted in a reduction of more than 90% in the platelet count, without significantly affecting the leucocyte or the erythrocyte counts.

## Results

### *Bone marrow embolism*

Neither the vehicles nor the surgical procedure to aspirate the bone marrow had any effect on cardiovascular state of the animals. The infusion of bone marrow emulsion (Figure 1) in the saline group ($n=12$) was consistently followed by a sharp rise in the systolic pressure of the right ventricle (RVSP) (36 ± 3 mmHg), a fall of mean arterial systemic pressure (MASP) (46 ± 5 mmHg) and a transient increase in HR (17 ± 4 beats min$^{-1}$). Additionally, the platelet count dropped (155,900 ± 19,000 mm$^{-3}$), and 9 out of 12 animals died from respiratory arrest within 10 min following the embolism. Animals which survived remained alive at the end of a 6 h observation period.

Quite remarkably, the thrombocytopenic rabbits ($n=6$) showed a substantial reduction of the RVSP and MASP responses to bone marrow embolism. In addition, their survival rate increased to 5 out of 6 animals. All these results were significantly different from those of the saline group ($P<0.05$).

Vagotomized rabbits ($n=7$) and those pretreated with MDL-72222 ($n=6$) showed a similar pattern of effects after the challenge with bone marrow emulsion: both procedures effectively blocked the fall in MASP and the change in HR, but the rise of RVSP and the drop in the platelet count remained unaffected. Most interestingly, the survival rate to embolism in the vagotomy group was 6 out of 7 and in the MDL-72222 group reached 100% ($n=6$).

By contrast, ketanserin pretreatment blunted the responses of the RVSP to the embolism, leaving intact the responses of the MASP and platelet count. The survival rate was not significantly different from the saline group.

The combination of ketanserin and MDL-72222 ($n=6$), reduced significantly the responses in RVSP, MASP, HR, but platelet count still fell significantly. The survival rate was also 100% ($n=7$).

Aspirin, either alone or in combination with ketanserin produced a marked protection against both the increase in RVSP and the fall in MASP, comparable to that seen in thrombocytopaenic rabbits. In these two groups the platelet count dropped to the same extent as in the saline group but the survival rate in both was 100%.

Table 1 shows the effects of several pretreatments on survival rate, haemodynamic responses and platelet count induced by bone marrow embolism in the rabbit.

*Intravenous 5-HT*

5-HT consistently produced an increase in RSVP but the pattern of effects on the MASP and HR were variable. In 4 out of 8 rabbits the MASP initially fell during 1–2 min and then rose. Two animals showed only hypotension and in the remaining 2 the MASP showed only a transient rise. The most common response of HR to 5-HT was bradycardia (5 out of 8 cases). Tachycardia was seen in 3 cases. Ketanserin pretreat-

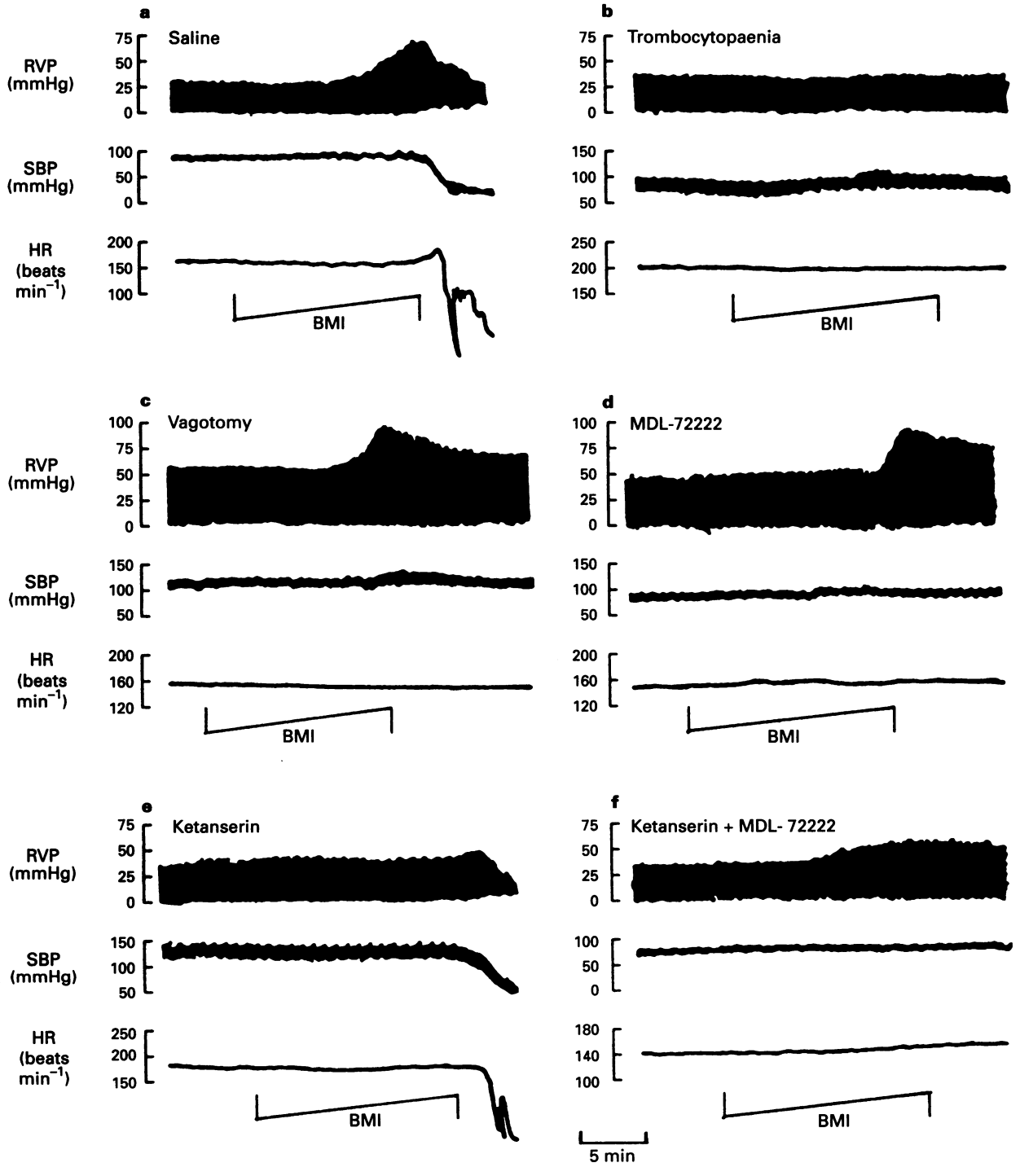


**Figure 1** Original recordings of the effects of i.v. bone marrow infusion (BMI) on the right ventricular pressure (RVP), systemic blood pressure (SBP) and heart rate (HR) after several pretreatments. Upper panel: saline only plus BMI (a), thrombocytopaenia (b); the middle panel: vagotomy (c) and 5-HT$_3$ antagonist [MDL-72222 0.3 mg kg$^{-1}$] (d); lower panel 5-HT$_2$ antagonist [ketanserin 0.3 mg kg$^{-1}$] (e) and both antagonists (f).

**Table 1** Effects of several pretreatments on survival rate, haemodynamic responses and platelet count induced by bone marrow embolism in the rabbit

| *Pretreatments* | *Rate of Survival* | *RVSP* (mmHg) *before* | *after* | *MASP* (mmHg) *before* | *after* | *HR* (beats min$^{-1}$) *before* | *after* | *PC* ($\times 10^3$ mm$^{-3}$) *before* | *after* |
|---|---|---|---|---|---|---|---|---|---|
| Saline | 3/12 | 29±1 | 65±4 | 86±3 | 40±6 | 187±11 | 204±7 | 348±24 | 196±26 |
| Thrombocytopaenic | 5/6* | 32±2 | 40±4* | 87±4 | 82±5* | 203±11 | 194±10 | – | – |
| Vagotomized | 6/7* | 34±2 | 69±2 | 85±4 | 89±13* | 185±11 | 186±10 | 295±35 | 180±20 |
| Ketanserin | 2/6 | 33±3 | 55±5* | 84±3 | 41±11 | 200±13 | – | 307±40 | 84±21* |
| MDL-72222 | 6/6* | 34±2 | 66±4 | 88±3 | 90±4* | 192±10 | 193±9 | 324±37 | 167±36 |
| Ketanserin + MDL-72222 | 7/7* | 33±3 | 51±3* | 82±3 | 87±5* | 178±9 | 185±9 | 300±12 | 203±22 |
| Aspirin | 7/7* | 30±2 | 42±3* | 82±1 | 76±6* | 224±12 | 228±13 | 352±29 | 212±26 |
| Aspirin + ketanserin | 6/6* | 32±2 | 44±3* | 80±4 | 79±11* | 186±6 | 188±6 | 281±29 | 177±32 |

Results are expressed as mean ± s.e.mean, $n = 6-12$ rabbits. RVSP = right ventricular systolic pressure, MASP = mean arterial systemic pressure, HR = heart rate and PC = platelet count.
* Statistically different from the saline group ($P < 0.05$).

**Table 2** Effects of several pretreatments on the haemodynamic responses of i.v. 5-HT (0.5 mg kg$^{-1}$) in the rabbit

| *Pretreatments* | *RVSP* (mmHg) *before* | *after* | *MASP* (mmHg) *before* | *after* | *HR* (beats min$^{-1}$) *before* | *after* |
|---|---|---|---|---|---|---|
| Saline | 30±2 | 65±3 | 89±3 | biphasic: ↓to 69±7, ↑to 108±8 | 179±9 | biphasic: ↓to 157±14, ↑to 185±16 |
| Vagotomized | 30±1 | 60±5 | 83±2 | biphasic: ↓to 61±5, ↑to 102±5 | 198±5 | biphasic: ↓to 165±8, ↑to 233±18 |
| Ketanserin | 32±4 | 44±5* | 89±4 | biphasic: ↓to 71±3, ↑to 92±6 | 204±10 | biphasic: ↓to 163±8, ↑to 210±12 |
| MDL-72222 | 30±3 | 58±5 | 87±5 | monophasic: ↑to 107±6 | 182±11 | monophasic: ↓to 155±11 |

Results are expressed as mean ± s.e.mean, $n = 6-8$ rabbits.
RVSP = right ventricular systolic pressure. MASP = mean arterial systemic pressure.
* Statistically different from the saline group ($P < 0.05$).

ment significantly reduced the responses of the RVSP to 5-HT but did not significantly modify the pattern of responses in HR and MASP. Pretreatment with MDL-72222 did not change the RVSP response to 5-HT but significantly prevented the fall of the MASP leaving intact its rise. Contrasting with the variable responses to 5-HT alone on the HR, it consistently produced bradycardia when given after MDL-72222 (Table 2).

### *Bilateral vagotomy plus electrical stimulation of the cephalic trunks of the vagal nerves*

Bilateral vagotomy by itself did not produce a significant change in MASP in the anaesthetized rabbit. However, the HR increased from 207±4 to 245±7 beats min$^{-1}$ after vagotomy reaching a plateau after 20 min.

In turn, electrical stimulation of the cephalic ends produced a fall in MASP of 31±2 mmHg with the first train and of 26±1 mmHg with the second train in the saline group. In the group treated with MDL-72222 between the two trains of electrical stimuli the corresponding values of MASP fall were 35±4 mmHg and 27±3 mmHg. None of the difference within and between groups was statistically significant. A transient decrease in HR from 228±4 to 205±4 beats min$^{-1}$ was observed after each train of stimuli in the saline group and a similar effect was repeatedly observed in the MDL-72222 group.

## Discussion and conclusions

This study elucidates the relative contribution of 5-HT$_2$ and 5-HT$_3$ receptors, in the mediation of direct and reflex vascular responses to 5-HT released following intrapulmonary entrapment of activated platelets. Indeed, our experiments confirm that cyclo-oxygenase products, most likely TXA$_2$, make a remarkable contribution to the vascular effects of platelet entrapment of the lung as observed in other models by Amezcua *et al.* (1991) and Rivkind *et al.* (1989). It is possible that both TXA$_2$ and 5-HT act synergistically in this condition, and that TXA$_2$ promotes the recruitment of platelets and facilitates the release reaction thus amplifying the actions of the latter. This point deserves elucidation. Direct constrictor actions of 5-HT in the pulmonary vascular territory are mostly due to 5-HT$_2$ receptor activation.

The systemic vascular effects of 5-HT released during bone marrow embolism are clearly mediated by the activation of reflex mechanisms in the lung initiated by 5-HT$_3$ receptors. The vagal nerves provide the afferent limb of this reflex, and the response seems to include a centrally integrated fall in the vasomotor tone.

These conclusions are supported by the observation that electric stimulation of the cephalic end of the cut vagi produced a fall in SBP. The site of action of the 5-HT$_3$ antagonism in the prevention of the systemic effects of 5-HT released in the lung is not centrally located, as MDL-72222 did not interfere with the hypotension induced by cephalic vagal stimulation. Indeed, the presence of 5-HT$_3$ receptors in the so called juxta-pulmonary capillary nerve terminals has been reported (McQueen, 1990) and their stimulation is followed by bradycardia and hypotension in rabbits (Thoren, 1979; Armstrong *et al.*, 1986). Although we cannot exclude a role for platelet activation outside the lung in our experiments it is reasonable to assume that the vast majority of activated platelets are trapped in the pulmonary circulation (Hechtman, 1978). On the other hand, 5-HT is largely metabolized in the lung (Vane, 1969; Junod, 1982), so the intervention of 5-HT$_3$ receptors beyond the lung may not contribute to a major extent in the reflex responses observed in our model.

The fact that thrombocytopaenic rabbits showed a nearly complete abolition of both the pulmonary and systemic responses to the embolism whereas 5-HT$_2$ and 5-HT$_3$ antagonists only partially prevented those changes strengthens the possibility that other mediators in addition to 5-HT are intervening in the process.

It is clearly shown in this study that the reflex responses elicited by 5-HT via 5-HT$_3$ receptors located in the lung account for the peripheral circulatory collapse that characterizes intrapulmonary entrapment of activated platelets. The reduction of the pulmonary venous return to the left atrium seems to be far less important in this respect, since after embolism the vagotomized animals showed an increase in pulmonary pressure similar to the saline group and yet no fall in the SBP was observed. We cannot, however, exclude a minor but significant effect of 5-HT in the heart when 5-HT was injected i.v., a location different from the release reaction of platelets in the lung during the embolism.

On the other hand, the striking protective effects of vagotomy and MDL-72222 demonstrate that a contributory factor to death through peripheral circulatory failure in our model is the result of a centrally mediated fall in vascular tone initiated by 5-HT$_3$ receptors in the lung. TXA$_2$ also plays a prominent role in this respect as shown by the protection afforded by aspirin. However, the effects of 5-HT$_3$ blockade and vagotomy, in the presence of an intact cyclo-oxygenase pathway indicates that TXA$_2$ release is not sufficient to produce the vascular changes but possibly part of its effects are due to an interaction with processes involving 5-HT released. One action of 5-HT could the enhancement of 5-HT released by platelets and another a synergistic one at effector levels.

This study encourages further clinical research into the mechanisms of the peripheral circulatory collapse observed in man after different kinds of pulmonary embolism.

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