Serotonin enhances platelet procoagulant properties and their activation induced during platelet tissue factor uptake

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Aims Circulating tissue factor (TF) has been linked to thrombus propagation. Our group demonstrated that platelets possess mechanisms to capture TF-rich microvesicles (TF-MVs). Serotonin facilitates the development of platelets with increased procoagulant activity. An enhanced platelet serotonin uptake has been identified with increased cardiovascular risk. We have investigated the involvement of serotonergic mechanisms facilitating the interaction of human platelets with TF-MVs. Inhibitory strategies aimed at blocking serotonin and coagulation mechanisms were also studied.

Methods and results Standard aggregometry, flow cytometry, electron microscopy, and thrombin generation assays were performed. TF-MVs induced platelet aggregation in heparinized platelet-rich plasma (PRP) samples; this aggregation was further accelerated by serotonin. In washed platelets, serotonin enhanced platelet aggregation to TF-MVs with a maximum peak of 55.9 ± 1.8 vs. 48.7 ± 2.1% (P < 0.05). Inhibitory strategies with a selective serotonin re-uptake inhibitor and with lepirudin decreased these aggregations. Ultrastructural analysis revealed that serotonin induced platelet pseudopodia formation, thus facilitating the engulfment of TF-MVs. In general, serotonin significantly enhanced (P < 0.05) thrombin generation and the expression of activation markers and procoagulant activity in platelets measured for TF-MVs alone.

Conclusion Serotonin enhances the interaction of platelets with TF-MVs, increases platelet activation, and potentiates their overall procoagulant activity. The present results could have significant implications in thrombus formation and in the thrombogenic profile of pathological situations with increased cardiovascular risk.

KEYWORDS
Serotonin; Tissue factor; Platelet activation; Procoagulant effect; Thrombogenicity

1. Introduction
Platelets are known to play a critical role in haemostasis and to contribute to the development of ischaemic complications in cardiovascular disease. They recognize and bind to proteins present in the subendothelium to form a platelet aggregate on a damaged surface. At the same time, tissue factor (TF) exposed at sites of vascular damage initiates the coagulation mechanism to lead thrombin generation that activates other platelets and generates a stable fibrin net. In recent years, some groups have also reported the existence of a circulating form of TF. This TF is associated with certain blood-circulating cells, such as monocytes, leucocytes, macrophages, platelets, and cell-derived microparticles. Also a soluble form of TF has been described.

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serotonin (5-HT₂A)\(^{11}\) in their surface and a 5-HT transporter (SERT) to re-uptake 5-HT rapidly, move it into platelet dense bodies,\(^{12}\) and secrete it during platelet activation.

Several studies suggest a connection between serotonergic mechanisms and cardiovascular events. Some studies have reported different polymorphisms in the gene for the 5-HT₂A receptor,\(^{13}\) and the SERT receptor,\(^{14}\) that are associated with an increased cardiovascular risk.\(^{15}\) Recently, Nishihira et al.\(^{16}\) have reported that intravenous injection of sarpogrelate, an inhibitor of the 5-HT₂A receptor, in a rabbit model significantly reduced the ex vivo platelet aggregation induced by ADP, thrombin, and collagen alone as well as with 5-HT, and significantly prevented occlusive thrombus formation in vivo. Other authors have also reported that increased platelet 5-HT could be effective in the control of bleeding in idiopathic thrombocytopenic purpura.\(^{17}\) There is also clinical evidence that depression is commonly present in patients with coronary heart disease\(^{18,19}\) and, in fact, it is considered an independent factor of cardiovascular risk.\(^{20}\) The strength of the evidence has raised the need to publish recommendations for the screening, referral, and treatment of depression and coronary heart disease.\(^{21}\)

Activation of platelets could result in the exposure of the stored TF, as well as the release of 5-HT from platelet granules and dense bodies, respectively. TF and 5-HT released from activated platelets in a damaged area or on a disrupted plaque could play a critical role in the development of acute thrombotic events. In the present work, we have investigated the potential involvement of serotonergic mechanisms in platelet uptake of TF-bearing microvesicles and their influence in the prothrombotic profile. Inhibitory strategies aimed to block serotonin and coagulation mechanisms have also been studied.

2. Methods

The investigation conforms with the principles outlined in the Declaration of Helsinki.\(^ {22}\) This study has been approved by our institutional Ethics Committee (04/2044).

2.1 Chemical reagents and antibodies

Low molecular weight heparin (LMWH) was purchased from Pharmacia (Framingham, MA, USA). TF from human placenta (Thromborel\(^ {\text{R}}\) S) was from Dade Behring (Marburg GmbH, Germany). Serotonin hydrochloride was from Fluka (Sigma-Aldrich, Steinheim, Buchs SG, Switzerland). Seropram (citralopram 4%) was from Lundbeck (Schweiz, AG). Lepirudin (Refudin\(^ {\text{R}}\)) was from Celgene Europe Ltd (Windsor, UK). Phosphate-buffered saline (PBS) was from Gibco BRL (Life Technologies, Barcelona, Spain). Thrombin generation was assessed with a fluorogenic assay (Technothrombin TGA) from Technoclone GmbH (Vienna, Austria).

Platelets were analysed by dual flow cytometry using combinations of antibodies or markers conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll protein (PerCP). The platelet population was gated/detected using an antibody to CD41a, from BD Biosciences (San Jose, CA, USA). Antibodies to CD62-P (clone CLBThromb/6) and CD63 (clone CLBGran/12) were from Immunotech (Marseille, France). Antibodies to CD36, CD62-P, and annexin V to detect anionic phospholipids were from Pharmingen (San Diego, CA, USA). Antibodies to CD36, CD62-P, and annexin V to detect anionic phospholipids were from Serotec Ltd (Oxford, UK).

2.2 Experimental design

Platelet-rich plasma (PRP) from blood anticoagulated with citrate/phosphate/dextrose (CPD) (19 mM) or with LMWH 200 U/mL was exposed to placentary human TF vesicles (TF-MVs, 0.11 nM) or to 5-HT (5 μM), in an aggregometer under stirring conditions. These same experiments were carried out with platelets pre-incubated with citalopram, a selective serotonin re-uptake inhibitor (SSRIs; 300 or 3000 nM), or with lepirudin (160 U/mL), a direct thrombin inhibitor. Thrombin generation was measured in aliquots of citrated PRP exposed to combinations of TF-MVs (0.11 nM) and 5-HT (5 μM). Washed platelet suspensions were exposed to TF-MVs, 5-HT (50 μM), or combined 5-HT + TF to measure platelet aggregation. Ultrastructural changes were analysed by transmission electron microscopy. Exposure of platelet activation antigens (CD36, CD62-P, and CD63) and procoagulant antigens (FvA, FNG, and vWF), as well as binding of annexin V (ANV) to the anionic phospholipids, were assessed by flow cytometry.

2.3 Isolation of platelets

Blood was obtained from healthy donors and collected into CPD at a final citrate concentration of 19 mM. In some cases, LMWH was used as an anticoagulant to achieve a final concentration equivalent to 200 U/mL. This concentration was determined in previous experiments to allow platelet aggregation, but not plasma coagulation. Platelets were separated by centrifugation (250 × g, 22 °C, 15 min) to obtain PRP. Depending on the experimental purposes, platelets were washed three times with equal volumes of citrate/citric acid/dextrose (93 mM sodium citrate, 7 mM citric acid, and 140 mM dextrose), pH 6.5, and containing 5 mM adenosine and 3 mM theophylline. The final pellet was resuspended at a concentration of 1.2 × 10⁸ platelets/mL in a Hanks’ balanced salt solution supplemented with dextrose (2.7 mM) and NaHCO₃ (4.1 mM), and maintained for 45 min at 37 °C before experiments were performed. Platelets were counted in a Beckman/Coulter MD II System Hematology Analyzer (Beckman Coulter, CA, USA).

2.4 Aggregation studies

Interaction of platelets in the PRP with the agonists was evaluated according to the classic procedure of Born in 1962, using platelet poor plasma (PPP) to set the 100% platelet aggregation. The platelet count was not adjusted. PPP was obtained by centrifugation of whole blood at 1050 × g (15 min, 22 °C). In aggregation studies with washed platelet suspensions, the same buffer (Hanks’ balanced salt solution supplemented with dextrose and bicarbonate, pH 7.2) was used to set the 100% of platelet aggregation. The modifications in turbidimetric patterns during the aggregation of platelets were continuously recorded for 10 min, in a four-channel Menarini PA 3210 Aggrecoagrecrometer (Menarini Diagnostic, Firenze), using siliconized glass cuvettes, at 37 °C and under continuous stirring at 1000 rpm. The results of these changes were expressed as percentage of maximal platelet aggregation.

Aliquots of 450 μL of PRP (CPD, LMWH) or platelet suspensions were exposed to 50 μL of TF-MVs obtained by reconstitution of the vials according to the commercial instructions to reach a final concentration of TF equivalent to 0.11 nM. The TF concentration was calculated according to a previously described method for the evaluation of whole blood procoagulant TF activity.\(^ {5,6}\)

These same studies were performed in the presence of 5-HT at a concentration of 5.0 μM for PRP samples and 50 μM for washed platelets, both chosen after titration. It has been described that the response to 5-HT is biphasic, low doses potentiate and high doses inhibit the effects of other agonists depending on the extra- cellular [Ca²⁺].\(^ {23}\) In the aggregation studies with suspensions of...
isolated platelets, 5-HT was incubated for 1 min at 37 °C, prior to the addition of TF-MVs.

Two different inhibitory strategies were used: (i) assessment of the involvement of serotoninergic mechanisms by conducting aggregation studies in the presence of citalopram, an SSRI, at two different final concentrations: one equivalent to clinical doses of 300 nM and a 10-fold higher equivalent to 3.0 μM. An incubation with citalopram for 2 min preceded aggregation experiments. (ii) Evaluation of the involvement of thrombin, using lepirudin at a clinical dose equivalent to 160 U/mL, which was incubated before the aggregation studies with TF-MVs and 5-HT together.

For sequential studies, washed platelet aggregations were stopped at different time points (0, 1, and 5 min) by addition of 0.1% glutaraldehyde for ultrastructural studies, or 0.3% paraformaldehyde for immunocytochemical studies, or to analyse activation markers and procoagulant antigens by flow cytometry.

2.5 Thrombin generation assay

Thrombin generation on citrated PRP was assessed with a fluorogenic assay. This assay is based on the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin over time upon activation of the coagulation cascade by TF and negatively charged phospholipids in plasma.25,26 In our experimental setting, we used a TF-free reagent, so that TF present is either the endogenous or on PRP aliquots or the TF-MVs added. We evaluated thrombin generation of citrated PRP samples alone and exposed to 5-HT (5 μM), TF-MVs (0.11 nM), or a combination of both agonists 5-HT + TF-MVs.

PRP aliquots of 40 μL, in the absence or presence of one or both platelet agonists, were inserted into a fluorescence plate, mixed with 50 μL of a reagent with the anionic phospholipids and with 30 μL of the fluorogenic substrate which triggers the assay. Fluorescence generated was measured at 1 min intervals for 90 min at a wavelength of 390/450 nm (excitation/emission). The evaluation program, TECHNOTHROMBIN® TGA for THERMO Fluoroskan Ascent Softwars (version 2.6) facilitated by Technocline, transforms the relative units of fluorescence into thrombin concentration (nM) and provides the time to achieve this peak of thrombin (min).

2.6 Ultrastructural studies on platelet–TF interactions previously incubated with 5-HT

Platelet suspensions previously incubated with 5-HT for 1 min and subsequently exposed to the TF-MVs were processed for ultrastructural studies. Platelet aggregations were stopped at 0, 1, and 5 min, by combining with equal volumes of 0.1% glutaraldehyde in White's saline27 for 5 min, and then centrifuged (800 × g, 22 °C, 5 min). The supernatant was removed and replaced with 3% glutaraldehyde in the same buffer. Pellets were post-fixed with 1% osmium tetroxide in distilled water containing 15 mg/mL potassium ferrocyanide (pH 7.4) for 90 min at 4 °C. After osmium fixation, samples were dehydrated in increasing ethanol gradient, then treated with propylene oxide and embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate to enhance contrast. Examination of grids was carried out in a Phillips (Mahwah, NJ, USA) 301 electron microscope.

2.7 Flow cytometry studies

Samples of washed platelet suspensions exposed to TF-MVs, 5-HT, or previously incubated with 5-HT for 1 min and then exposed to TF-MVs were analysed by flow cytometry using a FACSscan flow cytometer (Becton–Dickinson, Mountain View, CA, USA) at an excitation wavelength of 488 nm. Suspensions of washed platelets were analysed using dual-colour labelling with specific antibodies as described previously.28 Samples were added to polypropylene tubes pre-loaded with 50 μL PBS, pH 7.2, incubated with saturating concentrations of FITC-, PE-, or PerCP-conjugated antibodies for 15 min, and diluted with 1 mL PBS for immediate analysis. Platelets were differentiated by their characteristic forward vs. side scatter and by positivity for GPIb-IIIa. Histograms were composed from fluorescence data obtained in the logarithmic mode from 5000 events analysed in each sample. Antibodies to platelet activation antigens (CD36 and CD62-P, both conjugated to PE, and CD63 conjugated to FITC), to procoagulant antigens (FVa, FNG, and vWF, all conjugated to FITC), and the binding of PE-conjugated ANV to anionic phospholipids were analysed. For all studies, a negative control was performed with the matched IgG conjugated with PerCP, FITC, or PE.

Data were expressed as percentage of positive events to CD62-P, CD63, FVa, and IgG isotype. For this purpose, an analytical marker was set in the corresponding fluorescence channel to define 2% of the resting platelet population with the highest membrane fluorescence at the baseline level. This marker was used as a threshold to determine the proportion of platelets exhibiting immunofluorescence above this level in all subsequent samples. For the platelet antigens vWF, FNG, and CD36, data were expressed as the mean of the fluorescence in platelets.

2.8 Statistics

Data are expressed as mean ± standard error of the mean (SEM). One-way ANOVA test for independent experiments was applied when multiple comparisons were required, and Student's t-test for paired data was used for comparisons between two different conditions. The level of statistical significance was established at P < 0.05.

3. Results

3.1 Platelet aggregation studies

Aggregation studies performed with PRP obtained from citrated blood showed a mild aggregation to TF-MVs (5.6 ± 0.8%) and minimal reversible platelet aggregation (5.8 ± 2.3%) to 5-HT. Exposure to a combination of 5-HT + TF-MVs also resulted in a reversible aggregation of platelets but increased the percentage of maximal aggregation to 12.4 ± 2.2% (Figure 1A). On the other hand, PRP aliquots obtained from blood anticoagulated with LMWH aggregated irreversibly (89.1 ± 6.0%) after 2 min of TF-MVs addition. As in citrated PRP, 5-HT in samples anticoagulated with LMWH induced a reversible platelet aggregation (9.6 ± 4.3%) that was higher probably due to the presence of calcium. Exposure to 5-HT + TF-MVs resulted in a first peak equivalent to 14.3 ± 2.3% of platelet aggregation and accelerated the time to fully aggregate, but no statistically significant increase was detected. Figure 1 summarizes these aggregation profiles.

Previous incubation of LMWH PRP samples with the SSRI citalopram at a final concentration of 300 nM decreased platelet aggregation by 30% and delayed the synergistic effect observed by 5-HT and TF-MVs together. When 10-fold citalopram was used, a total inhibition of platelet aggregation was observed. Inhibitory strategies using lepirudin at a clinical concentration equivalent to 160 U/mL caused a statistical reduction of 75% (P < 0.05) in the aggregation profiles, suggesting a thrombin-mediated mechanism in the second wave of platelet aggregation, but did not affect the first wave. These results with inhibitory strategies are summarized in Figure 2A.

Experiments performed with washed platelets exposed to TF-MVs, under stirring conditions, revealed a reversible platelet aggregation with a maximum peak of 48.7 ± 2.1% (mean ± SEM, n = 10) at 1 min, which disappeared after 3 min. Addition of 5-HT alone caused a mild aggregation.
Previous incubation of platelets with 5-HT for 1 min followed by addition of the TF-MVs statistically increased (P < 0.05) the first peak of aggregation up to 55.9 ± 1.8% and caused a second irreversible wave of aggregation, but with aggregation levels ranging from 42.1 to 62.3% of maximal aggregation (P < 0.01 vs. TF-MVs and 5-HT) (Figure 1).

Incubation of platelet suspensions with citalopram (300 nM) caused slight inhibition on the aggregation profiles that appeared more evident when higher concentrations (3.0 μM) of the SSRI were used. Lepirudin (160 U/mL) did not affect the first wave of aggregation but decreased the following irreversible aggregation, reaching levels of 5–10% (P < 0.05) of maximal aggregation (Figure 2B).

### 3.2 Thrombin generation studies

Measure of thrombin generation in citrated PRP resulted in a maximum peak of thrombin of 270.3 ± 45.9 nM after 18.5 ± 0.6 min. The presence of 5-HT or TF-MVs alone in the PRP aliquots increased this peak to 331.2 ± 43.5 nM at 19.1 ± 0.6 min and 392.1 ± 42.9 nM (P < 0.05 vs. PRP alone) at 3.3 ± 0.1 min, respectively. However, the maximal levels of thrombin were detected in PRP samples exposed to a combination of 5-HT + TF-MVs showing a maximal thrombin concentration of 465.1 ± 52.8 nM at 3.5 ± 0.2 min (P < 0.05 vs. PRP alone and 5-HT).

### 3.3 Ultrastructural studies on platelet suspensions

Ultrastructural analysis was performed once previous incubation with 5-HT had finished, just at the moment TF-MVs were added and at 1 and 5 min upon TF-MVs addition. Incubation of platelets with 5-HT for 1 min caused a moderate pre-activation. Platelets presented pseudopodia accompanied in some cases with mild centralization of granules, but no signs of platelet degranulation were observed (Figure 3A). Washed platelets exposed to TF-MVs for 1 min appeared clearly activated and forming loosen aggregates. Activation was associated with loss of the discoid shape, generation of pseudopodia, partial degranulation, and moderate concentration of organelles in the central region. According to the aggregation profiles, platelets were aggregated after 5 min (Figure 3B) and only few of them showing signs of platelet activation were isolated.

An exhaustive electron microscopy analysis revealed that incubation with 5-HT facilitated that platelets pseudopodia surrounded TF-MVs facilitating their uptake by platelets. These vesicular structures corresponding to TF-MVs were

![Figure 1](image1.png) Platelet aggregation profiles induced by human TF-MV (0.11 nM, open circles), 5-HT (5 μM in PRP samples and 50 μM in washed platelet suspensions, closed circles), and a combination of both agonists (open squares). Aggregation profiles obtained with: (A) PRP from blood anticoagulated with CPD, (B) PRP from blood anticoagulated with LMWH (200 U/mL), and (C) washed platelet suspensions. In all cases (n = 12), the combined presence of both agonists showed enhanced platelet aggregation profiles. The fact that 5-HT caused higher aggregation in LMWH PRP than in CPD PRP suggests a role of calcium in serotonergic mechanisms.

![Figure 2](image2.png) Inhibitory strategies in platelet aggregation profiles induced by human 5HT + pTF (0.11 nM, open squares) using: (A) PRP from blood anticoagulated with LMWH (200 U/mL), and (B) washed platelet suspensions. Samples were pre-incubated for 2 min with: low concentration of SSRI (300 nM; open circles), high concentration of SSRI (3 μM; closed circles), and lepirudin (160 U/mL; open triangles). Inhibitory strategies showed a decrease on platelet aggregation profiles, suggesting a role of serotonergic mechanisms and thrombin generation.
visible in the OCS and α-granules for 5 min (Figure 3B). It appears that 5-HT pre-activates platelets and maintains the aggregating state during TF-MVs uptake confirming results of aggregation studies.

3.4 Flow cytometry analysis of platelet activation and procoagulant activity

Exposure of washed platelets to TF-MVs induced statistically significant expression of the activation antigens such as CD36 and CD62-P, and a moderate expression of CD63 which is more likely a sign of strong activation. All antigens showed a maximal peak of expression at 1 min, where platelet aggregation is highest, and a tendency to decrease afterwards. In these samples, the expression of procoagulant activity measured as binding of ANV to anionic phospholipids was moderate and the expression of FVa (P < 0.01), FNG (P < 0.05), and vWF (P < 0.01) from platelet α-granules resulted statistically increased in all cases, being maximal after 1 min.

Activation with 5-HT showed an increase in CD62-P levels (P < 0.05), but no significant changes of other activation markers were detected. The expression of procoagulant antigens increased statistically at 1 min measuring the higher levels at 5 min, specially FVa (P < 0.01) but also vWF and FNG (both with P < 0.05). Binding of ANV to platelets also increased after 5 min of 5-HT activation (P < 0.05), but exposure of anionic phospholipids was more moderate than with TF.

Platelets pre-incubated with 5-HT for 1 min and subsequently exposed to TF-MVs were markedly activated. A statistically significant increase of all the activation antigens studied was observed. Moreover, after activation with 5-HT + TF, these platelets showed a marked expression of procoagulant antigens: FVa (P < 0.01), FNG (P < 0.05), vWF (P < 0.01), and binding of ANV to the anionic phospholipids (P < 0.05). In all cases, levels measured were maximal after 1 min with a tendency to decrease after 5 min, but they never returned to basal values. All cytometry results are summarized in Figure 4.

4. Discussion

In the present work, we have observed an association between serotonergic mechanisms and TF uptake by platelets. Addition of 5-HT enhanced platelet activation and procoagulant properties induced by TF-MVs uptake, and favoured intraplatelet TF trafficking. These mechanisms could have important implications in thrombus formation and the thrombogenic profile of certain pathological conditions.

Previous experiments from our group already described that TF exposed on microvesicles can be internalized by platelets and that this process caused reversible aggregation with platelets returning to a resting state. In the presence of rFVIIa, however, platelets aggregate irreversibly. It could be speculated that TF carried by platelets may become exposed on the platelet surface after platelet activation and trigger the coagulation mechanisms during thrombus growth. In this work, we observe that in the presence of 5-HT during platelet uptake of TF-MVs, platelets become activated and aggregate irreversibly, as with rFVIIa, suggesting another potential mechanism involved in thrombus formation. Recently, studies in a fibroblast cell line revealed that FVIIa binding to cell surface TF induced the endocytosis of TF-FVIIa and mobilized intracellular TF from the Golgi store to the cell surface. The study revealed that receptors for soluble agonists like PAR-2 play a role in the internalization and mobilization of TF in fibroblasts and also show that the activation of PAR-1 and PAR-2, independently of FVIIa binding to TF, mobilizes TF and enhances TF expression on cell surface. Our present study suggests that serotonergic mechanisms could also potentiate the activation mechanisms associated with platelet TF uptake, and possibly to receptors for soluble agonists, but the involved mechanisms need to be further explored.

Serotonin (5-HT) has long been considered a weak soluble platelet agonist, but with the ability to potentiate the aggregation induced by other agonists. The differentiation between weak and strong agonists has been already challenged by Jin and Kunapuli. These authors suggested

![Figure 3](https://academic.oup.com/cardiovascres/article-abstract/84/2/309/323836/313)

**Figure 3** Ultrastructural studies in washed platelet suspensions after 1 min of incubation with 5-HT (50 µM) and following exposure to TF-MVs (0.11 nM) for 0 (A) and 5 min (B). The presence of 5-HT before addition of TF-MVs induced a pre-activated state on platelets causing generation of pseudopodia which allowed a more effective uptake and trafficking (arrow) of TF-MVs through the OCS membranes and also favoured the irreversibilization of platelet aggregation. Magnification bar corresponds to 0.5 µm.
that physiological responses of platelets may require simultaneous activation through weak and strong receptors, resulting in converging signal transduction pathways that would be responsible for the full functional platelet response. In the present work, we have shown that 5-HT alone does not cause full aggregation of a platelet suspension, although a weak, reversible aggregation wave was detected. Analysis of platelets showed activation signs, as confirmed by expression of some platelet activation markers (CD62-P) and procoagulant antigens (FVa and FNG). Notwithstanding, one of the most interesting observation was that previous incubation of platelets with 5-HT and subsequently exposure to TF-MVs enhanced platelet reactivity and procoagulant properties. One of the most significant effects is that the peak of maximal aggregation enhanced statistically and became irreversible. Several studies have evidenced that 5-HT is involved in the development of platelets with increased procoagulant activity known as coated platelets.34–37 Under our experimental conditions, increased expression of antigens associated with platelet activation, α-granule proteins, and exposure of anionic phospholipids in the surface of activated platelets suggests that though platelets take up TF and become activated, 5-HT could facilitate this state by promoting the generation of coated platelets. Consequently, activation of platelets containing 5-HT and TF-MVs in their granules would enhance their thrombogenic and procoagulant responses.

The two most direct implications of these observations would be in major depression and in cardiovascular disease, both highly prevalent conditions in our society. In major depression, an imbalance of serotonergic mechanisms is thought to participate in the pathogenesis of the disease. Enhanced platelet 5-HT uptake has been associated with increased cardiovascular risk.14,38 On the other hand, several studies have shown a link between increased risk of cardiovascular problems in clinically depressed patients39,38 and a reduction of this risk possibly due to treatment with selective 5-HT re-uptake inhibitors (SSRI).39 Interestingly, we observed that by addition of the SSRI citalopram, platelet aggregation was reduced. This observation is in agreement with a previous in vitro study of our group and with clinical studies that established a bleeding risk associated with the use of the antidepressants group of SSRI.41–43 One of the findings is that they may potentiate bleeding complications associated with the use of non-steroidal anti-inflammatory drugs. This effect also suggests a possible antithrombotic effect of SSRI that should be established in further clinical studies.

It is well known that cardiovascular events and inflammatory processes favour the secretion of TF-bearing microparticles.44 According to our previous studies, circulating TF would be taken up by platelets and stored within.45 Moreover, Muller et al. identified intraplatelet TF in the OCS membranes and matrix of α-granules in resting platelets that could be exposed on the cell surface after activation with collagen and thrombin, or secreted in platelet-derived TF-bearing microparticles. Our data suggest that TF expressed on the platelet surface together with 5-HT released from dense bodies could have implications in thrombus formation and in the thrombogenic profile of some pathological situations, causing an increased procoagulant response in the damaged area.

We suggest that serotonergic mechanisms potentiate platelet activation favouring the capture of TF-MVs, which could have significant implications in thrombus formation and propagation. This effect could be of interest under some pathological conditions in which TF-MVs are increased (i.e. acute inflammatory processes, diabetes, cancer, etc.) and could be taken up by platelets. Under these conditions, the presence of serotonin could also potentiate the platelet response increasing its thrombogenic profile. These results

Figure 4  Flow cytometry analysis of platelet activation-dependent (CD62-P, CD36, and vWF) and procoagulant activity (ANV, FVa, and FNG) markers after activation of washed platelets with TF-MV (0.11 nM), 5-HT (50 μM), or a combination of 5-HT + TF-MV. According to the aggregation profiles, a statistical increase in the expression of platelet antigens was detected. The higher values were always obtained with previous incubation of the platelet suspension with 5-HT for 1 min and following exposure to TF-MVs. *P < 0.05 vs. t = 0 min; **P < 0.05 vs. t = 1 min; (n = 6).

CD62-P

CD36

ANV

FVa

FNG

vWF

5-HT

5-HT+TF-MVs

5-HT+TF-MVs

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indirectly imply that pharmacological inhibitory strategies aimed to modulate 5-HT-mediated responses may offer a new target for the development of antithrombotic drugs.

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