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Biomedicine and Diseases: Review

Serotonin reuptake inhibitors and cardiovascular diseases: a platelet connection

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Abstract. Selective serotonin reuptake inhibitors (SSRIs) are a heterogeneous group of new antidepressants that cause a well documented acquired but reversible serotonin deficiency in blood platelets. Platelets are small, anucleate cells and are the only blood cells specialized in storing peripheral serotonin. Platelets are also an integral part of the hemostatic process that is initiated during pathologic thrombus formation in cardiovascular diseases. Serotonin release from platelets is important for functional hemostasis as indicated by congenital diseases with serotonin-deficient platelets that can lead to life-threatening bleeding problems. The postulate that SSRIs should have an impact on cardiovascular diseases is therefore well founded. Cardiovascular effects of SSRIs have indeed been shown in a number of studies investigating the effect of SSRIs in patients with psychosomatic comorbidity. SSRIs reduce the incidence of recurrent myocardial infarction (MI) in patients suffering from post-MI depression. In addition, SSRIs inhibit tight clot formation of platelets in vitro, which points to a direct anti-thrombotic or pro-fibrinolytic effect of SSRIs.

Key words. Platelets; serotonin; selective serotonin reuptake inhibitors; cardiovascular disease.

Introduction

A platelet connection between selective serotonin reuptake inhibitors (SSRIs) and cardiovascular diseases (CVDs) would undoubtedly originate in the exclusive ability of platelets to take up and transport serotonin. Serotonin is a potent biogenic amine exhibiting strong vasoactive properties, possibly through stimulation of serotonin receptors on endothelial cells and through nitric oxide production [1, 2]. Serotonin is widespread in nature, found in fruit and vegetables such as pineapple and tomato [3], as well as in the neuronal systems of all organisms from *Drosophila* [4] to humans [5]. Tryptophan is the amino acid precursor of serotonin. Tryptophan hydroxylase (tph) and amino acid decarboxylase convert this essential amino acid into serotonin [6, 7], whereby tryptophan hydroxylation is the rate-limiting step.

In birds and mammals, including humans, serotonin levels in the central nervous system represent only a small fraction of the total serotonin in the body. Serotonin is also independently produced in peripheral tissues [8]. In these organisms, enterochromaffin cells in the intestine produce serotonin and release it into the blood. Interestingly, two different tryptophan hydroxylase isoenzymes synthesize serotonin in serotonergic neurons of the raphe nuclei (tph2) and in peripheral tissues (tph1) [9]. Highly efficient uptake and transport systems for peripheral serotonin have evolved, namely platelet or thrombocyte dense granules [10]. Serotonin uptake into dense granules protects the organism from serotonin-induced uncontrolled, harmful vasoconstriction or vasodilation [11]. Although many aspects of platelet serotonin transport are well characterized, such as the serotonin (5-HT) transporter (5-HTT or SERT) [12], the serotonin receptor 5-

HT_{2A} [13] and dense granule formation and release [14–16], the full function and role of platelet serotonin transport is still unknown.

Platelets have been viewed and used as neuronal models of serotonin uptake and release for the past 50 years. Beginning in the 1970s, platelets and neurons were found to be similar with respect to serotonin uptake, and platelets were used as easily accessible neuronal models [17–20]. However, the interest in platelets as neuronal models diminished quickly because of the sensitivity of platelets to environmental stress [21–23], as well as the difficulty of measuring peripheral serotonin reproducibly [24, 25]. The discovery that SSRIs are effective antidepressants has revived such interest, since SSRIs block the reuptake of serotonin into neurons [26] as well as platelets [27–29]. To exploit this analogy, it has been suggested that pretreatment levels of platelet serotonin in samples from depressed patients might predict therapeutic outcome for at least some SSRIs [27]. Thus, the platelet model could be a useful clinical tool for monitoring the effect of antidepressants. It is by now well documented that prolonged intake of certain SSRIs, especially at high concentrations, leads to a significant decrease in platelet serotonin [28–31].

CVDs are conditions that affect the proper functioning of the heart and blood vessels, chief among which are myocardial infarction (MI, heart attack), cerebrovascular disease (stroke), transient ischemic attack (TIA) and peripheral vascular diseases. CVDs, principally heart disease and stroke, are the leading cause of death for both men and women among all racial and ethnic groups in developed countries [32]. Although CVDs can be treated or prevented, an estimated 17 million people die of CVDs each year, and at least 10% of the victims are between 35 and 64 years old.

Platelets clot in response to vessel wall injury at the acute stage of CVD, release their granule content [33] and exert both positive and negative feedback for platelet recruitment to the clot [34, 35]. Serotonin release during occlusive coronary thrombus formation has been found to increase clot stability and ischemia due to vasoconstriction [36, 37].

This review summarizes the published effects of SSRIs on platelets. Particular emphasis was placed on the inhibition of serotonin release and concomitant diminished interaction with endothelial cells, smooth muscle cells, platelets and plasma proteins, because these are all potential contributors to the cardiovascular effect of SSRIs. The controversial literature related to benefits and risks of SSRIs in patients with cardiovascular diseases led to warnings about the use of SSRIs in these patients. However, most studies investigated the effect of SSRIs on depressed patients with CVD, which significantly complicates the interpretation of these results.

Platelet serotonin

Looking for an agent in the blood responsible for hypertension, Maurice Rapport, Arda Green and Irvine Page discovered serotonin in 1948 [38] and named it for its presence in serum (sero-) and its vasoactive properties (-tonin). Serotonin was identified as 5-hydroxytryptamine (5-HT) and shown to be identical to enteramine that had been extracted from stomach and intestine [39]. The past 3 decades have seen an enormous increase in interest in and knowledge about the neurotransmitter serotonin. However, while interest in brain serotonin exploded, spurred by discoveries of its important roles in mood disorders, research on circulating serotonin has not kept pace. Initially, platelets were viewed as easily obtainable models for neurons because platelets share the serotonin receptor subtype 5-HT_{2A} and the expression of the serotonin transporter with neurons.

In humans, almost all circulating serotonin is transported by platelets in dense granules. It is long known that serotonin is a weak platelet agonist, but its effect is enhanced by ADP and epinephrine [40]. Two important issues related to serotonin as platelet agonist should be noted. First, under normal conditions the plasma levels of serotonin are very low, 0.1–10 ng/ml compared to a mean of 884 ± 202 ng/10^9 platelets measured by high-pressure liquid chromatography (HPLC) with electrochemical detection [31, 41, 42]. With a special gas chromatography-mass spectrometry method normal plasma serotonin levels were reported to be even lower, with a range of 0.05–0.3 ng/ml [43]. However, it was found that platelet-activating concentrations have to exceed 1 µM [44]. This means that the high serotonin concentrations required for platelet activation are only available after release from dense granules in response to other agonists. It has also been shown that strong activation is required in order for dense granule release to occur [45]. Second, loading of radiolabeled serotonin requires incubation of platelets with 2 µM concentrations of serotonin generally for 30 min at 37 °C. This procedure is not known to activate human platelets as it does not affect platelet morphology or platelet surface markers.

Figure 1 shows a schematic summary of the four most important interactions of peripheral serotonin after release from platelet stores: (1) interaction with endothelial cells, (2) direct and indirect interaction with smooth muscle cells, (3) platelet activation via positive feedback within the platelet clot and (4) binding to alpha-granule proteins. Among the possible responses of platelets to stimulation by serotonin are serotonylation of small GTPases of the Rab4 and RhoA family [46] and phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK 1/2) [47]. Although the involvement of small GTPases in dense granule release has long been discussed, their serotonylation has not been unequivocally demonstrated.

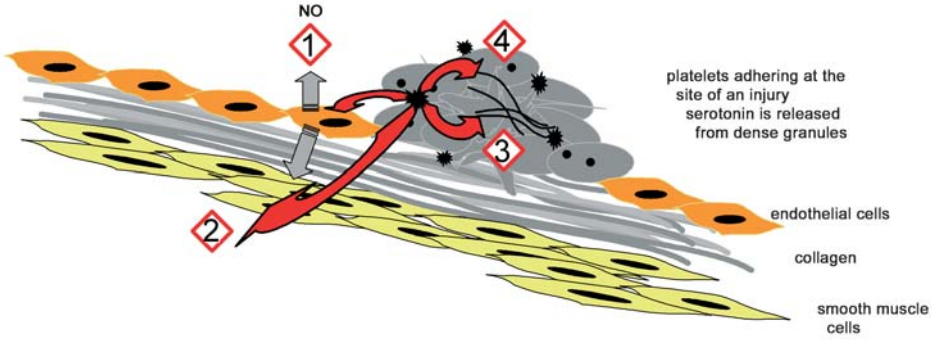


Figure 1. A schematic drawing of known functions exerted by circulating serotonin (5-hydroxytryptamine, 5-HT). Enterochromaffin cells release serotonin into the blood, where it is taken up by platelets and stored in platelet dense granules. During platelet aggregation, serotonin is released from these intracellular stores and acts on endothelial cells (1), which respond to serotonin by secretion of nitric oxide (NO), which was found to be equivalent to endothelium-derived relaxing factor. These substances act on smooth muscle cells and lead to vascular relaxation or constriction. Smooth muscle cells also express receptors for serotonin. Serotonin directly affects the tonus of large vessels through these receptors in particular at the site of injury (2). The $5\text{-}HT_{2A}$ receptors on platelets are stimulated by serotonin that is released from neighbouring platelets in a clot. Locally high serotonin concentrations initiate a positive feedback mechanism (3). Recently, serotonin binding to proteins released from alpha-granules was found to stabilize the platelet clot (4).

Further, serotonin-mediated tethering of proteins released from alpha-granules to the platelet surface has been demonstrated, which requires activation of platelets by collagen and thrombin [48, 49]. From this brief summary it is quite clear that any drug intervention that affects the serotonin content of platelets or the release from dense granules should have a major impact on hemostasis and thrombosis.

The influence of serotonin on the cardiovascular system depends both on its role as a neurotransmitter and as a neurohormone. For an excellent review on the importance of serotonin as regulator of cardiovascular function the reader is referred to Côté et al. [50]. In the heart, increased serotonin availability has been shown to produce arrhythmia, leading to heart block or to valvular fibroplasias [51]. Serotonin has also been suggested to regulate cardiac development [52]. Recently, an animal model with depleted peripheral serotonin provided the opportunity to explore the consequences that are related to lower levels of serotonin in cardiovascular function [9]. In this model the non-neuronal tph1 gene is disrupted, causing dramatic loss of peripheral serotonin, prolonged bleeding times and progressive heart failure in tph1–/– mice [53].

These results seem to contradict the reports that SSRIs reduce the risk for cardiovascular disease. However, while tph1 deletion prevents the peripheral synthesis of serotonin and thus reduces its concentration in circulation to 4–8% of normal values [50], SSRIs only inhibit serotonin uptake into platelets, leading to increased availability in plasma. Currently, the fate of this free serotonin is unknown, but binding to a plasma protein might be one possibility to control the direct vasoactive properties of serotonin. Since untreated depression was shown to be an independent risk factor for CVD, the link might be upregulated pro-thrombotic acute phase proteins such as fibrinogen. Nevertheless, exposure of mouse embryos to SSRIs inhibits serotonin uptake into the myocardium during the early stages of cardiac morphogenesis and also leads to cardiomyopathy [52].

Methods to measure serotonin are crucial to successful research into platelet serotonin and its function. Irreversible versus reversible changes in platelet serotonin content determine which methods of serotonin measurement are suitable. Irreversible release of platelet serotonin content during platelet aggregation affects all components of platelet dense granules in the same way because dense granules fuse with the plasma membrane and undergo exocytosis [54, 55]. The irreversible release of serotonin can therefore be assessed by indirect methods measuring ATP release [56], counting of dense granules on whole mounts, which reflects the calcium storage in dense granules [57], mepacrine fluorescence that is specific for either calcium or adenosine nucleotide concentrations [58], or the uranaffin reaction that was developed for the detection of adenine nucleotides in amine-containing granules [59]. However, platelets are capable of regulating the serotonin content of dense granules independently from other dense granule constituents. Drug intervention in serotonin uptake therefore only affects serotonin, while other dense granule constituents are unaffected. Direct detection of serotonin is required to determine these serotonin-specific changes for which HPLC with electrochemical or fluorescence detection has been widely used [60, 61].

Conflicting results have been published on the correlation between peripheral serotonin levels with depression and the effect of medication [62]. The measurement of peripheral serotonin as indicator for mood disorders has not been reproducible [63]. While some studies did not find

a correlation between peripheral serotonin and treatment, others found serotonin measures to correlate with treatment response [64]. An immunocytochemical assay for platelet serotonin using microscopy or flow cytometry has recently become available [41]. This assay may be suitable to answer the question whether peripheral serotonin could serve as a measure of mood disorders and treatment response.

The serotonin transporter

The serotonin (5-HT) transporter (5-HTT or SERT) is located in the membrane of serotonergic neurons, cells of the peripheral nervous system, epithelial cells and platelets. It is a key regulator of serotonergic neurotransmission [65], which is thought to contribute to many physiologic functions such as motor activity, food intake [66], sleep and reproductive activity, as well as cognition and emotional states including mood [67] and anxiety [68]. The transporter removes the neurotransmitter molecules that have been released into the synaptic cleft and returns them to the presynaptic terminals, where they are metabolized [69]. SERT belongs to the extended SLC6 gene family of Na^+/Cl^--dependent cell surface transport proteins characterized by 12 hydrophobic membrane-spanning domains [70]. Like other members of this transporter family, the SERT can operate in reverse with its function regulated by protein kinases. Its requirement for K^+ countertransport is unique [71].

The SERT is the prime target for SSRIs, which are well established antidepressant drugs [72–74]. The human SERT gene has been cloned, and its expression was characterized in different tissues, including human brain and blood platelets [75]. It appears identical in brain and platelets [76]. Both proteins are encoded by the same single-copy gene, which has been assigned to the human chromosome 17 [77]. It has been suggested that lymphocytes also possess a serotonergic storage system that can be affected by SSRIs [78]. Some studies found that resting lymphocytes express SERT [79], while others suggest significant transporter expression on activated cells, indicating that activated lymphocytes might remove serotonin from the blood to the lymphatic system [80].

Brain serotonin and the blood-brain barrier

Only the brain stem neurons of the raphe nuclei synthesize serotonin. Serotonin is involved in a diverse array of cognitive and behavioural functions because virtually every area of the brain receives projections from the raphe nuclei [65].

The blood-brain barrier (BBB) separates brain serotonin, and circulating serotonin and is the reason why the two compartments are discussed separately. The dogma persists that this specialized vascular shield can only be crossed by tryptophan, the amino acid precursor of serotonin [81], but under physiological conditions not by serotonin itself. However, animal studies have challenged this view by indicating that high concentrations of circulating serotonin could make the BBB permeable to serotonin [82, 83]. The demonstration of two genes for tryptophan hydroxylase, the enzyme for the rate-limiting step of biological serotonin synthesis, strengthens the view that the two pools of serotonin (in the brain and in circulating platelets) are generally independently regulated but does not exclude the possibility for exchange under extreme conditions [84]. There are even reports suggesting that treatment with SSRIs, which raises central serotonin, is linked to reductions in whole blood serotonin [63, 85].

The effect of SSRIs in the brain

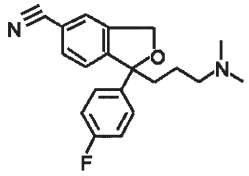
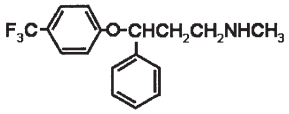
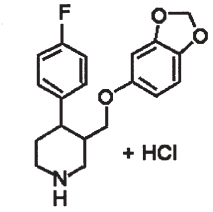
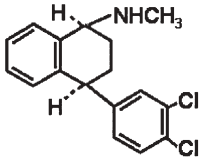
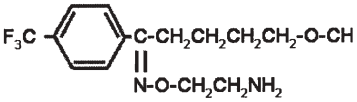
The treatment of depression with antidepressants is based on findings that depression is caused by reduced serotonin levels in the brain [86]. A serotonin hypothesis of mood disorders was put forward in 1963 [87]. The first reports of antidepressants affecting serotonin reuptake into neurons were published in the late 1960s, and their collective pharmacology was reviewed by Preskorn [88]. Characteristics of the five most widely used SSRIs, citalopram, fluoxetine, paroxetine, sertraline and fluvoxamine, are summarized in table 1 based on previously published data [89]. Despite the fact that these drugs belong to the same class, the SSRIs, significant structural and activity differences exist. Figure 2 shows the effect of SSRIs in blocking the reuptake of serotonin released into the synaptic cleft. This increases the availability of serotonin and prolongs signaling [90]. However, the increased presence of serotonin at post-synaptic receptors alone does not guarantee a clinically meaningful response.

The effect of SSRIs on platelets

SSRIs block serotonin uptake

Depending on the type of SSRI, it blocks the serotonin receptor 5-HT_{2A} and SERT with different affinity and selectivity (table 1). Serotonin transport via SERT is likely to be the prevalent process due to the low affinity of SSRIs to 5-HT_{2A} receptors. Thus, SSRIs interacting with SERT prevent the reuptake of serotonin into platelets. This leads to an increasingly serotonin-deficient platelet population as old platelets are removed and new platelets enter the circulation. Platelets are produced by megacaryocytes with dense granules devoid of serotonin. Platelets from patients treated with high doses of fluoxetine (40 mg/day) [91], fluvoxamine (300 mg/day) [92], paroxetine (60 mg/day), sertraline (250 mg/day) or citalo-

Table 1. SSRIs and their characteristics.

Substance	Structure	Dose (mg/day)	Affinity for SERT IC_{50}* values (nM)	Affinity for 5-HT2A IC_{50}* values (nM)	Circulation half life (days)
Citalopram		20 – 60	2	9000	1.5
Fluoxetine		20 – 80	8	2000	2 – 4
Paroxetine		20 – 60	0.4	30000	1
Sertraline		50 – 200	0.2	8000	1
Fluvoxamine		100 – 300	4	8000	0.5 – 1

* IC_{50}: inhibitory concentration 50, i.e. the SSRI concentration required for 50% inhibition.

pram (60 mg/day) [93] for 6 weeks are therefore highly depleted of intracellular serotonin. Interestingly, based on platelet immunocytochemistry, this acquired serotonin deficiency is indistinguishable from congenital serotonin disorders such as Hermansky-Pudlak syndrome [41]. However, SSRI-treated patients rarely develop bleeding problems. This is consistent with the postulated increased risk for CVD in untreated depression and suggests an underlying pro-thrombotic disturbance in depression [94].

SSRIs block serotonin release

SSRIs have an additional effect on platelets because they also prevent the release of serotonin from those platelets that still contain the biogenic amine. This leads to reduced aggregation responses to ADP and epinephrine and disaggregation of the loosely formed aggregates.

It has been shown that substituted amphetamines such as MDMA (Ecstasy) have acute and long-term effects on neuronal SERT because they cause PKC to translocate. The SSRI fluoxetine abolished this response [95]. In addition to $P2X_1$ and $P2Y_{12}$, ADP stimulates platelets through $P2Y_1$, a G_q-protein-coupled ADP receptor expressed on platelets and neurons [96–98]. In platelets, this signaling pathway activates protein kinase C (PKC) and raises intracellular calcium levels, which normally stimulate SERT to facilitate serotonin efflux. Ligand binding to SERT abolishes both SERT functions, efflux and reuptake. ADP stimulation of the $P2Y_{12}$ receptor might amplify platelet aggregation and secretion but does not initiate these processes. At present, the involvement of the $P2Y_{12}$ signaling pathway through G_i protein and adenylyl cyclase inhibition does not seem to play a role for the inhibition of serotonin release by SSRIs. A link

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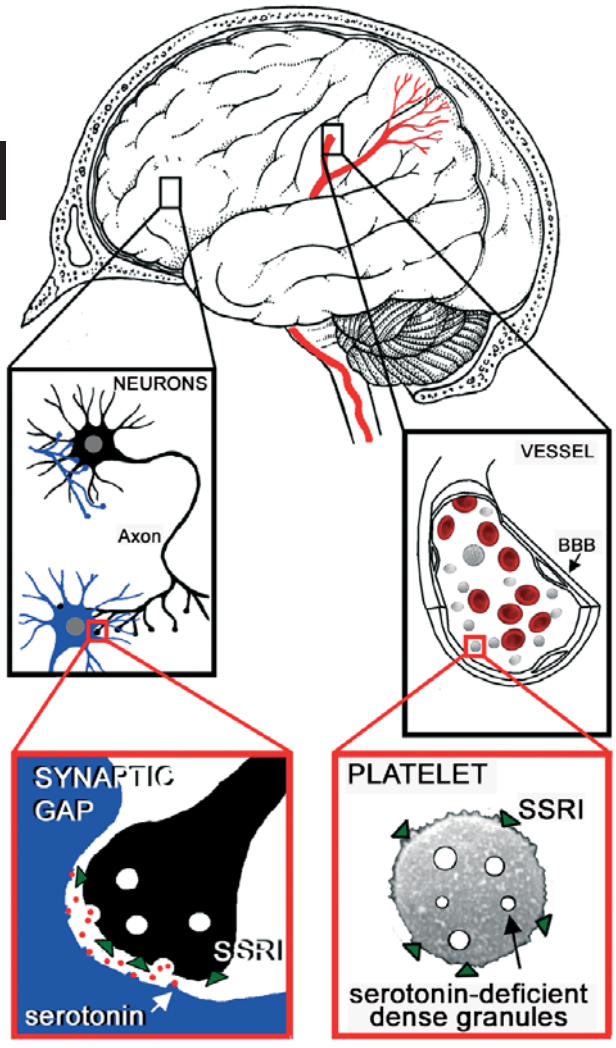

Figure 2. A schematic drawing of the direct effect of SSRIs on neurons and platelets. Circulating serotonin is considered an independent pool from serotonin in the central nervous system. Under normal conditions the BBB, which is a special tight lining of the vasculature in the brain, is only permeable to tryptophan but not serotonin. However, under stress the BBB permeability for serotonin has been shown to increase significantly. When serotonergic neurons are excited, they release serotonin into the synaptic gap to produce a post-synaptic signal. The half-life of serotonin in the synaptic cleft is very short, facilitated by serotonin recycling through a reuptake and repackaging mechanism that begins with serotonin binding at the pre-synaptic transporter. Depression is characterized by reduced serotonin concentrations in the brain. SSRIs can cross the BBB. The presence of SSRIs blocks the pre-synaptic reuptake of serotonin, leading to higher serotonin levels in the synaptic cleft. Similarly, SSRIs block the uptake of serotonin into platelet dense granules.

between the platelet serotonin transporter and the mechanism of platelet clot formation might have important implications in light of the suggested increased risk of cardiovascular problems in clinically depressed patients and the risk reduction possibly offered by SSRI treatment.

Cardiovascular diseases and the role of platelets

According to the World Health Organization, CVDs such as myocardial infarction, cerebrovascular diseases, transient ischemic attacks and peripheral vascular diseases account for 17 million deaths every year. A substantial number of these deaths can be attributed to tobacco smoking, which increases the risk of dying from coronary heart disease and cerebrovascular disease 2–3-fold. Physical inactivity and poor diet are other risk factors which increase individual risks of cardiovascular diseases.

Platelets play a central role in the hemostatic process and consequently are similarly involved in the pathological counterpart, thrombosis [99]. Drugs currently used for antiplatelet therapy interfere with platelet activation. Glycoprotein IIb-IIIa (GPIIb-IIIa) antagonists have gained particularly widespread acceptance in the treatment of acute thrombotic conditions [100, 101]. The activated form of GPIIb-IIIa is the binding site for fibrinogen and as such is required for platelet crosslinking during hemostasis and thrombosis [102].

SSRIs and CVDs

Only a few studies examined cardiovascular side-effects of SSRIs in patients with pre-existing cardiovascular disease. These studies show a favourable cardiovascular profile [103–106]. In the current primary care setting, SSRIs are considered to be more suitable for depressed patients with a recent history of heart disease than any other group of antidepressants. Clearly, all these studies focused on the treatment of depression rather than CVD.

Fluoxetine was marketed in the U.S. in February 1988. In comparative studies in patients without CVD, fluoxetine has been shown to produce a modest, but significant decrease in heart rate. The drug had no effect on the PR interval, the interval in an electrocardiogram caused by arterial contraction, or the QRS complex, the interval caused by the contractions of left and right ventricles obtained from electrocardiography measurements. In comparison to amitriptyline, fluoxetine produced less orthostatic hypotension [107]. The drug has not been studied in patients with CVD alone, and caution is recommended if it is prescribed for this depressed population.

A recent case report describes the occurrence of a transient ischemic attack (TIA) temporally related to the initiation of paroxetine. A 57-year-old Caucasian male with a history of intermittent atrial fibrillation and hypercholesterolemia developed a transient ischemic attack, and was hospitalized and given anticoagulation treatment. The presenting symptoms resolved, but recurred when paroxetine was restarted 2 days later. An objective causality assessment revealed that the transient ischemic attack was probably an adverse event resulting from use of

paroxetine [108]. Such anecdotal reports should be assessed with caution since it is known that many peripheral parameters are abnormal in depressed patients. A number of immunological and acute phase markers have been found significantly elevated in depression, and some such as fibrinogen also play a key role in CVD [109].

Very recently, reports have been published suggesting that depression is an independent risk factor for CVD [110, 111] and ischemic heart disease [109, 112]. In this context, SSRIs have been investigated as antithrombotic agents [113]. The use of sertraline was suggested to be a safe and effective treatment for recurrent depression in patients with recent myocardial infarction or unstable angina [114]. Reports suggesting that certain SSRIs cause bleeding problems in some patients have been very controversial [115–118].

Although highly variable bleeding tendencies are symptomatic for congenital serotonin deficiencies, the role of serotonin in clot formation is not clear. It has been shown that even fibrinogen-coated latex spheres form tight aggregates in vitro after the addition of thrombin [119]. This finding might suggest that serotonin is less important for aggregate formation than for aggregate stability and the prevention of premature clot lysis. In addition, if serotonin is not released from dense granules, it cannot stimulate platelets to release their alpha-granule contents. Thus, the inhibition of serotonin uptake into platelets would reduce the concentration of this clot-stabilizing factor in a thrombus and might favour clot lysis.

Platelet-endothelial activation plays a key role in the progression of acute coronary syndromes, including acute myocardial infarction and unstable angina, with serotonin undoubtedly involved as a key agonist [120]. Because of this connection the use of antidepressant drugs in patients with heart disease was expected to be beneficial [121].

A possible mechanism for the anti-thrombotic effect of SSRIs is outlined in figure 3C. Addition of SSRIs to normal platelets has no effect on thrombin-induced platelet aggregation but inhibits the secondary wave of aggregation in response to ADP and significantly reduces dense granule release. A phase contrast micrograph of platelets stimulated with 10 µM ADP shows that platelets are only loosely attached to each other (fig. 3A). The corresponding fluorescence micrograph showing Alexa488-labeled serotonin indicates that serotonin is still located inside dense granules, which are seen as bright green dots. ADP activates platelets through two G-protein-coupled ($P2Y_1$, $P2Y_{12}$) and one ion-gated receptor (P2X). The $P2Y_{12}$ ADP receptor is G_i-coupled and by inhibiting adenylyl cyclase (AC) reduces the amount of cyclic AMP (cAMP) which promotes platelet activation without a causal relationship to aggregation [122]. Because ion-dependent transporters such as the SERT are not known to be directly coupled to G proteins, this pathway might not be involved in SSRI-mediated inhibition of serotonin release from platelets

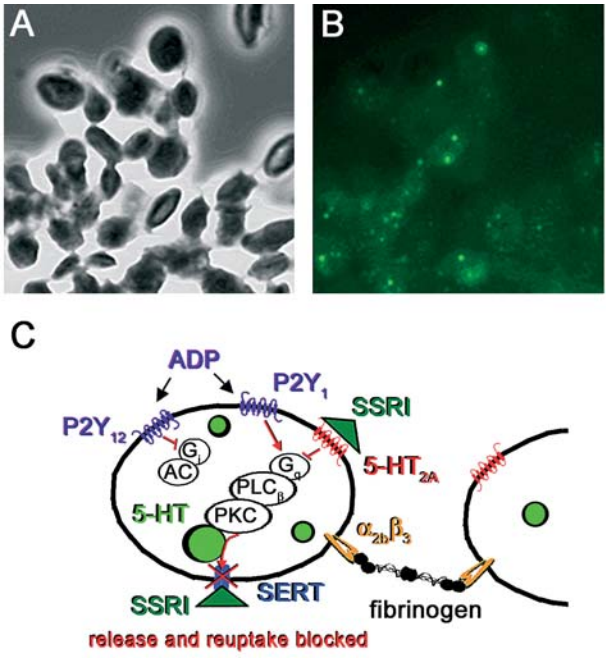

Figure 3. Effect of SSRIs on serotonin content and function of normal platelets in vitro. The addition of SSRIs to normal platelets prior to activation with a weak agonist such as ADP (10 µM) or epinephrine (10 µM) inhibits in vitro dense granule release and allows only loose aggregates to form. This is indicated by the loose assembly of primarily discoid platelets, as determined with phase contrast microscopy (*A*), and the serotonin fluorescence contained in dense granules indicated by the bright dots in the corresponding fluorescence micrograph (*B*). ADP activation of $P2Y_1$ and intracellular signaling via increased PKC activity concomitant with a raise of intracellular calcium normally results in granule secretion. 5-HT_{2A} signaling is shared with $P2Y_1$, and SSRI binding might have an inhibitory effect on this signaling pathway (*C*). More important, however, SSRIs bind to SERT and prevent PKC activation and serotonin release by the transporter. The lack of serotonin secretion reduces positive feedback stimulation of platelets. In addition, serotonin is not available to associate with fibrinogen and possibly other proteins to facilitate clot stabilization.

stimulated with ADP. The $P2Y_1$ ADP receptor pathway is shared by the serotonin 5-HT_{2A} receptor. Interaction of SSRIs with the serotonin receptor 5-HT_{2A}, which is G_q-coupled, could inhibit the activation of phospholipase C β (PLC_β), which in turn would increase intracellular Ca^{2+}, activate protein kinase C (PKC) and cause dense granule secretion. However, in view of the low affinity of the SSRIs for the 5-HT_{2A} receptor, it is much more likely that the SSRIs directly block the PKC-stimulated release of serotonin via SERT [123]. This possibility is also supported by the observation of primary aggregation of platelets after addition of ADP, but experimental proof of this hypothesis is still needed. Addition of ADP to normal platelets in the presence of SSRIs causes microaggregation and primary aggregation but no or very little dense granule release. This leads to the formation of loose and disaggregating clots in vitro (fig. 3B). Protein phospho-

rylation by PKC has been implicated in the control of neurotransmitter release and various forms of synaptic plasticity [124] and is therefore likely to play a role in platelet dense granule release [54].
It is also apparent that various antidepressants regulate the expression of G proteins in several brain regions after chronic administration [125]. In some brain cells PKC concentrations were increased in response to serotonin binding to the 5-HT_{2A} receptor [124]. In anucleate platelets, changes in expression of G proteins are not likely to be significant, but post-translational changes can affect signal transduction and their ability to activate second messenger systems.

Discussion

Regardless of the effect of SSRIs on brain serotonin and depression, an anti-thrombotic or pro-fibrinolytic effect of SSRIs could be postulated based on the current knowledge about platelet serotonin biochemistry. Platelets play an important role in cardiovascular diseases, in particular in acute situations involving thrombus formation. As known from congenital deficiencies and animal models, serotonin is required for functional hemostasis. Therefore, any drug intervention affecting platelet serotonin will have an effect on CVDs.
Interestingly, SSRIs have not entered the market as cardiovascular drugs. In fact, the proposed cardiovascular benefit of SSRIs, the changes of platelet serotonin levels in patients with depression and the effect of treatment with SSRIs on platelets and platelet aggregation are still considered side effects to their antidepressant activity. Controversial literature dealing with correlations between platelet serotonin and scoring measures of depression has further hampered the value of platelet serotonin as a diagnostic marker for depression. Serotonin-specific immunocytochemistry was used to visualize serotonin in acquired deficiencies as a consequence of the SSRI treatment of depression [31]. A better understanding of the interaction of SSRIs with platelets is particularly important in light of recent reports on the link between depression and acute coronary events [110, 112, 114, 126].
Although the number of patients suffering from depression concomitant to CVDs is high, it might be misleading to assume that the cardiovascular effects of SSRIs in psychosomatic patients is the same as in the absence of psychotic problems. In addition, the psychological side effects of SSRIs in the absence of depression might be negligible. Clinical trials testing the effect of SSRIs on normal volunteers support this assumption since SSRIs did not show any psychotic effects in normal control subjects [127, 128].
Reports in the literature on the cardiovascular effect of SSRIs are controversial. Reports on increased platelet activation with clinical depression [94], anti-thrombotic effects of SSRIs [110, 129], as well as the rare occurrence of bleeding problems with SSRI treatment [130–134] have been published. The presence of fluoxetine, paroxetine or citalopram inhibits the secondary wave of aggregation in response to ADP, and significantly decreases the release of both ATP and serotonin in normal PRP in vitro. The same phenomenon was seen with platelets from patients treated with SSRIs. Therefore, SSRIs inhibit the release of serotonin during platelet aggregation, in addition to their much better characterized function as serotonin reuptake inhibitors [31]. The inhibition of dense granule release by SSRIs is in accordance with other reports suggesting that SSRIs have anti-thrombotic [135] or pro-fibrinolytic [136] activity.
Serotonin released from dense granules might be important in downstream events during clot formation and retraction, as suggested by the role of serotonin in collagen- and thrombin-activated (COAT) platelets [48]. A mechanism has been described that involves transglutaminase-mediated conjugation of serotonin to released alpha-granule proteins such as fibrinogen and thrombospondin [49]. Fibrinogen is a highly abundant plasma protein (2–4 g/l) that has been found to associate with serotonylated proteins on the surface of COAT platelets. It is also thought that fibrinogen itself might be serotonylated. Association of serotonin with fibrinogen could have several possible roles: (i) it could affect fibrinogen binding to its receptor on the platelet surface, activated glycoprotein IIb-IIIa, also known as integrin $\alpha_{IIb}\beta_3$; (ii) serotonin could affect fibrinogen and/or fibrin as targets for factor XIIIa crosslinking and thus regulate clot retraction; (iii) serotonin could affect plasminogen cleavage and thereby regulate fibrinolysis. Because SSRIs change the level of available serotonin in plasma, drug intervention with SSRIs is likely to affect the association of fibrinogen and serotonin and therefore clot stability.
Although SSRIs show cardiovascular benefits, the possibility for undesirable side effects when SSRIs cross the BBB might limit their application in patients with CVD. Clearly, the future goal in this area of research will be the design of SSRIs that are excluded from the brain but interact with the platelet serotonin system.

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