

# Selective serotonin reuptake inhibitors inhibit glycoprotein VI-mediated platelet aggregation through the influence of the interaction between FcR $\gamma$ and Syk

Yu-Lun Tseng

Department of Psychiatry, China Medical University, Taichung, Taiwan

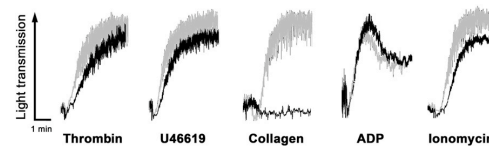
## Introduction

Platelets are recognized as a peripheral model for central serotonergic neurons because they share similarity in the reuptake, storage and metabolism of serotonin. The antidepressants selective serotonin reuptake inhibitors (SSRIs) block the reuptake of serotonin through serotonin transporter in neurons as well as platelets. It has been reported that the beneficial effect of SSRIs on the reduction of cardiovascular disease in depression patients are raised by the depletion of platelet serotonin level and the subsequent reduction of platelet activation. However, the mechanism of the effect of acute treatment of SSRIs remains unknown. We currently reported that an SSRI, citalopram, exerts an agonist-dependent role in inhibiting the aggregation in response to collagen and convulxin, indicating that the transport of serotonin regulates the activation of glycoprotein (GP) VI-mediated pathways. Therefore, we further clarify the mechanism of the inhibitory effect of SSRIs on GPVI-mediated pathway of platelet aggregation.

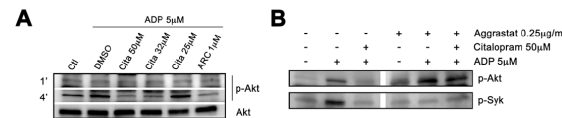
## Methods

Blood from healthy donors was collected by venipuncture into sodium citrate (9:1) and centrifuged to prepare platelet-rich plasma. The antiplatelet effect of SSRIs was determined by platelet aggregometer. The expression of GPIIb/IIIa and P-selection on platelets was examined by flow cytometry. The influence of SSRIs on the molecules of GPVI-dependent signal transduction pathways was determined by Western immunoblot.

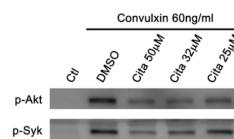
## Results



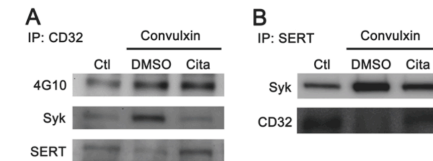
**Fig. 1.** Inhibitory effects of citalopram on platelet aggregation. (A) Human platelet suspension was preincubated with buffer (gray lines) or citalopram (50  $\mu$ M, black lines) at 37 °C for 3 min, and then thrombin (1 U/ml), U46619 (10  $\mu$ M), collagen (10  $\mu$ g/ml), ADP (20  $\mu$ M) plus fibrinogen (200  $\mu$ g/ml) or iononycin (10  $\mu$ M) were added to trigger aggregation.



**Fig. 2.** Inhibitory effects of citalopram on the signal transductions of platelets activated by ADP under stirring conditions. (A) Human PRP was pretreated with DMSO, various concentrations of citalopram (Cita) or AR-C66096 (ARC) at 37°C for 3 min and then ADP was added to trigger aggregation for next 1 or 4 min. Lysates of platelets were immunoblotted as stated. (B) In the absence or presence of Aggrastat (0.25 mg/ml), PRP was pretreated with DMSO or citalopram at 37°C for 3 min prior to stimulation with ADP. Lysates of platelets were immunoblotted as stated.

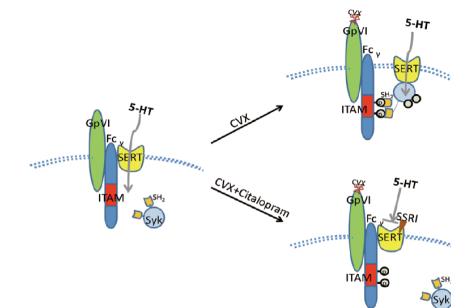


**Fig. 3.** Inhibitory effects of citalopram on GPVI-mediated signal transductions of platelets under stirring conditions. Human isolated platelets were pretreated with DMSO or various concentrations of citalopram (Cita) at 37°C for 3 min and then convulxin was added to trigger aggregation for 4 min. Lysates of platelets were immunoblotted as stated.



**Fig. 4.** Inhibitory effects of citalopram on GPVI-mediated signal transductions of platelets under stirring conditions. Human isolated platelets were pretreated with DMSO or various concentrations of citalopram (Cita) at 37°C for 3 min and then convulxin was added to trigger aggregation for 4 min. Lysates of platelets were immunoblotted as stated.

## Conclusion



Schematic illustration of the mechanism of SSRI in regulating GPVI signaling pathways. Note the phosphorylation of FcR $\gamma$ II was not influenced by the addition of SSRI. SSRI influenced the disassociation between FcR $\gamma$ II and SERT, and the association between FcR $\gamma$ II and Syk triggered by convulxin (CVX).