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# The influence of ACEA – a selective cannabinoid $CB_1$ receptor agonist on whole blood and platelet-poor plasma serotonin concentrations

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Through the $CB_1$ receptor cannabinoids modulate serotonin (5-hydroxytryptamine, 5-HT) release in the central nervous system which is connected with some of their pharmacological effects, especially antidepressant activity. 5-HT has many important physiological functions also in the periphery, particularly in the circulatory system and digestive tract. 5-HT dysfunction may be involved in some diseases pathogenesis including hypertension, migraine, cardiac disorders, cerebral ischemia or peripheral vascular diseases. Cannabinoids possible influence on 5-HT release in peripheral tissues may be clinically significant. The aim of the present study was to investigate the influence of ACEA (arachidonyl-2-chloroethylamide), a selective cannabinoid $CB_1$ receptor agonist on whole blood (WB) and platelet-poor plasma (PPP) 5-HT levels. The experiments were carried out on male and female Wistar rats. ACEA (3 mg/kg i.p.) was given alone and in combination with a selective $CB_1$ receptor antagonist AM 251 (3 mg/kg i.p.). Concentrations of 5-HT in WB and PPP were determined by enzyme-linked immunosorbent assay (Serotonin ELISA). ACEA significantly decreased concentration of 5-HT in WB (to 61%, $p < 0.02$) and its effect was blocked by AM 251. ACEA also reduced of 5-HT in PPP (to 62%) however, the difference was statistically insignificant. Research results reveal that due to $CB_1$ receptor stimulation, ACEA reduces 5-HT contents in bloodstream. This effect is probably the result of inhibition of 5-HT release from gastrointestinal tract.

## 1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a hydroxylated and decarboxylated derivative of the amino acid tryptophan. 5-HT serves many diverse physiological functions, such as the regulation of appetite, sleep, mood, cognition, sexual behavior, cardiovascular and gastrointestinal functions via an interaction with multiple 5-HT receptors (Barnes and Sharp 1999; Beattie and Smith 2008; Hoyer et al. 2002; Villalón and Centurión 2007). To date, 14 5-HT receptors, belonging to seven families (5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_5$, 5-$HT_6$ and 5-$HT_7$), have been identified (Hannon and Hoyer 2008; Hoyer et al. 2002; Martin and Humphrey 1994). In mammals, including humans, 5-HT levels in the central nervous system (CNS) represent only a small fraction of the total 5-HT in the body. 5-HT is also independently produced in the peripheral tissues. In periphery 5-HT is found predominantly in the gastrointestinal tract (80% of body 5-HT), both in enterochromaffin cells (95%) and enteric neurones (5%), where it is known to play a significant role in the control of gastrointestinal motility, sensation, and secretion (Sanger 2008; Kim and Camilleri 2000; Spiller 2001). In the bloodstream, more than 99% of 5-HT is stored in platelets which actively take it up using a serotonin transporter (SERT) identical to that found in the nervous tissue (Lesch et al. 1993; Ni and Watts 2006). 5-HT, released from activated platelets, induces smooth muscle cell contraction and proliferation but stimulates endothelial cells to release vasodilating substances (Ellis et al. 1995; Jähnichen et al. 2005; Schoeffter and Hoyer 1990; Villalón and Centurión 2007) and acts (via 5-$HT_{2A}$ receptors) as a "helper agonist" of platelet aggregation in humans (Nagatomo et al. 2004; Nishihira et al. 2006). Altered concentrations of circulating 5-HT have been implicated in several pathologic conditions including hypertension, migraine, cardiac disorders, cerebral ischemia (Dempsie and MacLean 2008; Doggrell 2003; Ramage and Villalón 2008; Robertson 1991; Villalón and Centurión 2007) and peripheral vascular diseases (Coffman and Cohen 1994; Nakamura et al. 2001; Pietraszek et al. 1993; Rydzewski et al. 1996).

Maccarone et al. (2003) reported that 5-HT and the endocannabinoid 2-arachidonoylglycerol (2-AG) could mutually reinforce their receptor binding on platelet surface. 2-AG is an endogenous lipid that acts through the activation of G-protein-coupled cannabinoid receptors and is essential for many physiological processes. In the cardiovascular system 2-AG is generated by both activated endothelial cells and platelets, and participates in the vascular control and thrombosis. 2-AG is the most abundant endocannabinoid in platelets (Maccarone et al. 2001; Randall 2007). Rat platelets contain 2-AG only, whereas in humans, another endocannabinoid anandamide (AEA) occurs. Its concentration in blood platelets, however, is twenty times lower than that of 2-AG (Maccarone et al. 1999). Under physiological concentrations, 2-AG activates platelets and interferes with other aggregation activators.

2-AG activates platelets by a non-$CB_1$/non-$CB_2$ "platelet type" cannabinoid receptor ($CB_{PT}$) which gets blocked by both SR 141716A, a $CB_1$ receptor antagonist and SR 144528, a $CB_2$ receptor antagonist (Baldassarri et al. 2008; Maccarone et al. 2001). AEA is an unlikely agonist of platelets *in vivo*, but it can rather act as a coagonist in combination with other classical aggregating agents. In contrast to 2-AG, AEA does not operate through cannabinoid receptors (Maccarone et al. 1999).

Reports concerning the influence of $\Delta^9$-tetrahydrocannabinol (THC) and other exogenous cannabinoid receptor agonists are not consistent. Formokong et al. (1989) demonstrated that THC and other phytocannabinoids inhibited blood platelets aggregation. They also reduced 5-HT release. However, the restriction was only partial and did not correlate with aggregation inhibition. Its antiaggregative activity was also observed by Levy et al. (1976), but this time, the influence on 5-HT release was not reported. In contrast, Deusch et al. (2004) demonstrated that exposure of human platelets to THC resulted in platelet activation, which would favour thromboembolism.

Cannabinoids are known to modulate 5-HT release in the central nervous system (CNS) by the $CB_1$ receptor which is strictly connected with some of their pharmacological effects such as antidepressant-like activity in particular (Gobbi at al. 2005; Hill and Gorzalka 2005). Experimental data suggest that this receptor may also regulate serotonin release from peripheral tissue depositories (Hu et al. 2007).

## 2. Investigations and results

The aim of this investigation was to study the influence of ACEA, a selective $CB_1$ receptor agonist on 5-HT concentration in whole blood (WB) and platelet-poor plasma (PPP). Such an experimental system allows the simultaneous assessment of blood platelet activity. 5-HT concentration in PPP is a good criterion of 5-HT extracellular pool (Ortiz and Artigas 1992) whereas the whole blood concentration seems representative for its contents in platelets (Takada et al. 1995).

In the control group, 5-HT average concentrations amounted to $1630.9 \pm 202.01$ ng/ml in WB and $55.5 \pm 10.62$ ng/ml in PPP. ACEA at a dose of 3 mg/kg i.p. reduced 5-HT concentration in WB to $994.4 \pm 174.84$ ng/ml (which amounts to 61% in comparison with the control, $p < 0.02$). The effect of ACEA was counteracted by a selective cannabinoid $CB_1$ receptor antagonist AM 251 (3 mg/kg i.p.) (Fig. 1). To a similar degree, ACEA decreased 5-HT concentration in PPP (to 62%) however, the difference was not statistically significant (Fig. 2). AM 251 itself influenced 5-HT concentration neither in WB nor in PPP. 5-HT concentration in platelet-poor plasma/5-HT concentration in whole blood (PPP/WB) in all examined groups did not differ significantly from the PPP/WB ratio of the control group (Fig. 3).

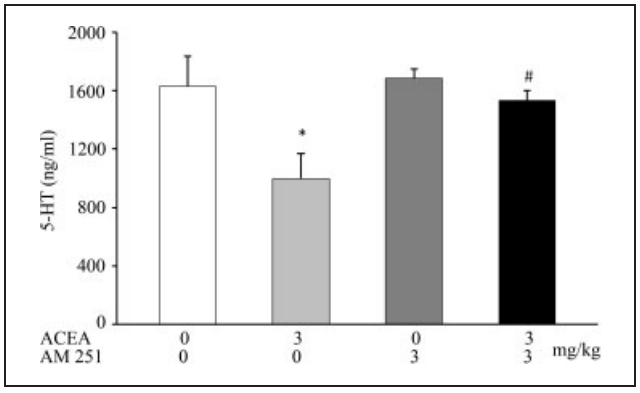


Fig. 1: Effect of ACEA given alone or in combination with AM 251 on whole blood 5-HT concentration. Results are presented as means ± SEM of 6–7 animals for each experimental group. * $P < 0.02$ vs. control and # $P < 0.02$ vs. ACEA (Dunnett's test)

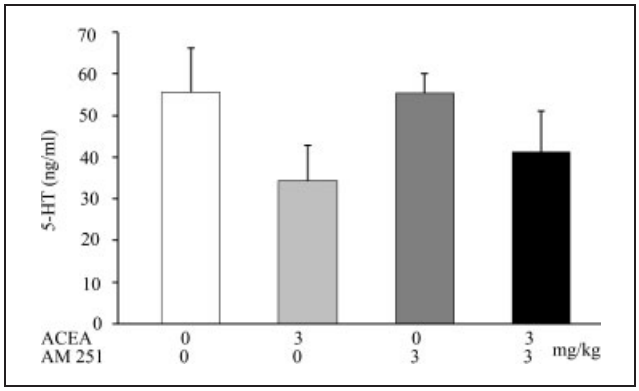


Fig. 2: Effect of ACEA given alone or in combination with AM 251 on platelet poor plasma 5-HT concentration. Results are presented as means ± SEM of 6–7 animals for each experimental group

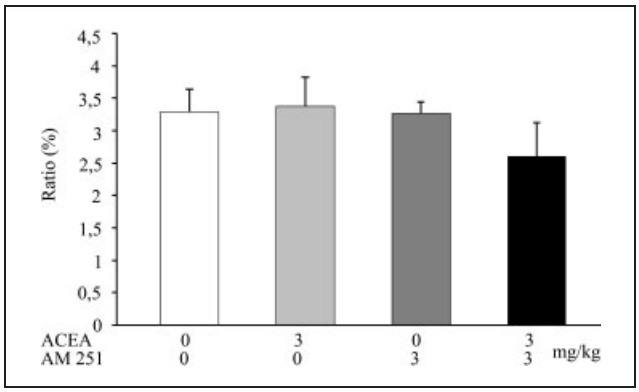


Fig. 3: Effect of ACEA given alone or in combination with AM 251 on PPP/WB ratio of 5-HT concentration. Results are presented as means ± SEM of 6–7 animals for each experimental group

## 3. Discussion

The surveys revealed that ACEA – a selective agonist of the $CB_1$ cannabinoid receptor – reduced 5-HT concentration in WB down to 61% ($p < 0.02$). This effect was counteracted by AM 251 – a selective antagonist of $CB_1$ receptor. Quite similarly, however statistically not significantly, ACEA reduced 5-HT concentration in PPP. The decrease of 5-HT concentration in WB (which stands for the contents of 5-HT in platelets) could suggest either its uptake by platelet reduction or platelet activation. Not only was no increase found, but this monoamine level in PPP proved to be lowered as well. Besides, PPP/WB ratio after ACEA administration was close to this parameter value in the control group. On this basis, one may assume that 5-HT total pool in the bloodstream has been reduced. As the blood was sampled shortly after ACEA administration (1 h) and due to the fact that ACEA did not influence the PPP/WB ratio, the decrease may be presumed to be caused mainly by the decrease of 5-HT release in the gastrointestinal tract (from enterochromaffin cells and enteric neurons) which is the main source of monoamine in blood.

The gastrointestinal tract of many species, including humans, contains an endocannabinoid system where endocannabinoids (anandamide and 2-AG) are synthesized locally

and act on $CB_1$ and $CB_2$ receptors, modulating a variety of functions (Coutts and Izzo 2004; Izzo and Camilleri 2008). $CB_1$ receptors, which are known to play the main role in the digestive system, are present in neurons of the enteric nervous system and in sensory terminals of vagal and spinal neurons and regulate the release of several neurotransmitters (Duncan et al. 2005; Hu et al. 2007).

Inhibition of 5-HT release in the gastrointestinal tract is connected with $CB_1$ receptor stimulation and is an important mechanism of cannabinoids antiemetic activity. Hu et al. (2007) confirmed these observations in their studies and showed that $CB_1$ receptor agonists inhibit vomiting induced by Staphylococcal enterotoxin in house musk shrew (*Suncus murinus*) by 5-HT release limitation in the intestine and this effect is reversed by a $CB_1$ receptor antagonist.

ACEA could reduce 5-HT release from the intestine into the bloodstream and consequently limited its contents in blood platelets. 5-HT contents restriction in blood platelets cannot be associated with ACEA influence on 5-HT uptake or release by blood platelets because in PPP, not 5-HT increase but its drop was detected. Our observation is consistent with the results achieved by Maccarrone et al. (2003) who found that 2-AG does not influence SERT activity. In turn, Velenovská and Fisar (2007) observed that activity of SERT was acutely affected by cannabinoids only at high drug concentrations. Similarly, cannabinoids were not found to increase 5-HT release from the platelets. On the contrary, in some *in vitro* tests, 5-HT release was inhibited by exogenous cannabinoids (Formokong et al. 1989; Volfe et al. 1985).

ACEA effect was suppressed by AM 251 – a selective antagonist of $CB_1$ receptor which, altogether with ACEA receptor selectivity, suggests that 5-HT contents decrease in the bloodstream after $CB_1$ receptor stimulation.

In conclusion, the results of this study suggest that, similarly to CNS, $CB_1$ receptor agonists may inhibit 5-HT release in peripheral tissues.

## 4. Experimental

### *4.1. Animals*

The studies were carried out on male and female Wistar rats weighing 250–300 g. The animals were kept in a colony room at a temperature of $21 \pm 2$ °C under 12/12 h light/dark cycle (lights on at 7 a.m.), with food and water freely available. The experimental procedures were approved by the Local Ethics Committee and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### *4.2. Chemicals*

ACEA (arachidonyl-2-chloroethylamide, ethanol solution 5 mg/ml, Tocris), AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide, Tocris), Cremophor EL (Sigma). ACEA was diluted with Cremophor EL : saline (1 : 14) and AM 251 was dissolved in a mixture of ethanol : Cremophor EL : saline (1 : 1 : 18). ACEA and AM 251 were administered intraperitoneally (i.p.) at a dose of 3 mg/kg, 1 h before blood collection. Control rats received solvents. Injection volumes were 4 ml/kg. The blood was sampled under thiopental anesthesia (80 mg/kg i.p.) by an intracardiac puncture and mixed with 3.13% trisodium citrate (v/v ratio 9 : 1).

### *4.3. 5-HT concentration assay*

#### *4.3.1. Whole blood 5-HT concentration assay*

Deionised water (4 ml) was added to 1 ml of whole blood and the samples were left for 10 min to allow blood cells lysis (Żółtowski et al. 2002). Thereafter, the samples were centrifuged at room temperature for 15 min at 1000 g.

#### *4.3.2. Platelet-poor plasma 5-HT concentration assay*

Collected blood was centrifuged at room temperature for 10 min at 200 g. Subsequently, the resulting plasma was centrifuged at 4 °C, for 10 min at 4500 g, to separate PPP from the platelet pellet.

Concentrations of 5-HT (ng/ml) in whole blood and platelet-poor plasma were determined by enzyme-linked immunosorbent assay (Serotonin ELISA, IBL, Hamburg) according to the manufacturer's instructions. The ratio of the platelet-poor plasma concentration of 5-HT to that of whole blood was calculated with the following formula: PPP/WB × 100%.

### *4.4. Statistics*

The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test as a *post-hoc*. The accepted level of significance was $P < 0.05$.

**References**


Baldassarri S, Bertoni A, Bagarotti A, Sarasso C, Zanfa M, Catani MV, Avigliano L, Maccarrone M, Torti M, Sinigaglia F (2008) The endocannabinoid 2-arachidonoylglycerol activates human platelets through non-CB1/CB2 receptors. J Thromb Haemost 6: 1772–1779.

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38: 1083–1152.

Beattie DT, Smith JA (2008) Serotonin pharmacology in the gastrointestinal tract: a review. Naunyn Schmiedebergs Arch Pharmacol 377: 181–203.

Coffman JD, Cohen RA (1994) Plasma levels of 5-hydroxytryptamine during sympathetic stimulation and in Raynaud's phenomenon. Clin Sci (Lond) 86: 269–273.

Coutts AA, Izzo AA (2004) The gastrointestinal pharmacology of cannabinoids: an update. Curr Opin Pharmacol 4: 572–579.

Dempsie Y, MacLean MR (2008) Pulmonary hypertension: therapeutic targets within the serotonin system. Br J Pharmacol 155: 455–562.

Deusch E, Kress HG, Kraft B, Kozek-Langenecker SA (2004) The procoagulatory effects of $\Delta^9$-tetrahydrocannabinol in human platelets. Anesth Analg 99: 1127–1130.

Doggrell SA (2003) The role of 5-HT on the cardiovascular and renal systems and the clinical potential of 5-HT modulation. Expert Opin Investig Drugs 12: 805–823.

Duncan M, Davison JS, Sharkey KA (2005) Review article: endocannabinoids and their receptors in the enteric nervous system. Aliment Pharmacol Ther 22: 667–683.

Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS (1995) Mediation by 5-hydroxytryptamine2B receptors of endothelium-dependent relaxation in rat jugular vein. Br J Pharmacol 114: 400–404.

Formokong EA, Evans AT, Evans FJ (1989) The inhibitory effects of cannabinoids, the active constituents of Cannabis sativa L. on human and rabbit platelet aggregation. J Pharm Pharmacol 41: 705–709.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D (2005) Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci USA 102: 18620–18625.

Hannon J, Hoyer D (2008) Molecular biology of 5-HT receptors. Behav Brain Res 195: 198–213.

Hill MN, Gorzalka BB (2005) Pharmacological enhancement of cannabinoid $CB_1$ receptor activity elicits an antidepressant-like response in rat forced swim test. Eur Neuropsychopharmacol 15: 593–599.

Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71: 533–554.

Hu DL, Zhu G, Mori F, Omoe K, Okada M, Wakabayashi K, Kaneko S, Shinagawa K, Nakane A (2007) Staphylococcal enterotoxin induces emesis through increasing serotonin release in intestine and it is downregulated by cannabinoid receptor 1. Cell Microbiol 9: 2267–2277.

Izzo AA, Camilleri M (2008) Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects Gut 57: 1140–1155.

Jähnichen S, Glusa E, Pertz HH (2005) Evidence for $5\text{-HT}_{2B}$ and $5\text{-HT}_7$ receptor-mediated relaxation in pulmonary arteries of weaned pigs. Naunyn Schmiedebergs Arch Pharmacol 371: 89–98.

Kim DY, Camilleri M (2000) Serotonin: a mediator of the brain-gut connection. Am J Gastroenterol 95: 2698–2709.

Lesch KP, Wolozin BL, Murphy DL, Reiderer P (1993). Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. J Neurochem 60: 2319–2322.

Levy R, Schurr A, Nathan I, Dvilanski A, Livne A (1976) Impairment of ADP-induced platelet aggregation by hashish components. Thromb Haemost 36: 634–640.

Maccarrone M, Bari M, Menichelli A, Del Principe D, Finazzi-Agrò A (1999) Anandamide activates human platelets through a pathway independent of the arachidonate cascade. FEBS Lett 447: 277–282.

Maccarrone M, Bari M, Menichelli A, Giuliani E, Del Principe D, Finazzi-Agrò A (2001) Human platelets bind and degrade 2-arachidonoylglycerol, which activates these cells through a cannabinoid receptor. Eur J Biochem 266: 819–825.

Maccarrone M, Bari M, Principe DD, Finazzi-Agrò A (2003) Activation of human platelets by 2-arachidonoylglycerol is enhanced by serotonin. Thromb Haemost 89: 340–347.

Martin GR, Humphrey PP (1994) Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. Neuropharmacology 33: 261–273.

Nagatomo T, Rashid M, Abul Muntasir H, Komiyama T (2004) Functions of 5-HT2A receptor and its antagonist in the cardiovascular system. Pharmacol Ther 104: 59–81.

Nakamura K, Kariyazono H, Masuda H, Sakata R, Yamada K (2001) Effects of sarpogrelate hydrochloride on adenosine diphosphate- or collagen-induced platelet responses in arteriosclerosis obliterans. Blood Coagul Fibrinolysis 12: 391–397.

Ni W, Watts SW (2006) 5-Hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). Clin Exp Pharmacol Physiol 33: 575–583.

Nishihira K, Yamashita A, Tanaka N, Kawamoto R, Imamura T, Yamamoto R, Eto T, Asada Y (2006) Inhibition of 5-hydroxytryptamine receptor prevents occlusive thrombus formation on neointima of the rabbit femoral artery. J Thromb Haemost 4: 247–255.

Ortiz J, Artigas F (1992) Effects of monoamine uptake inhibitors on extracellular and platelet 5-hydroxytryptamine in rat blood: different effects of clomipramine and fluoxetine. Br J Pharmacol 105: 941–946.

Pietraszek MH, Choudhury NA, Baba S, Sakaguchi S, Hachiya T, Urano T, Takada Y, Takada A (1993) Serotonin as a factor involved in pathophysiology of thromboangiitis obliterans. Int Angiol 12: 9–12.

Ramage AG, Villalón CM (2008) 5-Hydroxytryptamine and cardiovascular regulation. Trends Pharmacol Sci 29: 472–481.

Randall MD (2007) Endocannabinoids and the haematological system. Br J Pharmacol 152: 671–675.

Robertson JI (1991) Serotonergic type-2 (5-HT2) antagonists: a novel class of cardiovascular drugs. J Cardiovasc Pharmacol 17 (Suppl 5): S48–S53.

Rydzewski A, Urano T, Hachiya T, Kaneko H, Baba S, Takada Y, Takada A (1996) The effect of a 5-HT2 receptor antagonist sarpogrelate (MCI-9042) treatment on platelet function in Buerger's disease. Thromb Res 84: 445–452.

Sanger GJ (2008) 5-Hydroxytryptamine and the gastrointestinal tract: where next? Trends Pharmacol Sci 29: 465–471.

Schoeffter P, Hoyer D (1990) 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT1D receptor subtype. J Pharmacol Exp Ther 252: 387–395.

Spiller RC (2001) Effects of serotonin on intestinal secretion and motility. Curr Opin Gastroenterol 17: 99–103.

Takada Y, Ihara H, Urano T, Takada A (1995) Changes in blood and plasma serotonergic measurements in rats-effect of nicotine and/or exposure to different stresses. Thromb Res 80: 307–316.

Velenovská M, Fisar Z (2007) Effect of cannabinoids on platelet serotonin uptake. Addict Biol 12: 158–166.

Villalón CM, Centurión D (2007) Cardiovascular responses produced by 5-hydroxytryptamine: a pharmacological update on the receptors/mechanisms involved and therapeutic implications. Naunyn Schmiedebergs Arch Pharmacol 376: 45–63.

Volfe Z, Dvilansky A, Nathan I (1985) Cannabinoids block release of serotonin from platelets induced by plasma from migraine patients. Int J Clin Pharm 5: 243–246.

Żółtowski R, Pawlak R, Matys T, Pietraszek M, Buczko W (2002) Propranolol modifies platelet serotoninergic mechanisms in rats. J Physiol Pharmacol 53: 265–274.