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RESEARCH NOTE

The metabolism of serotonin in neuronal cells in culture and platelets

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Abstract The aim of this study is to find a relationship between serotonin (5-HT) and its metabolite 5-hydroxy indol acetic acid (5-HIAA) in hippocampus, frontal neocortex and platelets. Serotonin and 5-HIAA were measured in cultured neurons and compared with those produced by human platelets. The cortical neuronal 5-HIAA/serotonin ratio was 4.7 and for hippocampal neurons it was 3.2. In human platelets, this ratio was 1.35 suggesting that the highest serotonin metabolism occurs in the frontal neocortex followed by the hippocampus and platelets. In the presence of 0.3 μM of *p*-chlorophenylalanine both cultured neurons and platelets exhibited an approximately 50% decrease in serotonin and 5-HIAA concentration suggesting similarities in the metabolic profile in both preparations. In addition, we found that serotonin by itself does not play any role in platelet aggregation but potentiates this phenomenon in the presence of calcium ionophore A23187. This synergistic interaction between serotonin (2–5 μM) and A23187 (0.5–2 μM) was inhibited by serotonin receptor blockers [methysergide (IC_{50} = 18 μM) and cyproheptadine (IC_{50}, 20 μM)] and calcium channel blockers (verapamil and diltiazem, IC_{50} = 20 and 40 μM, respectively) that indicate both mechanisms are receptor mediated. Similarly, U73122, an inhibitor of phospholipase C (PLC), blocked the synergistic effect of serotonin and ionophore at an IC_{50} value of 9.2 μM. Wortmannin, a phosphoinositide 3-kinase (PI 3-K) inhibitor, also blocked the response (IC_{50} = 2.6 μM) by inhibiting respiratory burst. However, neither genistein, a tyrosine-specific protein kinase inhibitor, nor chelerythrine, a protein kinase C (PKC) inhibitor, affected aggregation. Our results are strongly suggestive of a synergistic interaction between serotonin type-2 and Ca-ionophore via a PLC/Ca signalling pathway.

Keywords Calcium ionophore-A23187 · Serotonin · Cultured neurons · 5-Hydroxy indol acetic acid (5-HIAA) · *p*-Chlorophenylalanine · Platelet aggregation · Calcium channel blockers · Serotonin receptor antagonist

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Introduction

Serotonin (5-hydroxytryptamine, serotonin) exerts diverse effects depending on the tissue involved and the receptor subtype mediating its action. The molecular sites of serotonin binding, re-uptake and auto-regulation have proved to be viable targets for pharmacological intervention in various disorders including acute and chronic pain, migraine, anxiety, depression, schizophrenia, hypertension and emesis (Bonate 1991; Glennon 1987; Fatima-Shad 2006). Serotonin is stored in subcellular storage vesicles at the terminal branches of neurons (Fatima-Shad and Barry 1998). Intracellular serotonin is metabolized by mitochondrial monoamine oxidase (MAO) into 5-hydroxyindoleacetic acid (5-HIAA). In addition, cerebrospinal fluid (CSF) 5-HIAA is excreted into the plasma and ultimately in the urine, and has been used as a biological marker for serotonin turnover (Tamir and Gershon 1990). However, the role of serotonin in platelet aggregation is not understood.

We have previously shown that platelet aggregation agonists, when paired at subthreshold concentrations, initiate aggregation. Combinations evaluated previously include arachidonic acid and adenosine di phosphate (ADP), thrombin

and ADP, epinephrine and ADP, and serotonin and epinephrine (Murphy 1990). Serotonin is of special interest because platelets take up this amine and release it during thrombus formation and hemostasis (Shah and Saeed 1995). Serotonin type 2A receptors, which couple with guanine nucleotide binding proteins (Gq-phospholipase C pathway), have been identified in platelets (Sanders-Bush and Mayer 1996; De Clerck et al. 1984). A23187, a calcium ionophore, rapidly enhances phosphorylation of tyrosine in platelet proteins (Watson and Arkinstall 1994), and alters the charge distribution in the plasma membrane (Vostal and Shalman 1993). In the present study, our primary goal was to determine whether serotonin potentiates the effect of low dose ionophore with regard to human platelet aggregation. We report here that although serotonin has little aggregatory effect of its own, it markedly potentiates aggregation produced by A23187.

Prior studies in our laboratory have shown that synergisms between other platelet agonists result from actions on multiple signalling pathways (Murphy 1990; Williamson et al. 1995; Saeed et al. 2001). Therefore, in search for a mechanism for this synergism, we studied the effects of inhibitors of five enzymes implicated to various degrees with intraplatelet signalling. In addition, platelets were exposed to serotonin receptor blockers and calcium channel blockers to examine the possibilities that serotonin receptors on platelets or membrane calcium channels might be involved in the aggregation synergism. Our working hypothesis evolved around the accumulative evidence (Fatima-Shad 2006; Dinan 1996; Berk et al. 2000) that platelet can act as peripheral markers for neuronal activities.

Materials and methods

Enzyme linked immunoassay (ELISA) Kits for serotonin and 5-HIAA, Puck's saline, E.M.E.M, 5-hydroxytryptamine, *p*-chlorophenylalanine, wortmannin, genistein, methysergide, cyproheptadine, indomethacin, diltiazem, verapamil, and A23187 were purchased from Sigma Chemical Co., St Louis, USA. Chelerythrine was obtained from Calbiochem–Novabiochem, UK. U73122 was purchased from Research Biochemicals International, Natick, MA, USA. All other chemicals used were of highest purity grade available.

Tissue culture

Neuronal cultures were prepared as described earlier (Fatima-Shad and Barry 1998). Briefly, five 1-day-old rat pups were quickly decapitated (every time replacing a new filter paper on the dissecting stage to avoid contamination). The hippocampi and frontal neocortex were removed quickly by using tooth picks and placed in ice-cold Puck's saline (a special dissecting medium that was aliquoted before hand in a 3 ml measure and kept in a refrigerator, as the sodium bicarbonate in the solution will lose its buffering properties below 8°C). The hippocampal cells were mechanically dissociated by gentle trituration through a narrow-bored fire-polished Pasteur pipette with 30–40 passages in 3 ml cold Puck's saline. It was important for the Puck's saline to be very cold at the beginning of the dissection and also for the hippocampi to be removed very quickly so as to retain the viability of the maximal number of cells. Dissociated cells were counted in a haemocytometer and their viability was checked by exclusion of Trypan blue. Usually 4×10^5 viable cells were distributed into poly-L-lysinated air dried 35 mm plastic plates containing freshly prepared 2 ml of modified "E.M.E.M. plus", containing 85% EMEM + 10% fetal calf serum + 2% penicillin/streptomycin + 2% L-glutamine + 1% fungizone. The plates were then incubated at 37°C in an atmosphere of humidified air with 5% CO_2. The day after the tissue-culture preparation, the medium in each plate was exchanged for fresh "E.M.E.M. plus" and their volume was reduced to 1 ml/dish. This was found to be beneficial to carefully pipette out the superficial layer of the cell medium contaminated with blood cells on the day after culture induction and then after every 3–4 days.

Estimation of serotonin and 5-HIAA using ELISA

For the measurement of concentration in the cultured neurons, platelet-rich plasma (PRP) and platelet-poor plasma (PPP), the serotonin ELISA Kit was used, which provides materials for the quantitative measurement of serotonin in serum, plasma, urine, tissue homogenates and tissue culture supernatants. Assay procedure followed the basic principle of competitive ELISA, whereby there is competition between a biotinylated and non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample.

Briefly, 50 µl of each standard, acylated control mammalian cultured hippocampus, cortex and PRP and samples were added to the appropriate wells. Then 50 µl of serotonin biotin was added to the wells. The plate was then shaken carefully after adding 50 µl of serotonin antiserum into the wells. The sealed plate was then incubated overnight (16–20 h) at 2–8°C. Afterwards, each well was washed three times with buffer and the plates were inverted on a clean blotting paper to remove any remaining liquid. Then 150 µl of enzyme conjugate was pipetted into each well. Plates were then sealed with an adhesive foil for 120 min at room temperature on an orbital shaker (500 rpm). Each well was washed again three times with

buffer and 200 µl *p*-nitrophenyl phosphate (*p*NPP) substrate solution were added to each well and the plates incubated at room temperature for 60 min on an orbital shaker. The substrate reaction was stopped by adding 50 µl of pNPP stop solution into the wells. The contents were then mixed by gently shaking the plate. The optical density was read at 405 nm with a micro titre reader within 60 min after stopping the substrate reaction.

Serotonin and its metabolite 5-HIAA concentrations were determined with and without incubation with 0.3 µM of *p*-chlorophenylalanine in cultured hippocampal and neocortical neurons as well as in platelets by using ELISA techniques

Preparation of human platelets

Human male and female volunteers aged between 20 to 30 years and were medicine-free for at least 2 weeks gave their informed consent to participate in the study. The study was approved by the Human Ethics Committee of the International Center for Chemical and Biological Sciences, University of Karachi. All subjects were free of any physical illness, as documented by a general clinical examination and by normal blood and urine tests.

Blood was drawn from the antecubital vein and mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at $260\times g$ for 15 min at 20°C to obtain platelet-rich plasma (PRP). All aggregation studies were carried out in PRP with platelet counts between 2.5 and 3×10^8 ml^{-1} at 37°C.

Measurement of platelet aggregation

Aggregation was measured using a Dual Channel Lumi-aggregometer with 0.45 ml aliquots of PRP. Enzyme inhibitors or blocking agents dissolved in normal saline were incubated for 1 min before challenge with A23187 (0.5 µM) plus serotonin (2 µM). This latter treatment combination was used as the control response against which IC_{50} value was calculated. Aggregation was recorded for 4 min additionally, so that total exposure time of the platelets to inhibitors was 5 min.

Data evaluation

Inhibitory IC_{50} concentrations expressed as means ± SEM were derived from six replicated dose–response curves, five doses per curve. Serotonin and its metabolite 5-HIAA were estimated five times with and without *p*-chlorophenylalanine and were expressed as mean ± SEM.

Results

Levels of serotonin and 5-HIAA

In cultured hippocampal and frontal neocortical neurons the levels of serotonin and 5-HIAA were measured and a ratio was calculated. Serotonin and 5-HIAA were measured and compared with each other and with that of human platelets. 5-HIAA/serotonin ratio in the frontal neocortical neurons was 4.7, in hippocampal neurons it was 3.2 and in human platelets 1.35. This ratio remained constant in the presence of 0.3 µM of *p*-chlorophenylalanine for both hippocampal and cortical cultured neurons as well as in platelets (Table 1). *p*-Chlorophenylalanine, a selective and irreversible inhibitor of tryptophan hydroxylase, a rate-limiting enzyme in the biosynthesis of serotonin, acts pharmacologically to deplete endogenous levels of serotonin and the resultant differences between treated and non-treated serotonin levels were significant ($P < 0.01$), and also for 5-HIAA ($P < 0.05$).

Serotonin, calcium and platelet aggregation

The results given in Fig. 1 show representative tracings from experiments showing the effect of: panel A: synergistic interaction of subthreshold concentrations of serotonin and calcium ionophore used as the control for subsequent panels. Panels B to D: inhibitory effects of methysergide, verapamil or wortmannin pretreatments, respectively, on the synergistic interaction of serotonin and A23187.

Table 1 Concentration of serotonin and 5-HIAA in cultured cortical and hippocampal tissues and platelets with and without 0.3 µM of *p*-chlorophenylalanine

	Hippocampus	Hippocampus plus[a]	Cortex	Cortex plus[a]	Platelets	Platelets plus[a]
Serotonin	72.4 ± 2.6 ng/ml ($n = 5$)	36.1 ± 0.6 ng/ml ($n = 5$)	77.5 ± 1.8 ng/ml ($n = 5$)	38.1 ± 0.4 ng/ml ($n = 5$)	248.7 ± 2.6 ng/ml ($n = 5$)	128.2 ± 0.6 ng/ml ($n = 5$)
5-HIAA	230.6 ± 13.6 ng/ml ($n = 5$)	120.5 ± 6.4 ng/l ($n = 5$)	361.72 ± 21.2 ng/ml ($n = 5$)	170.5 ± 9.8 ng/ml ($n = 5$)	335.4 ± 2.72 ng/ml ($n = 5$)	167.6 ± 1.1 ng/ml ($n = 5$)
5-HIAA/serotonin ratio	3.2	3.3	4.7	4.5	1.35	1.30

[a] Platelets with 0.3 µM of *p*-chlorophenylalanine

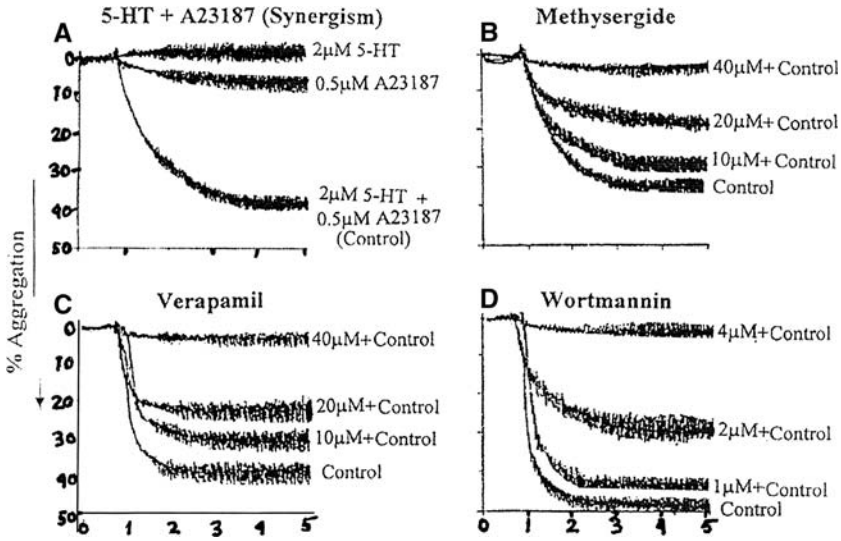

Fig. 1 Representative tracing showing the effect of various inhibitors of serotonin and Calcium ionophore A23187

Serotonin and calcium ionophore inhibitors

Most of the agents used for inhibiting serotonin and calcium channel activities exhibit a significant blocking action on calcium ionophore induced platelet aggregation (Table 2).

Discussion

Serotonin is a potent, often inhibitory, neuro-modulator, which is effective locally at the neural sites of its release. Its levels in CSF are too low for detection. The serotonin metabolic product, 5-HIAA, was measured because it is stable and is thought to reflect accurately the rate of serotonin turnover in brain tissue and platelets. We observed similarities in the metabolism in cultured neurons and platelets indicating a similar metabolic profile of the breakdown of serotonin in both preparations. We postulated that changes in the availability of serotonin and its metabolite may in turn reflect the modification of serotonin receptor activities.

Table 2 Effect of various inhibitors on platelet aggregation induced by 5-HT and A23187 (mean ± SEM; $n = 6$)

Inhibitors	IC50 μM ± SE	Possible site of action
Methysergide	18 ± 2.5	5-HT2 receptors
Cyproheptadine	20 ± 3.2	5-HT2 receptors
Verapamil	19 ± 2.5	Calcium channels
Diltiazem	40 ± 1.2	Calcium channels
U73122	9.2 ± 0.17	PLC
Wortmannin	2.6 ± 0.07	PI 3-K
Genistein	No response[a]	TPK
Chelerythrine	No response[a]	PKC

[a] Concentration up to 10 μM

Serotonin/5-HIAA ratio not only indicates the availability of endogenous serotonin but also the rate of catabolism and resultant availability of this biogenic amine.

Serotonin levels in the brain are highly dependent on levels of 5-hydroxytrytophan (5-HTP) in the central nervous system. 5-HTP easily crosses the blood–brain barrier, not requiring the presence of a transport molecule (Shah et al. 2000; Sebekova et al. 2001). 5-HTP, serotonin and glutamate co-activities in neurons as well as platelets are well established in schizophrenia and other mental disorders (Dinan 1996; Aghajanian and Marek 2000; Berk et al. 2000). The rationale for these comparative studies is to examine if platelets could be used as a peripheral model of central nervous system monoaminergic neuronal function.

It is well known that human platelets take up, store and release serotonin. Moreover serotonin type 2A receptors on platelet membranes are linked to phosphoinositide breakdown (Berk 1999; De Chaffoy de Courcelles et al. 1985). These receptors are coupled to guanine nucleotide binding proteins (G-proteins), especially Gq/PLC (De Chaffoy de Courcelles et al. 1985). When the receptors are activated, Gqα subunits in the membrane interact with PLC, which generates inositol 1,4,5 triphosphate (IP3) and diacylglycerol. IP3, in turn, releases calcium from intracellular stores and enhances calcium influx (Shah and Saeed 1995; Beigon et al. 1987). A23187 increases the influx of calcium through calcium channels directly. In the present study, serotonin alone did not act as a platelet activating agent, but it amplified aggregation produced by subthreshold concentrations of the ionophore. This observation suggests that serotonin sensitizes platelets to A23187, perhaps by binding to serotonin type 2A receptors. These receptors may also be responsible for autocrine responses to serotonin released from platelets during activation by calcium ionophore, for example, A23187. In fact, 5HT receptor antagonists, methysergide and cyproheptadine (Berridge 1993),

counteracted the aggregating effect of serotonin plus A23187 at IC_{50} values of 18 and 20 μM respectively. These data suggest that the serotonin type 2A receptors described by other investigators (Sanders-Bush and Mayer 1996; De Clerck et al. 1984) could play a potentiating role in human platelet aggregation produced by a variety of activating stimuli.

Calcium channel blocking agents like diltiazem or verapamil, inhibited serotonin and A23187-induced synergism. Results with the channel blockers are consistent with the enduring proposal that calcium influx causes aggregation (Shah and Saeed 1995; Mylecharane 1991; Vinge et al. 1988). However, interpretations of results obtained with verapamil and diltiazem must take into account observations of other workers that L-type calcium channel blockers, at concentrations used here may also block serotonin receptors. Verapamil at 0.1–10 μM has been reported to block serotonin type 2A receptors on renal mesangial cells (Ware et al. 1986) and serotonin type 3 receptors on neuroblastoma and embryonic renal cells (Goppelt-Struebe and Stroebel 1997). Other investigators (Hargreaves 1996), working with response activators different from those used in this study, have reported that indomethacin almost completely inhibited platelet aggregation and thromboxane A_2 (TXA_2) formation. Thus our observation that the serotonin/A23187 synergism was also inhibited by indomethacin provides additional evidence that cyclooxygenase (COX) pathway may be involved in aggregation (Saeed et al. 2001; Stichteno et al. 1997).

The finding that pretreatment of PRP with U73122, a frequently used tool for inhibiting PLC prevents the synergistic response is consistent with the report that intact and functional PLC is required for aggregation (Shah et al. 1999). Similarly, wortmannin, a selective PI 3-kinase inhibitor (Offermanns et al. 1997), prevented serotonin and A23187 mediated synergism at an IC_{50} value of 2.6 μM. However, a recent review (Ui et al. 1995) has called attention to a need for caution in interpreting responses evoked by these intracellular enzyme inhibitors because of a lack of specificity in certain preparations and at higher concentrations. For example, at higher doses, wortmannin inhibits calcium-channel activity and myosin light chain kinase (MLCK) activity (Murphy 1990; Taylor and Broad 1998). Thus, the possibility cannot be excluded that wortmannin may have exerted some of its actions by a direct effect on either calcium channels or MLCK.

Although chelerythrine has been shown to inhibit aggregation evoked by adrenaline (Hashimoto et al. 1992), it was inactive in our study at concentrations up to 10 μM. However, protein kinase C, the target for chelerythrine, has several isoforms (Shah et al. 1996), which may contribute to variability in response to inhibitors depending on their concentration specificity. Genistein, an inhibitor of another kinase, tyrosine-specific protein kinase (Akiyama et al. 1987; Wilkinson and Hallam 1994), was also inactive against the serotonin/A23187 response, suggesting that the aggregatory combination exerts selective effects as opposed to a blanket effect on all intraplatelet signalling pathways. In conclusion, our results point to a novel aspect of platelet aggregation mediated by an interaction between serotonin and A23187. This process for aggregation seems to require products of the Gq receptor-signalling pathway (IP3, PIP3 and calcium) and perhaps the tyrosine kinase linked receptors (which also produce PIP3). Complexity and redundancy in aggregatory regulation suggest that multiple signalling controls are used to govern the complex contributions of platelets to hemostasis.

In summary, similar neuronal and platelet serotonin metabolic activities and, in turn, availability of the serotonin could modify receptor function. This modified serotonin receptor activity may lead to the changes in the calcium channel activities and hence PLC/Ca signalling cascade and the degree of platelet aggregation could prove to be a useful indicator for the level of neuronal apoptosis.

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References

Aghajanian GK, Marek GJ (2000) 5-HT model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Rev 31:302–312

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 262:5592–5595

Berk M (1999) Lamotrigine and the treatment of mania in bipolar disorder. Eur Neuropsychopharmacol 9(4):S119–S123

Berk M, Plein H, Belsham B (2000) The specificity of platelet glutamate receptor supersensitivity in psychotic disorders. Life Sci 66:2427–2432

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. Nature 361:315–325

Biegon A, Weizman A, Karp L, Ram A, Tiano S, Wolf M (1987) Serotonin 5-HT2 receptor binding on blood platelets. A peripheral marker for depression? Life Sci 41:2485–2492

Bonate PL (1991) 5-HT receptor subtypes: functional, physiological, and clinical correlates. Clin Neuropharmacol 14:1–16

De Chaffoy de Courcelles DC, Leysen JE, De Clerk F, Van Belle H, Janssen PAJ (1985) Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S2 receptor sites. J Biol Chem 260:7603–7608

De Clerck F, Xhonneux B, Leysen J, Janssen PAJ (1984) Evidence for functional 5-HT2 receptor sites on human platelets. Biochem Pharmacol 33:2807–2811

Dinan TG (1996) 5-HT and the regulation of hypothalamic–pituitary–adrenal axis function. Life Sci 58:1683–1694

Fatima-Shad K (2006) Effect of D-serine on the serotonin receptors of human platelets. Exp Brain Res 173:353–356

Fatima-Shad K, Barry PH (1998) Morphological and electrical characteristics of post-natal hippocampal neurons in culture: the presence of bicuculline- and strychnine-sensitive IPSPs. Tissue Cell 30:236–250

Glennon RA (1987) Central 5-HT receptors as targets for drug research. J Med Chem 30:1–12

Goppelt-Struebe M, Stroebel M (1997) Mechanisms of serotonin-induced Ca^{2+} response in mesangial cells. Naunyn-Schmiedebergs Arch Pharmacol 356:240–247

Hargreaves AC, Gunthorpe MJ, Taylor CW, Lummis SC (1996) Direct inhibition of 5-hydroxytryptamine-3 receptors by antagonists of L-type Ca^{2+} channels. Mol Pharmacol 50:1284–1294

Hashimoto Y, Ogihara A, Nakanishi S (1992) Two thrombin-activated Ca^{2+} channels in human platelets. J Biol Chem 267:17078–17081

Murphy DL (1990) Peripheral indices of central 5-HT function in humans. Ann NY Acad Sci 600:282–295

Mylecharane E J (1991) 5-HT2 receptor antagonists and migraine therapy. J Neurol 238:45–52

Offermanns S, Toombs CF, Hu YH, Simon MI (1997) Defective platelet activation in Gaq-deficient mice. Nature 389:183–186

Saeed SA, Connor JD, Rasheed H, Gilani AH, Lodhi S, Ali SS et al (2001) Wortmannin inhibits platelet aggregation produced by γ-aminobutyric acid and calcium ionophore, A23187. Res Comm Mol Pathol Pharmacol 109:87–94

Sanders-Bush E, Mayer SE (1996) 5-Hydroxytryptamine (serotonin) receptor agonists and antagonists. In: Hardman JG, Limbird LE (eds) The pharmacological basis of therapeutics, chap 11, 9th edn. McGraw-Hill, New York, pp 249–263

Sebekova K, Spustova V, Opatrny K Jr, Dzurik R (2001) 5-HT and 5-hydroxyindole-acetic acid. Bratisl Lek Listy 102:351–356

Shah BH, Lashari I, Rana S, Saeed O, Rasheed H, Saeed SA (2000) Synergistic interaction of adrenaline and histamine in human platelet aggregation is mediated through activation of phospholipase C, MAP kinase and cyclooxygenase pathways. Pharmacol Res 42:479–83

Shah BH, Nawaz Z, Pertani SA, Roomi A, Mahmood H, Saeed SA, Gilani AH (1999) Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca2+ signaling. Biochem Pharmacol 58:1167–1172

Shah BH, Saeed SA (1995) Phosphtidylinositol 3-kinase inhibitor, wortmannin, inhibits 5-hydroxytryptamine mediated potentiation of platelet aggregation induced by epinephrine. Res Commun Mol Pathol Pharmacol 89:157–164

Shah BH, Shamim G, Khan S, Saeed SA (1996) Protein kinase C inhibitor, chelerythrine, potentiates the adrenaline mediated aggregation of human platelets through calcium influx. Biochem Mol Biol Int 38:1135–1141

Stichteno DO, Wagner B, Frolich JC (1997) Effect of meloxicam and indomethacin on cyclooxygenase pathways in healthy volunteers. J Invest Med 45:44–49

Tamir H, Gershon MD (1990) 5-HT-storing secretory vesicles. Ann NY Acad Sci 600:53–66

Taylor CW, Broad LM (1998) Pharmacological analysis of intracellular Ca^{2+} signalling: problems and pitfalls. Trends Pharmacol Sci 19:370–375

Ui M, Okada T, Hazeki K, Hazeki O (1995) Wortmannin as a unique probe for an intracellular signalling protein, phosphoinositide 3-kinase. Trends Biochem Sci 20:303–307

Vinge E, Anderson TLG, Larsson B (1988) Effects of some calcium antagonists on aggregation by adrenaline and serotonin and on alpha-adrenoceptor radioligand binding in human platelets. Acta Physiol Scand 133:407–416

Vostal JR, Shalman NR (1993) Venculin is a major platelet protein that undergoes Ca2+-dependent tyrosine phosphorylation. Biochem J 294:675–680

Ware JA, Johnson PC, Smith M, Salzman E (1986) Inhibition of human platelet aggregation and cytoplasmic calcium response by calcium antagonists: studies with aequorin and quin 2. Circ Res 59:39–42

Watson S, Arkinstall S (1994) The G-protein linked receptors: facts book. Academic, New York, pp 42–172

Wilkinson SE, Hallam TJ (1994) Protein kinase C: is its pivotal role in cellular activation overstated? Trends Pharmacol Sci 15:53–57

Williamson P, Bovers EM, Sweets EF, Comfurius P, Schlegel RA, Zwaal RFA (1995) Continuous analysis of the mechanisms of activated transbilayer lipid movement in platelets. Biochemistry 34:10448–10455