Biochemical Pharmacology, Vol. 40, No. 2, pp. 309–314, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

5-HYDROXYTRYPTAMINE AND THE METABOLISM OF ARACHIDONIC ACID BY THE LIPOXYGENASE AND CYCLOOXYGENASE OF WASHED HUMAN PLATELETS

YVONNE P. DRAGAN and EARL F. ELLIS*

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, U.S.A.

(*Received* 27 *June* 1988; *accepted* 4 *December* 1989)

Abstract—During secondary aggregation, platelets release 5-hydroxytryptamine (5-HT) from their dense granule stores concurrent with arachidonic acid (AA) metabolism. To examine the hypothesis that released 5-HT has a modulatory effect on the metabolism of AA by platelets, we incubated nonaggregating washed human platelets with 5-HT in the presence of [^{3}H]AA. Stimulation with 10^{-4} M 5-HT, followed by incubation with 3 μM AA and 1 μCi [^{3}H]AA for 5 min, resulted in a decrease in the formation of thromboxane B_2 (TxB_2) and 12-hydroxyheptadecatrienoic acid (HHT, $P < 0.05$). The same treatment conditions and stimulation with 10^{-7} to 10^{-4} M 5-HT resulted in an elevation of 12-hydroxyeicosatetraenoic acid (12-HETE) formation ($P < 0.05$). Treatment with the monoamine uptake inhibitor imipramine (20 μM) further increased the stimulation of 12-HETE formation observed in the presence of 10^{-4} M 5-HT, suggesting that 5-HT may act at the platelet surface. A 5-HT_{1A} receptor agonist, 8-hydroxy-dipropylaminotetralin (DPAT, 10^{-6} to 10^{-4} M) stimulated the formation of platelet cyclooxygenase (CO) products, whereas (±)1-(2,5-dimethoxy-4-iodo phenyl)-amino propane hydrochloride (DOI, 10^{-6} to 10^{-4} M), a 5-HT_2 receptor agonist, had no significant effect on CO product formation. In addition, the 5-HT_2 receptor antagonist ketanserin (10^{-7} M) did not block the changes in CO or lipoxygenase metabolism induced by 5-HT. Since both DOI and DPAT stimulated 12-HETE formation whereas ketanserin was unable to reverse the 5-HT-enhanced 12-HETE formation, it seems unlikely that the stimulation of a 5-HT_2 receptor is responsible for this action of 5-HT on platelets. We conclude that 5-HT depresses CO product formation while increasing 12-HETE formation through interaction with a platelet serotonergic binding site other than the 5-HT_2 receptor.

Perfusion or incubation of nervous and vascular tissue with 5-hydroxytryptamine (5-HT) has been shown to increase prostaglandin (PG) biosynthesis [1–6]. Additionally, 5-HT has been associated indirectly with increased prostaglandin biosynthesis since it stimulates arachidonic acid (AA) release by several tissues [7] and is known to increase the rate of phosphatidylinositol (PI) turnover in platelets [8].

Although 5-HT is known to increase cyclooxygenase (CO) metabolism in several biological systems, studies examining the effect of 5-HT on the formation of all of the platelet AA metabolites, including 12-hydroxyeicosatetraenoic acid (12-HETE) have not been reported. To address whether 5-HT affects platelet AA metabolism, we used washed human platelets incubated with [^{3}H]AA and stimulated with concentrations of 5-HT that are attainable in plasma under normal and pathophysiological conditions.

MATERIALS AND METHODS

Materials. 5-HT was purchased from the Sigma Chemical Co. (St. Louis, MO). The serotonin analogs DOI [±1-(2,5-dimethoxy-4-iodo phenyl)-amino propane hydrochloride] and DPAT (8-hydroxy-dipropylaminotetralin) were purchased from Research Biochemicals Inc. (Natick, MA). Ketanserin was a gift from Janssen Pharmaceutica (Beerse, Belgium). Imipramine was a gift from the CIBA Pharmaceutical Co. (Summit, NJ).

Glass-distilled methanol and ethyl acetate were purchased from Burdick & Jackson (Muskegon, MI). Water was deionized and purified with a Milli-R/Q Water Purification System (Millipore, Bedford, MA). Authentic tritiated AA and AA metabolite standards were obtained from New England Nuclear (Boston, MA).

Platelet preparation. Blood (50 mL) was drawn from the antecubital vein of healthy adult human volunteers who had not ingested any medication in the preceding 10 days. The blood was drawn into a syringe containing 5 mL of 3.8% (w/v) trisodium citrate, pH 7.4, and washed platelets were prepared by the method of Hamberg *et al.* [9]. The final platelet resuspension was in Krebs–Henseleit buffer (pH 7.4) made without calcium.

Incubation and extraction procedure. Aliquots of washed platelets (0.5 mL, 1×10^8 platelets) were equilibrated in siliconized glass cuvettes in a dual channel Payton aggregometer (Payton Electronics, Buffalo, NY) at 37° for 3 min prior to drug addition. Aggregation was monitored throughout each incubation, and none of the experimental incubations resulted in secondary aggregation. Unlabeled arachidonic acid (NuCheck Prep, Elysian, MN) at a final concentration of 3 μM AA and 1 μCi [^{3}H]AA (240 Ci/mmol) were combined, prepared as the

* Send all correspondence and reprint requests to: Dr. Earl Ellis, Box 613, MCV Station, Richmond, VA 23298.

sodium salt, and employed as the AA substrate. We have shown previously that this AA concentration permits detection of both increases or decreases in lipoxygenase and cyclooxygenase product formation [10].

In the control condition, 10 μL of the AA substrate was added to the platelet suspension. Pretreatment with 5-HT was begun 15 sec before addition of the AA substrate, and ketanserin or imipramine was given 15 sec before 5-HT. Aggregation was monitored during the 5-min incubation and none of the treatments induced secondary aggregation. The incubation was terminated after 5 min by the addition of 0.4 mL acetone. The platelet suspension was then placed on ice and the pH lowered to 3 with formic acid. Next, the reaction mixture was extracted twice with 3 mL of ethyl acetate, and the extracts were pooled and dried under nitrogen. The residue was dissolved in 2 mL methanol and filtered through a 0.45 μm filter (Millipore).

HPLC analysis. The sample (0.5 mL) was injected by a WISP 710B autosampler onto a 30 cm × 3.9 mm μBondapak C_{18} 10 μm particle size reverse-phase column with a phenyl Corasil packed guard column (Waters Associates, Milford, MA). Elution was accomplished in less than 1 hr with two solvents which were pumped at a total flow rate of 2 mL/min. Two model 6000A Waters pumps (Waters Associates) were employed. Solvent A was distilled water containing 0.1% glacial acetic acid and 0.15% ammonium hydroxide, pH 6.2. Solvent B was methanol.

The gradient elution was controlled by a Waters 720 System Controller (Waters Associates). Initial conditions were 45% methanol and 55% solvent A. The elution program consisted of a 10-min isocratic elution at initial conditions, followed by a 20-min linear gradient from 45 to 85% methanol. The column was next eluted with 100% methanol for 10 min, followed by a 10-min re-equilibration at initial conditions before the next sample was injected. The column eluate was directed into an on-line radiometric detector (Ramona LS from IN/US, Fairfield, NJ). Within the detector the eluant was split, and half of the eluant was mixed with scintillation fluid (Budget Solv, RPI, Mt. Prospect, IL) in a 3:1 ratio of scintillant to eluant. Peak identity was established by comparison with the chromatographic mobility of authentic radiolabeled standards. The retention times of the standards were: thromboxane B_2 (TxB_2), 8 min; 12-hydroxyheptadecatrienoic acid (HHT), 22 min; 12-HETE, 26.5 min; and AA, 31 min. HHT was identified by comparison with a standard that had been identified as HHT by gas chromatography/mass spectrometry. Data are presented as the percentage of counts recovered in each peak compared to the total counts recovered. Since 25% of the AA radiolabel is lost as malondialdehyde (MDA) during the generation of HHT, the percentage of counts recovered as HHT was multiplied by 1.33. Cyclooxygenase product formation is presented as the sum of the counts recovered in the TxB_2 and HHT peaks.

Statistical analyses. An ANOVA was performed with the various treatment groups. When a significant F ratio was obtained, a Duncan's procedure was employed; otherwise a Student's *t*- or a modified Student's *t*-test was performed. In all cases, the null hypothesis was rejected at P values greater than 0.05. Data for each experiment are presented as the mean ± SE of 3–7 experiments, each of which was performed in duplicate.

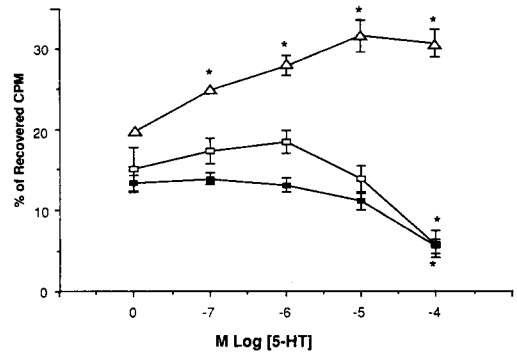

Fig. 1. Effect of 5-HT on AA metabolism in washed human platelets. Platelets (10^8/0.5 mL) were incubated in the presence of 3 μM AA and 1 μCi [^{3}H]AA with or without 10^{-7} to 10^{-4} M 5-HT. Following a 5-min incubation, AA and its metabolites were extracted into acidified ethyl acetate and subjected to reverse-phase HPLC. Values for each experiment are the mean ± SE of counts recovered from 3 to 6 platelet preparations, each of which was performed in duplicate. Key: (★) $P < 0.05$ compared to preparations not treated with 5-HT. Symbols: (△) 12-HETE, (□) TxB_2, and (■) HHT.

RESULTS

We examined the effect of a 15-sec stimulation with 5-HT prior to the addition of the 3 μM AA and 1 μCi [^{3}H]AA substrate on washed human platelet metabolism of AA. The effect of 5-HT on platelet 12-HETE formation has not been examined previously and Fig. 1 shows that the addition of 10^{-7} M to 10^{-4} M 5-HT resulted in increased 12-HETE formation ($P < 0.05$). As also shown in Fig. 1, a decline in the production of both TxB_2 and HHT was observed when platelets were stimulated with 10^{-4} M 5-HT ($P < 0.05$). No statistically significant effect on cyclooxygenase formation was observed for the other concentrations of 5-HT tested (10^{-7} to 10^{-5} M), although a trend toward decreased cyclooxygenase activity was observed with 10^{-5} M 5-HT.

Imipramine is a monoamine uptake blocker which has been shown to block 5-HT uptake in human platelets [11]. At a concentration of 20 μM, imipramine has been shown to block 95% of the uptake of 100 μM 5-HT. To determine whether the 5-HT-induced changes in AA metabolism occurred through an action of 5-HT inside the platelet or at the platelet surface, we added imipramine to the platelet incubation 15 sec before the 5-HT addition. As shown in Fig. 2, 10^{-4} M 5-HT alone or imipramine alone increased the formation of 12-HETE. Also, the effect of imipramine plus 5-HT was not greater than the effect of imipramine alone unless a concentration of 10^{-4} M 5-HT was utilized.

Platelet morphological responses to 5-HT can be

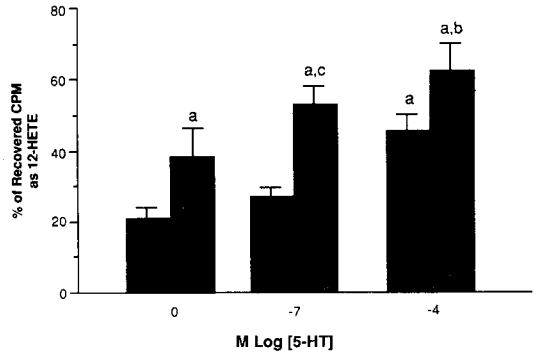

Fig. 2. Effect of imipramine and 5-HT on 12-HETE formation in washed human platelets. Platelets were washed and diluted to 10^8/0.5 mL incubation. Imipramine (2×10^{-5} M) followed 15 sec later by 5-HT (10^{-7} to 10^{-4} M) was added to the platelet preparation. Next, the AA substrate (3 μM AA and 1 μCi [^{3}H]AA) was added. After a 5-min incubation, AA and its metabolites were extracted into ethyl acetate and prepared for reverse-phase HPLC. Values for each experiment are the mean ± SE of 3 platelet preparations, each of which was performed in duplicate. Key: (a) significantly different from untreated platelets ($P < 0.05$), (b) significantly different from imipramine treatment alone ($P < 0.05$), and (c) significantly different from treatment with the same 5-HT concentration alone ($P < 0.05$). Columns: (■) 5-HT, and (▨) 5-HT plus imipramine.

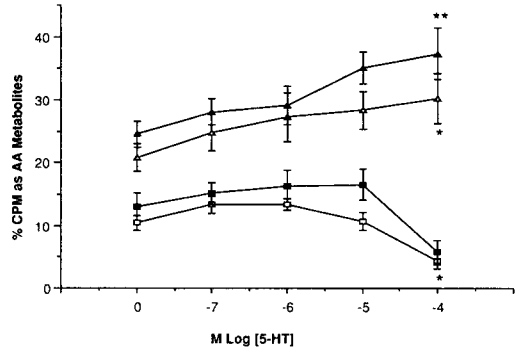

Fig. 3. Effect of ketanserin on the 5-HT-induced changes in arachidonic acid metabolism in washed human platelets. Platelets (10^8/0.5 mL) were incubated in the presence of AA substrate (3 μM AA and 1 μCi [^{3}H]AA) with 5-HT (10^{-7} to 10^{-4} M). Following a 5-min incubation, AA and its metabolites were extracted and subjected to reverse-phase HPLC. Values for each experiment are the mean percentage of counts recovered as arachidonic acid metabolites ± SE of 4–6 platelet preparations, each of which was performed in duplicate. Key: (★) $P < 0.05$ compared to preparations not treated with 5-HT, and (★★) $P < 0.05$ compared to treatment with ketanserin but without 5-HT. Symbols: TxB_2 + HHT product formation with 5-HT alone (□), and with 5-HT treatment in the presence of 10^{-7} M ketanserin (■); 12-HETE formation with 5-HT alone (△), and with 5-HT treatment in the presence of 10^{-7} M ketanserin (▲).

inhibited with 5-HT_2 receptor antagonists including ketanserin. Since 5-HT_2 receptor stimulation increases the rate of PI turnover and therefore may increase the availability of a precursor of AA, we examined the effect of ketanserin on AA metabolism. The concentration of ketanserin which we employed has been demonstrated to inhibit the 5-HT-induced morphological [12] and biochemical [13] changes observed in human platelets. Figure 3 shows that ketanserin had no effect on the inhibition of cyclooxygenase metabolism by 5-HT. Figure 3 also shows that 5-HT (10^{-4} M), in the presence and absence of ketanserin, increased 12-HETE formation compared to the unstimulated control ($P < 0.05$). Overall, the data show that a 15-sec ketanserin pretreatment did not alter significantly the effect of 5-HT alone on platelet AA metabolism.

To probe further the mechanism by which 5-HT alters platelet AA metabolism, we examined the effects of DOI, a 5-HT_2 receptor agonist, and DPAT, a 5-HT_{1A} receptor agonist, on platelet AA metabolism. Figure 4A shows that DPAT (10^{-6} and 10^{-5} M) stimulated cyclooxygenase product formation ($P < 0.05$) compared to the unstimulated control. Figure 4B shows that DOI did not alter significantly cyclooxygenase metabolism compared to the untreated control, although the trend at the higher concentration was similar to the decreased product formation observed at higher concentrations of 5-HT. At the 10^{-4} concentration, 5-HT, DOI, and DPAT all differed from one another ($P < 0.05$) with respect to their effect on cyclooxygenase product formation. Figure 5 shows that both DOI and DPAT concentration-dependently stimulated 12-HETE formation ($P < 0.05$) compared to platelets to which neither 5-HT nor its analogs had been added. Furthermore, 10^{-4} M DOI or DPAT stimulated 12-HETE formation to a greater extent than did 10^{-4} M 5-HT ($P < 0.05$).

DISCUSSION

Our finding that 5-HT altered platelet AA metabolism differs from the results obtained by de Clerck *et al.* [14] who used cat plasma and employed malondialdehyde generation as the sole measure of prostaglandin biosynthesis. In addition, our study differs from theirs in that we used a nonaggregatory concentration of AA which was below the saturation level of the cyclooxygenase, thus enabling us to detect both increases and decreases in the cyclooxygenase and lipoxygenase pathway [10].

The effects of 5-HT on human platelet AA metabolism which we observed could occur through at least three mechanisms: (1) an enhanced release of AA, (2) a direct effect on the fatty acid oxygenases, or (3) by interaction with a 5-HT binding site. Stimulation of platelet serotonergic receptors leads to an increase in numerous intracellular processes which could result in an increased release of AA. This includes the formation of phosphatidic acid (PA) and diacylglycerol (DAG) and the release of intracellular calcium by enhanced phosphatidyl inositol (PI) turnover [7, 8, 15–17]. In fact, the platelet PI response to ADP, epinephrine, A23187, and collagen is at least partially blocked by cyclooxygenase inhibition, thus providing evidence for the potential involvement of AA turnover in the platelet response to 5-HT [12]. Our studies, which utilize an exogenous

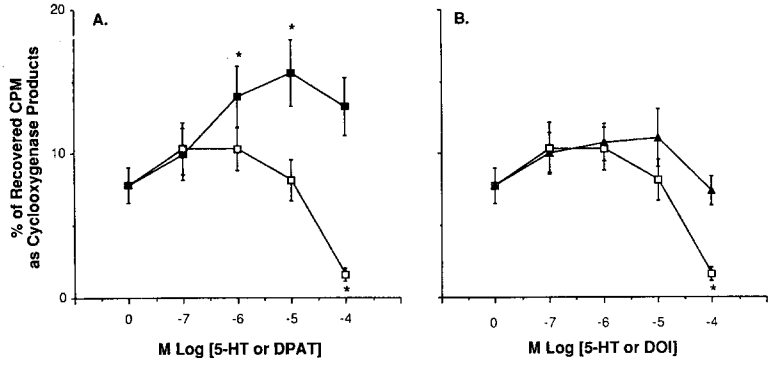

Fig. 4. Effect of 5-HT and two of its analogs (DPAT and DOI) on cyclooxygenase product formation in washed human platelets. Values for each experiment are the mean ± SE of 6–7 platelet preparations, each of which was performed in duplicate. Key: (★) $P < 0.05$ compared to preparations treated with 5-HT at the concentration at which the 5-HT analog was tested. Panel A: effect of the 5-HT_{1A} analog DPAT (10^{-7} to 10^{-4} M) on the formation of cyclooxygenase product formation (TxB_2 and HHT). Panel B: effect of the 5-HT_2 analog DOI on the formation of cyclooxygenase product formation (TxB_2 and HHT). Symbols: (□) 5-HT treatment (10^{-7} to 10^{-4} M), (■) DPAT (10^{-7} to10^{-4} M) treatment, and (▲) DOI (10^{-7} to 10^{-4} M) treatment.

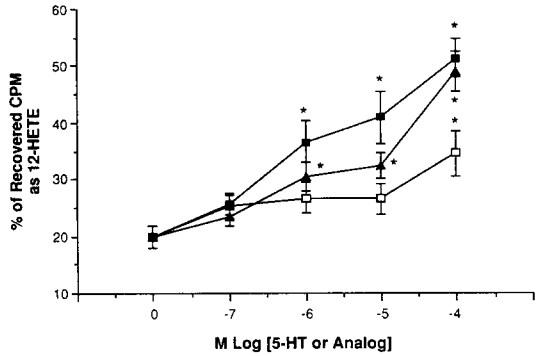

Fig. 5. Effect of 5-HT and two of its analogs on 12-HETE formation in washed human platelets. Values for each experiment are given as the mean ± SE of 4–6 platelet preparations, each of which was performed in duplicate. Key: (★) $P < 0.05$ compared to preparations not treated with 5-HT. Symbols: (□) 5-HT treatment (10^{-7} to 10^{-4} M), (■) DPAT (10^{-7} to 10^{-4} M) treatment, and (▲) DOI (10^{-7} to 10^{-4} M) treatment.

3 μM AA substrate, bypass the release step in AA metabolism. Therefore, it is unlikely that the changes in AA metabolism which we observed following 5-HT stimulation were due to an enhanced release of endogenous AA.

Several lines of evidence suggest that 5-HT can act directly upon fatty acid oxygenases. Serotonin has been shown to increase the rate of cyclooxygenase turnover in bovine seminal vesicle homogenates [18, 19]. Harada and Kawamura [20] have suggested that high concentrations of 5-HT result in a depression of cyclooxygenase activity. Thus, a direct interaction of 5-HT with the platelet cyclooxygenase enzyme could, at least partially, explain the trend toward stimulation of cyclooxygenase activity at 10^{-7} to 10^{-5} M 5-HT and the significantly decreased cyclooxygenase activity which we observed in incubations with 10^{-4} M 5-HT. A direct action of 5-HT on the platelet lipoxygenase can be excluded as a possible mechanism for the stimulation of 12-HETE formation which we observed in platelets treated with 10^{-7} to 10^{-4} M 5-HT, since imipramine, which blocks the uptake of 5-HT into the platelet, increased the stimulatory action of 5-HT on 12-HETE formation.

The stimulation of 12-HETE formation, which we observed in the presence of imipramine even without addition of 5-HT, is a novel finding. Imipramine itself may provoke 12-HETE formation or, alternatively, imipramine may enhance the action of any 5-HT previously released by the washed platelets. Since Born and Gillson [21] have shown that 5-HT can be released by platelets during the washing procedure, it is possible that imipramine acts in our untreated platelet incubations by allowing the released 5-HT to stimulate 12-HETE formation. The possible presence of 5-HT in the washed platelet incubation medium suggests that studies of AA metabolism in washed platelets may be biased toward an increased lipoxygenase and a depressed cyclooxygenase activity, compared to that which would be observed if 5-HT were not spontaneously released and present in the medium. Our results with imipramine suggest that 5-HT acts at the platelet surface to enhance 12-HETE formation. These findings in platelets imply that 5-HT release by other tissues such as the brain, which is known to form 12-HETE [22], may result in an increase in 12-HETE synthesis. Since imipramine may enhance the formation of neuronal 12-HETE in response to 5-HT, one might surmise that one mechanism of action of imipramine in the treatment of depression may involve 5-HT stimulation of brain 12-HETE levels. To our knowledge, the effect of lipoxygenase products on mammalian behavior has not been investigated.

Platelets possess two surface binding sites for 5-HT, a 5-HT_2 receptor and the 5-HT uptake carrier [23–26]. Recent evidence also suggests that platelets

possess primarily 5-HT_2-type receptors analogous to those found in rat cerebral cortex and on bovine vascular smooth muscle [27]. The morphological responses of platelets to 5-HT and the proaggregatory activity of 5-HT are blocked in a concentration-dependent manner by selective 5-HT_2 antagonists [28]. In addition, the 5-HT_2 agonist, DOI, and its congeners, induce aggregation in cat platelets [29]. Thus, the presence and physiological relevance of platelet 5-HT_2 receptors are well documented.

Although interaction of 5-HT with 5-HT_2 receptors is believed to be the mechanism of 5-HT-induced platelet activation, several of the 5-HT-induced platelet responses are not inhibited by 5-HT_2 antagonists. AA metabolism is not involved in the morphological changes observed in human platelets exposed to 5-HT. For example, addition of 5-HT to human platelets does not result in irreversible aggregation. In fact, 5-HT does not induce the release of TxB_2 or ATP except in previously sensitized platelets [24]. 5-HT is, however, proaggregatory and 5-HT addition amplifies human platelet aggregatory responses to ADP, epinephrine, norepinephrine, and collagen [12]. We have found that ketanserin does not alter the 5-HT-induced depression of platelet cyclooxygenase activity. This lack of inhibition by ketanserin and the high concentration of 5-HT necessary to induce the decrease in cyclooxygenase products suggest that this action of 5-HT is not receptor mediated. Since DPAT does not stimulate the 5-HT_2 receptor, the stimulation of 12-HETE formation observed with 5-HT and both DPAT and DOI suggests that this stimulation is not due to interaction with the 5-HT_2 site. It might be suggested that the concentration of ketanserin we employed was insufficient to inhibit the actions of 5-HT. This seems unlikely as the 10^{-7} M concentration of ketanserin which we used has been used by other investigators to inhibit other biochemical effects of 5-HT [12, 13]. A second reason for our inability to inhibit a potentially 5-HT_2-mediated action of 5-HT pertains to other actions of ketanserin. Very recent work by Leysen *et al.* [30] describes the ability of ketanserin to enhance the release of 5-HT from platelets. Their results suggest that the inhibitory action of ketanserin at 5-HT_2 sites may be overcome by a ketanserin-induced release of 5-HT from its platelet stores.

Several biological systems [31–33] possess two types of serotonergic receptors, one of which acts via elevation of cyclic AMP (5-HT_1) and the other which acts by elevation of intracellular calcium levels (5-HT_2). Since binding studies provide evidence that 5-HT_1 sites are not found on platelet membranes [34], it is unlikely that the alterations in AA metabolite distribution which we observed are due to 5-HT_1 receptor activation.

The other serotonergic binding site found on platelets is the 5-HT transporter [34, 35]. Recent work by Ieni and Myerson [36] reports that the 5-HT_{1A} agonist, DPAT, binds to this site. Previous studies on the effects of 5-HT_{1A} have not examined the effect of this agonist on AA metabolism. We found that DPAT increased the formation of CO products, whereas 5-HT depressed their formation. At the lower 5-HT concentrations employed in our study, a trend toward increased CO product formation was observed; this trend may be due to a 5-HT_{1A}-like conformation or action of 5-HT.

We found DPAT as well as DOI to enhance 12-HETE formation by interaction with a site which is insensitive to ketanserin. Since the transporter would recognize both 5-HT_{1A} and 5-HT_2 agonists, the stimulation of 12-HETE formation which we observed with 5-HT, DOI, and DPAT may be due to interaction with the 5-HT transporter.

In conclusion, the effect of 5-HT on platelet AA metabolism may be due to the action of 5-HT at a serotonergic binding site other than the 5-HT_2 receptor. The increase in 12-HETE formation which we observed with 5-HT, DOI, and DPAT may occur through interaction with the 5-HT transporter which recognizes different 5-HT conformations.

Acknowledgements—We thank Richard J. Police and Sallie A. Holt for their assistance throughout this study. These studies were supported by NS07288, NS12587, and NS23432. In addition, these studies were supported by a grant-in-aid from the American Heart Association. Dr. Earl Ellis is an Established Investigator of the American Heart Association and a recipient of a Javits Neuroscience Investigator Award.

REFERENCES

1. Alabaster VA and Bahkle YS, The release of biologically active substances from isolated lungs by 5-hydroxytryptamine. *Br J Pharmacol* **40**: 583P–583P, 1970.
2. Coughlin SR, Moskowitz MA, Antoniades HN and Levine L, Serotonin receptor-mediated stimulation of bovine smooth muscle cell prostacyclin synthesis and its modulation by platelet-derived growth factor. *Proc Natl Acad Sci USA* **78**: 7134–7138, 1981.
3. Coughlin SR, Moskowitz MA and Levine L, Identification of a serotonin type 2 receptor linked to prostacyclin synthesis in vascular smooth muscle cells. *Biochem Pharmacol* **33**: 692–695, 1984.
4. Holmes SW, The spontaneous release of prostaglandins into the cerebral ventricles of the dog and the effect of external factors on this release. *Br J Pharmacol* **38**: 653–658, 1970.
5. Leslie CA, Prostaglandin biosynthesis and metabolism in rat brain slices. *Res Commun Chem Pathol Pharmacol* **14**: 455–469, 1976.
6. Ramwell PW, Shaw JE and Jessup R, Spontaneous and evoked release of prostaglandins from frog spinal cord. *Am J Physiol* **211**: 998–1004, 1966.
7. Conn PJ and Sanders-Bush E, Biochemical characterization of serotonin stimulated phosphoinositide turnover. *Life Sci* **38**: 663–669, 1986.
8. de Chaffoy de Courcelles D, Roevens P, Van Belle H and de Clerck F, The synergistic effect of serotonin and epinephrine on the human platelet at the level of signal transduction. *FEBS Lett* **219**: 283–288, 1987.
9. Hamberg M, Svensson J and Samuelsson B, Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. *Proc Natl Acad Sci USA* **71**: 3824–3828, 1974.
10. Dragan YP and Ellis EF, Optimization of an assay for studying the effects of agents on cyclooxygenase and lipoxygenase metabolism in washed human platelets. *Prostaglandins Leukotrienes Essential Fatty Acids*, in press, 1990.
11. Reimers H, Adenine nucleotides in blood platelets. In: *The Platelets: Physiology and Pharmacology* (Ed.

Longnecker GL), pp. 85–112. Academic Press, New York, 1985.
12. de Clerck F, Xhonneux B and van de Wiele R, Biochemical mechanisms in 5-hydroxytryptamine-induced human platelet aggregation. *Agents Actions* **17**: 220–228, 1985.
13. Erne P and Pletscher A, Rapid intracellular release of calcium in human platelets by stimulation of 5-HT_2-receptors. *Br J Pharmacol* **84**: 545–549, 1985.
14. de Clerck F, Xhonneux B, Leysen J and Janssen P, The involvement of 5-HT_2-receptor sites in the activation of cat platelets. *Thromb Res* **33**: 305–321, 1984.
15. Conn PJ and Sanders-Bush E, Serotonin-stimulated phosphoinositide turnover: Mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J Pharmacol Exp Ther* **234**: 195–203, 1985.
16. Kendall D and Nahorski SR, 5-Hydroxytryptamine stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: Pharmacological characterization and effects of antidepressants. *J Pharmacol Exp Ther* **233**: 473–479, 1985.
17. Litosch I, Wallis C and Fain JN, 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary gland. *J Biol Chem* **260**: 5464–5471, 1985.
18. Sih CJ, Takeguchi C and Foss P, Mechanisms of prostaglandin biosynthesis. III. Catecholamines and serotonin as cofactors. *J Am Chem Soc* **92**: 6670, 1970.
19. Takeguchi C, Kohno E and Sih CJ, Mechanism of prostaglandin biosynthesis. I. Characterization and assay of bovine prostaglandin synthase. *Biochemistry* **10**: 2372–2376, 1971.
20. Harada Y and Kawamura MKM, Is platelet 5-HT involved in prostaglandin endoperoxide generation as an endogenous tryptophan-like cofactor? *Jpn J Pharmacol* **43(S)**: 88P, 1987.
21. Born G and Gillson RE, Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J Physiol (Lond)* **146**: 472–491, 1959.
22. Adesuyi S, Cockrell C, Gamache D and Ellis E, Lipoxygenase metabolism of arachidonic acid in brain. *J Neurochem* **45**: 770–776, 1985.
23. Bevan J and Hepinstall S, Effects of ketanserin and mepyramine on platelet aggregation and on the uptake of 5-hydroxytryptamine into platelets. *Thromb Res* **30**: 415–423, 1983.
24. de Clerck F, David J-L and Janssen PAJ, Inhibition of 5-hydroxytryptamine-induced and -amplified human platelet aggregation by ketanserin (R 41 468), a selective 5-HT_2 receptor antagonist. *Agents Actions* **12**: 388–397, 1982.
25. Kim BK, Steiner M and Baldini MG, Serotonin binding sites of human blood platelets. *Anal Biochem* **106**: 92–98, 1980.
26. Lampugnani MA, DeGaetano G and Rossi EC, Functional distinction between serotonin uptake and serotonin-induced shape change receptors in rat platelets. *Biochim Biophys Acta* **693**: 22–26, 1982.
27. de Clerck F, Somers Y and Van Gorp L, Platelet–vessel wall interactions in hemostasis: Implication of 5-hydroxytryptamine. *Agents Actions* **15**: 627–635, 1984.
28. Leysen J, de Chaffoy de Courcelles D, de Clerck F, Niemegeers C and Van Nueten J, Serotonin-S2 receptor binding sites and functional correlates. *Neuropharmacology* **23**: 1493–1501, 1984.
29. Seggel MR, Qureshi GD and Glennon RA, Effect of 5-HT_2 selective agonists on cat platelet aggregation. *Life Sci* **41**: 1077–1081, 1987.
30. Leysen JE, Eens A, Gommeren W, van Gompel P, Wynants J and Janssen PAJ, Identification of non-serotonergic [^{3}H]ketanserin binding sites associated with nerve terminals in rat brain and with platelets; Relation with release of biogenic amine metabolites induced by ketanserin- and tetrabenzine-like drugs. *J Pharmacol Exp Ther* **244**: 310–322, 1988.
31. Barbaccia ML, Brunello N, Chaung D and Costa E, Serotonin-elicited amplification of guanylate cyclase activity in hippocampal membranes from adult rats. *J Neurochem* **40**: 1671–1679, 1983.
32. Berridge MJ and Heslop JP, Separate 5-hydroxytryptamine receptors on the salivary gland of the blowfly are linked to the generation of either cyclic adenosine 3′,5′-monophosphate or calcium signals. *Br J Pharmacol* **73**: 729–738, 1981.
33. MacDermont J, Higashida H, Wilson SP, Matsuzawa A, Minna J and Nirenberg M, Adenylate cyclase and acetylcholine release regulated by separate serotonin receptors of somatic cell hybrids. *Proc Natl Acad Sci USA* **76**: 1135–1139, 1979.
34. MacBride PA, Mann JJ, McEwen B and Biejon A, Characterization of serotonin binding sites on human platelets. *Life Sci* **33**: 2033–2041, 1983.
35. Peters JR and Grahame-Smith DG, Human platelet 5-HT receptors: Characterization and functional association. *Eur J Pharmacol* **68**: 243–256, 1980.
36. Ieni JR and Myerson LR, The 5-HT_{1A} receptor probe [^{3}H]-8-OH-DPAT labels the 5-HT transporter in human platelets. *Life Sci* **42**: 311–320, 1988.