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EFFECTS OF CLOFIBRATE AND 6-SUBSTITUTED CHROMAN ANALOGS ON HUMAN PLATELET FUNCTION:

MECHANISM OF INHIBITORY ACTION*

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Abstract—The effects of clofibrate (CPIB) and two related cyclic analogs, 6-chlorochroman-2-carboxylic acid (CCCA) and 6-phenylchroman-2-carboxylic acid (PCCA), on human platelet function were evaluated. CPIB, CCCA and PCCA all inhibited platelet activation, i.e. aggregation and secretion of [^{14}C]serotonin induced by ADP, epinephrine, collagen and thrombin, in a concentration-dependent manner. PCCA was at least fifty-two times more effective as an inhibitor of ADP-, epinephrine- and collagen-induced platelet activation and only 2-fold more effective as an inhibitor of thrombin-induced platelet activation when compared with CPIB or CCCA. Only PCCA inhibited platelet aggregation and [^{14}C]serotonin secretion induced by arachidonic acid (AA) in a concentration-dependent manner. CPIB and CCCA did not inhibit AA-induced platelet activation. In fact, both of these agents had a potentiating effect on the onset of platelet aggregation by AA. All three compounds inhibited thrombin-induced release of [^{3}H]arachidonic acid ([^{3}H]AA) from platelet phospholipids and thrombin-mediated malondialdehyde (MDA) production. Only PCCA, however, inhibited AA-induced MDA production. These results indicate that CPIB, CCCA and PCCA all inhibit platelet activation by inhibiting prostaglandin biosynthesis. PCCA blocked AA-induced platelet activation, and this additional inhibitory action of PCCA appears to be responsible for its comparatively higher inhibitory potency. A comparison of the structure-activity relationship of the inhibitors indicated that replacement of the chloro group by a phenyl group produced a compound (PCCA) that was a potent inhibitor of prostaglandin biosynthesis and was thereby a more effective antiaggregatory agent than either CPIB or CCCA.

Clofibrate is a well-known hypolipidemic drug [1–3]. It has also been shown to inhibit platelet aggregation and the secretion of granular materials [4–6]. The dual antilipemic and antiaggregatory action of clofibrate makes it a potentially effective and unique drug for the treatment and/or prevention of thromboembolic disorders. A number of studies, however, have shown that clofibrate not only failed to significantly lower the rate of mortality in patients suffering from ischemic heart disease (IHD), but also increased the numbers of IHD-unrelated death(s) [7]. More recently, there have been some suggestions that clofibrate may also possess hepatotoxic and carcinogenic activities [8]. Some European countries are withdrawing the drug from the market and the FDA has recommended restricted use of clofibrate [9].

In our search for agents that may have dual antilipidemic and antiaggregatory effects, we synthesized numerous analogs [10–12], among which were the related cyclic 6-substituted chromans, namely 6-chlorochroman-2-carboxylic acid (CCCA) and 6-phenylchroman-2-carboxylic acid (PCCA). Their effects on human platelet aggregation and serotonin secretion, as well as their differing modes of action, are the subject of this paper.

MATERIALS AND METHODS

Collection of blood and preparation of platelet-rich plasma. Blood was collected from normal human volunteers who reported to be without medication for at least 10 days prior to blood drawing. Citrate buffer (0.11 M) [13] or acid-citrate dextrose solution (ACD) [14] was used as an anticoagulant in ratios of 1:9 (v/v) or 1:6 (v/v) respectively. Citrated blood was centrifuged at 120 *g* for 15 min at room temperature. Platelet-rich plasma (PRP) was transferred to polypropylene tubes and stored under an atmosphere of 8% CO_2 in air to avoid changes in plasma pH [15]. The remaining blood sample was centrifuged at 1100 *g* for 15 min at room temperature to obtain platelet-poor plasma (PPP).

Preparation of washed platelet suspensions. The blood collected into ACD solution was centrifuged to obtain PRP as mentioned above. PRP was centrifuged at 1100 *g* for 10 min and the platelet pellet

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was resuspended in modified Tyrode's solution without calcium, pH 6.5 [16]. Apyrase (EC 3.6.1.5) (34 μg/ml) was added to the platelet suspension to prevent accumulation of ADP [17]. The platelet suspension was kept at 37° for 5 min and then centrifuged. Two percent EGTA [ethyleneglycolbis (amino-ethylether)tetra-acetate] was added (1:9, v/v) just before centrifugation during the first wash only. The washing procedure was repeated three times and the final platelet pellet was resuspended in a modified Tyrode's solution, pH 7.4 [16].

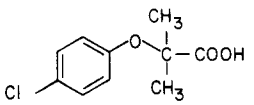
Platelet enumeration. Platelets were counted by phase-contrast microscopy [18]. The platelet count was adjusted to 3×10^8/ml for aggregation and secretion studies and to 1×10^9/ml for metabolic studies. Platelet aggregation studies were performed according to the turbidometric method of Born [19], as modified by Mustard *et al.* [20] using a Chronolog model 330 aggregometer (Haverton, PA).

Secretion of platelet materials. The secretion of platelet contents from the dense granules was measured by monitoring the secretion of radioactivity from platelets prelabeled with [^{14}C]serotonin ([^{14}C]-5-HT). Platelets were incubated with [^{14}C]-5-HT (0.2 μCi/3 × 10^8 platelets) for 15 min during the third wash. Platelets were washed once more to remove the radioactivity from the medium and then resuspended in modified Tyrode's solution, pH 7.4. Imipramine was added to block re-uptake of released serotonin. The secretion of [^{14}C]-5-HT from platelets was determined by centrifuging the samples at 1200 *g* for 1 min in a microfuge and counting an aliquot (100 μl) of the supernatant fraction in a liquid scintillation counter. The secretion data were calculated as the percentage of radioactivity in platelets. The effect of inhibitors is expressed as the percentage of inhibition of the maximum secretable radioactivity against the log molar concentrations of each inhibitor.

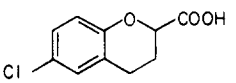
Loss of lactic acid dehydrogenase (LDH) from platelet cytoplasm was also monitored in preliminary experiments to discriminate between the secretion of granular contents and lysis of platelets. Only up to 5 percent of total LDH appeared in extracellular medium with the highest dose of inducers tested.

Release of [^{3}H]*arachidonic acid* ([^{3}H]*AA*) *from platelet phospholipids.* Portions of platelets (6.0 ml) were incubated with 1.0 ml of [^{3}H]AA-albumin (6 μCi) prepared as described by Rittenhouse-Simmons *et al.* [21], at 37° for 15 min during the third wash. After one additional washing, platelets were resuspended in a modified Tyrode's solution, pH 7.4. The uptake of [^{3}H]AA was determined from the difference in the amount of radioactivity in the platelet suspension and that in the platelet supernatant fraction. Aliquots (1 ml) of [^{3}H]AA-labeled platelets were incubated either with diluent, or drug and thrombin (5 units/ml) for 10 min. In preliminary studies, various doses of thrombin ranging from 0.1 to 5.0 units/ml of platelets were evaluated for the maximal release of [^{3}H]AA from platelets. The release of [^{3}H]AA was monitored according to Bills *et al.* [22]. After incubation, 2.67 ml of EDTA (0.1 M) was added to each tube and subsamples were taken for radiometric analysis. The mixtures were transferred to glass stoppered conical tubes containing 4.8 ml of ice-cold chloroform–methanol (1:2, v/v) and mixed thoroughly. Equal volumes (1.6 ml) of chloroform and of 0.1 M EDTA were added to each tube and samples were mixed vigorously every 5 min for 30 min. Samples were then centrifuged at 1200 *g* for 10 min at 4°. The aqueous phase was removed and acidified with four drops of formic acid. To the aqueous phase, 6.67 ml of chloroform-methanol (5:1, v/v) was added. After vigorous shaking and standing, the organic phases were separated, pooled, and concentrated to 2 ml by evaporation under a stream of nitrogen at 37°.

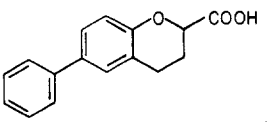
Lipid extracts were applied onto silicic acid (0.5 g) columns and the following fractions were eluted: (1) neutral lipids with 10 ml chloroform; (2) prostaglandin fraction with 10 ml of 5% methanol in chloroform; and (3) phospholipid fraction with 6 ml of methanol followed by 4 ml of 1% water in methanol. The collected fractions were evaporated to dryness under nitrogen and the residue was dissolved in a scintillation solution. The radioactivity was measured in a liquid scintillation counter, and release of [^{3}H]AA from platelet phospholipids was calculated as the difference of radioactivity in the



2-METHYL-2-(4′-CHLOROPHENOXY)-PROPIONIC ACID



6-CHLOROCHROMAN-2-CARBOXYLIC ACID



6-PHENYLCHROMAN-2-CARBOXYLIC ACID

Fig. 1. Chemical structures of 2-methyl-2-(4′-chlorophenoxy)-propionic acid, CPIB (I); 6-chlorochroman-2-carboxylic acid, CCCA (II); and phenylchroman-2-carboxylic acid, PCCA (III).

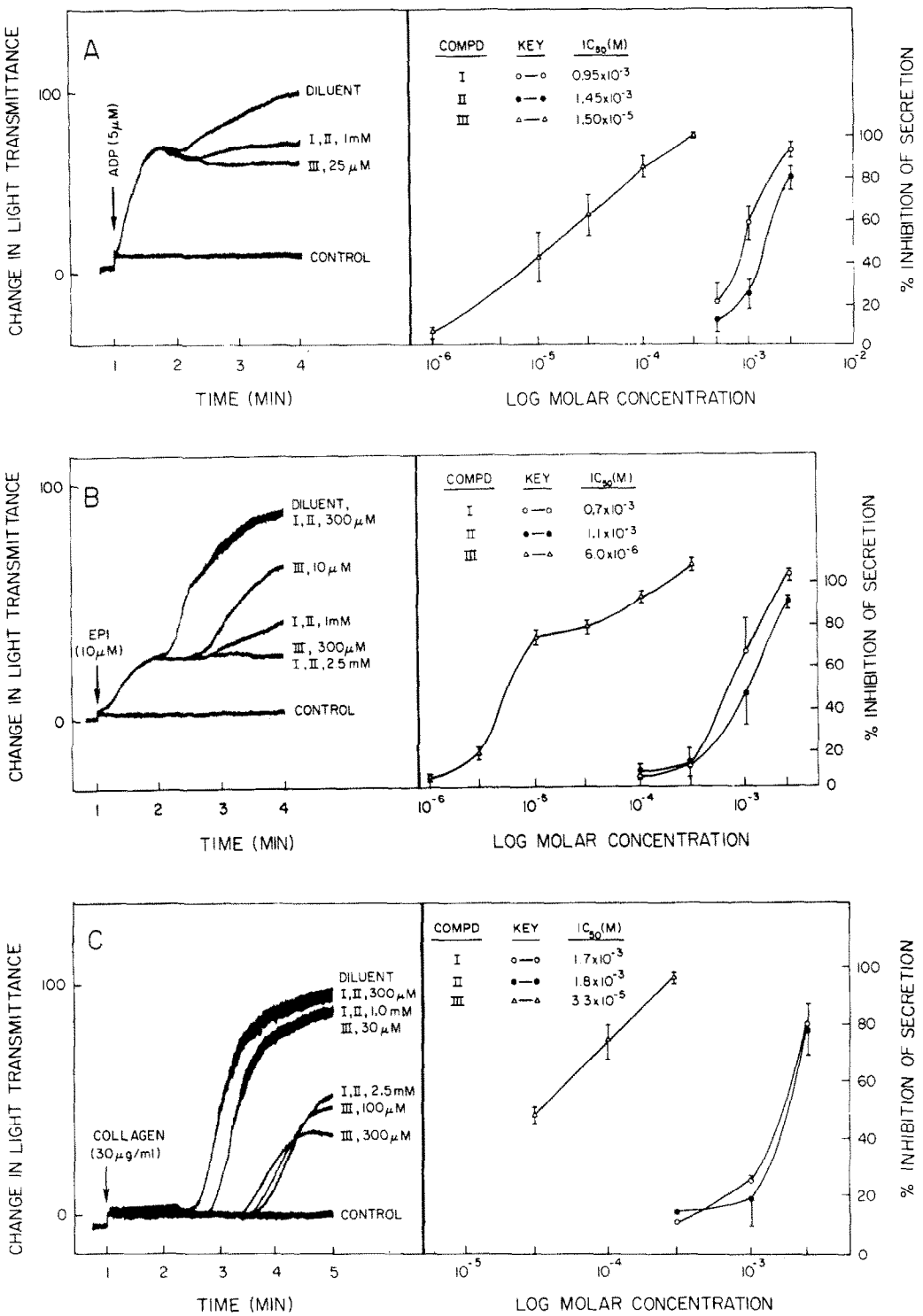


Fig. 2. Effects of CPIB (I), CCCA (II), and PCCA (III) on human platelet aggregation (left panels) and secretion of [^{14}C]-5-HT (right panels). Panel A: Imipramine (2.5 μM), diluent or different concentrations of either I, II or III (shown beside tracing) were added to PRP 1 min before the addition of ADP (5 μM). Panel B: Imipramine (2.5 μM), diluent or different concentrations of either I, II or III (shown beside each tracing) were added to PRP 1 min before the addition of epinephrine (10 μM). Panel C: Imipramine (2.5 μM), diluent or different concentrations of either I, II or III were added to PRP 1 min before the addition of collagen (30 μg/ml). Superimposed tracings of platelet aggregation are representative of four to six observations. The data on the secretion of [^{14}C]-5-HT are expressed as a percentage of inhibition of the secretion against the log molar concentrations of inhibitors. Each value is the mean ±S.E.M. of four or more experiments. An increased change in light transmittance (ordinate) indicates increased aggregation.

phospholipid fraction of control samples (diluent only) and test samples (diluent plus thrombin).

Measurement of malondialdehyde (MDA). Prostaglandin biosynthesis in platelets was monitored by measuring the amount of MDA produced [23]. Platelets incubated with either diluent, thrombin, or AA were centrifuged at 12,000 *g* for 1 min. An aliquot of the supernatant fraction (0.3 ml) was mixed with 0.3 ml of 100% trichloroacetic acid (TCA). After centrifugation, 0.2 ml of the supernatant fraction was heated with 0.2 ml of 0.53% 2-thiobarbituric acid reagent at 70° for 30 min. After cooling at room temperature, the volume was brought to 1 ml with distilled water and the amount of MDA present was monitored by the absorbancy at 532 nm.

Materials. *Para*-chlorophenoxyisobutyric acid (CPIB) was a gift from Ayerst Research Laboratories, New York, NY. 6-Chlorochroman-2-carboxylic acid (CCCA) and 6-phenylchroman-2-carboxylic acid (PCCA) were synthesized using methodology reported earlier [10, 11]. Structures of CPIB, CCCA, and PCCA are given in Fig. 1. Imipramine-hydrochloride was a gift from the CIBA Geigy Corp., Bayonne NJ. Epinphrine bitartrate, adenosine diphosphate (ADP), bovine thrombin, apyrase (EC 3.6.1.5), delipidated albumin, arachidonic acid, and an enzymatic assay kit for LDH were purchased from the Sigma Chemical Co., St. Louis, MO. Collagen was a soluble preparation from Worthington Biochemicals, Freehold, NJ. [^{14}C]-5-Hydroxytryptamine creatinine sulfate ([^{14}C]-5-HT, sp. act. 57 mCi/mmole) and [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid ([^{3}H]AA, sp. act. 61 Ci/mmole) were supplied by the Amersham Corp., Arlington Heights, IL, and the New England Nuclear Corp., Boston, MA, respectively.

Statistics. Data for each compound were calculated as inhibitory concentrations-50% (IC_{50}) against the aggregatory and biochemical responses measured. Differences between means were compared by Student's *t*-test using the 5 percent level of significance ($P < 0.05$).

RESULTS

Effects of CPIB, CCCA and PCCA on platelet aggregation and the secretion of [^{14}C]-5-HT induced by ADP, epinephrine and collagen. The inhibitory effects *in vitro* of CPIB, CCCA and PCCA on human platelet aggregation and the secretion of [^{14}C]-5-HT in PRP induced by ADP, epinephrine and collagen are shown in Fig. 2. CPIB, CCCA and PCCA all inhibited the second phase of aggregation induced by either ADP or epinephrine, and the aggregation induced by collagen, in a concentration-dependent manner (Fig. 2A–C). A comparison of the relative inhibitory potencies of these compounds shows that CPIB and CCCA were nearly equipotent as inhibitors of platelet aggregation induced by either ADP, epinephrine or collagen. In contrast, PCCA was from 52- to 183-fold more effective as an inhibitor of platelet aggregation when compared to either CPIB or CCCA. The inhibitory effects of these agents on the secretion of [^{14}C]-5-HT induced by ADP, epinephrine and collagen are given in the right-hand panels of Fig. 2A–C respectively. The inhibitory concentration-response curves for CPIB and CCCA against ADP, epinephrine and collagen are almost superimposable. By contrast, the concentration inhibition curve for PCCA is shifted significantly to the left regardless of the inducer of the secretion reaction (Fig. 2A–C). The calculated IC_{50}

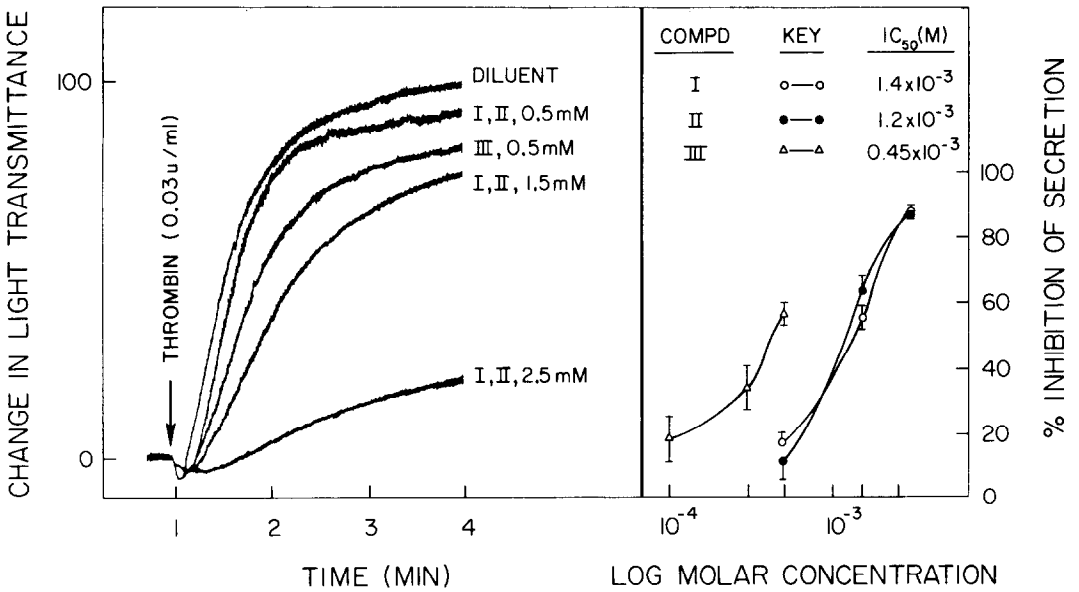


Fig. 3. Effects of CPIB (I), CCCA (II) and PCCA (III) on platelet aggregation (left panel) and the secretion of [^{14}C]-5-HT (right panel) caused by thrombin. Imipramine (2.5 μM), diluent or different concentrations of either I, II, or III were added to washed human platelets 1 min before the addition of thrombin (0.03 units/ml). Superimposed tracings of platelet aggregation are representative of four to six observations. The data on the secretion of [^{14}C]-5-HT are expressed as a percentage of inhibition of the secretion against log molar concentrations of the inhibitors. Each value is the mean ±S.E.M. of four or more experiments.

values (the concentration of an inhibitor to decrease the secretion of [^{14}C]-5-HT by 50 percent) for CPIB, CCCA and PCCA against ADP, epinephrine and collagen are also presented. Only 15, 6 or 33 μM PCCA was needed to inhibit by 50 percent the secretion of [^{14}C]-5-HT produced by either ADP, epinephrine or collagen respectively. On the other hand, higher concentrations of CPIB (950, 700 or 1700 μM) and CCCA (1450, 1100 or 1800 μM) were required to achieve a 50 percent inhibition of the secretion of [^{14}C]-5-HT induced by ADP, epinephrine and collagen respectively. PCCA, therefore, was from 52- to 183-fold more effective as an inhibitor of the platelet secretion reaction when compared with either CPIB or CCCA.

Effects of CPIB, CCCA and PCCA on platelet aggregation and the secretion of [^{14}C]-5-HT induced by thrombin. All three agents inhibited platelet aggregation by thrombin (Fig. 3). Comparison of the inhibitory responses of CPIB, CCCA and PCCA at a concentration of 500 μM showed that PCCA was again a more effective inhibitor. CPIB, CCCA and PCCA all inhibited thrombin-induced secretion of [^{14}C]-5-HT in a concentration-dependent manner (Fig. 3). On the basis of IC_{50} values, PCCA was found to be at least 2-fold more potent as an inhibitor when compared with either CPIB or CCCA.

Effects of CPIB, CCCA and PCCA on platelet aggregation and the secretion of [^{14}C]-5-HT induced by AA. ADP, epinephrine and low doses of collagen and thrombin cause platelet aggregation and the secretion of 5-HT by activating phospholipase A_2 (PLA_2), which then provides free AA that stimulates platelet aggregation as a result of its conversion to prostaglandin endoperoxides and thromboxane A_2 [24, 25]. Exogenous AA stimulates platelets without involving PLA_2. The effects of CPIB, CCCA and PCCA on AA-induced platelet aggregation and secretion of [^{14}C]-5-HT were examined to investigate whether these compounds acted prior to and/or subsequent to the production of AA. As shown in Fig. 4, CPIB and CCCA in concentrations up to 2.5 mM did not inhibit AA-induced platelet aggregation in PRP. In contrast, PCCA inhibited both platelet aggregation and the secretion of [^{14}C]-5-HT in a concentration-dependent manner and at concentrations that were considerably lower than those of CPIB or CCCA. Moreover, the inhibitory concentration–response curve for PCCA was nearly superimposable on the curve generated using aspirin ($IC_{50} \simeq 50$ μM; data not presented). CPIB and CCCA has a potentiating effect on the onset of AA-induced aggregation even though they inhibited the secretion of [^{14}C]-5-HT by 30 percent at 2.5 mM (Fig. 4).

Effects of CPIB, CCCA and PCCA on thrombin-induced release of [^{3}H]AA from platelet phospholipids. Release of AA from platelet phospholipids is a prerequisite for platelet secretion and aggregation, at least in the prostaglandin-dependent mechanism of platelet activation. We therefore examined the effects of CPIB, CCCA and PCCA on the release of [^{3}H]AA from platelets prelabeled with [^{3}H]AA. All three compounds significantly inhibited the release of [^{3}H]AA from platelet phospholipids (Table 1). Both CPIB and CCCA at a concentration of 2.5 mM decreased the release of [^{3}H]AA by 43 percent. One-fifth of this concentration of PCCA (0.5 mM) inhibited the release of [^{3}H]AA by 38 percent.

Effects of CPIB, CCCA and PCCA on thrombin and AA-induced production of malondialdehyde (MDA). Inhibition of platelet activation and the induced release of [^{3}H]AA by thrombin indicated that these inhibitors may block platelet secretion and aggregation by inhibiting the biosynthesis of prostaglandins. This possibility was examined by measuring the production of MDA, a byproduct of prostaglandin biosynthesis induced by thrombin and AA. All

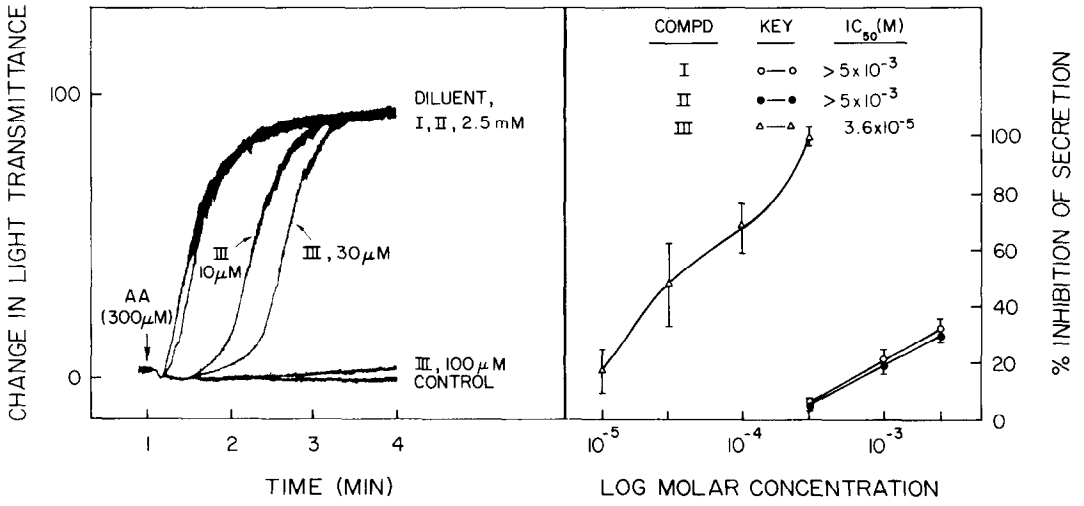


Fig. 4. Effects of CPIB (I), CCCA (II) and PCCA (III) on human platelet aggregation (left panel) and on the secretion of [^{14}C]-5-HT (right panel) stimulated by arachidonic acid. Imipramine (2.5 μM), diluent or different concentrations of either I, II or III were added to PRP 1 min before the addition of arachidonic acid (300 μM). Superimposed tracings of platelet aggregation are representative of four to six observations. The data on the secretion of [^{14}C]-5-HT are expressed as a percentage of inhibition of the secretion against log molar concentrations of inhibitors. Each value is the mean ±S.E.M. of four or more experiments.

Table 1. Effects of CPIB, CCCA and PCCA on thrombin-induced release of [^{3}H]arachidonic acid from platelet phospholipids*

Inhibitors	(mM)	% Inhibition†
CPIB	1.25	13.1 ± 3.0‡
	2.50	43.2 ± 2.9‡
CCCA	1.25	12.7 ± 6.1
	2.50	43.0 ± 2.9‡
PCCA	0.25	28.4 ± 7.5‡
	0.50	38.6 ± 3.6‡

* Inhibitors were added 1 min before addition of thrombin (5 units/ml).

† Values are the means ± S.E.M. of four experiments.

‡ Values were significantly different ($P < 0.05$) from the thrombin-treated sample.

three compounds inhibited thrombin-induced MDA generation in an increasing order of inhibitory potency (Table 2). The more potent PCCA inhibited MDA generation by 85 percent at 0.5 mM, whereas CCCA and CPIB at 1.25 mM only inhibited the formation of MDA by 65 and 50 percent respectively. In experiments using AA, CPIB (2.5 mM) and CCCA (2.5 mM) had no or very little (10 percent) inhibitory effect, whereas PCCA (0.5 mM) blocked AA-induced MDA generation by 37 percent (Table 2).

DISCUSSION

Two cyclic chroman analogs, CCCA and PCCA, were evaluated for their antiaggregatory effects *in vitro* in comparison with CPIB. The observations that CPIB inhibited epinephrine-, ADP-, collagen- and thrombin-induced secretion of [^{14}C]-5-HT and platelet aggregation are in agreement with earlier reports [4–6]. PCCA was a 52- to 183-fold more potent inhibitor of platelet [^{14}C]-5-HT secretion induced by either epinephrine, ADP or collagen as compared to CPIB or CCCA. In contrast, PCCA was only two times more effective as an inhibitor against thrombin-induced [^{14}C]-5-HT secretion. This difference may have resulted from the ability of thrombin to induce platelet secretion by more than one mechanism [26].

A major pathway of the platelet secretion depicted in Fig. 5 depends upon the biosynthesis of prostaglandin endoperoxides and thromboxane A_2 [24, 25]. The differential effects of CPIB, CCCA and PCCA on this pathway were examined to explore their antiaggregatory mechanisms. Each of these compounds inhibited thrombin-induced release of AA from platelet phospholipids (Table 1) as well as thrombin-mediated prostaglandin synthesis as assessed by MDA generation (Table 2). These observations suggest that these compounds inhibit platelet secretion, at least in part, by inhibition of the release of endogenous AA and, consequently, prostaglandin biosynthesis.

The ability of PCCA to block the conversion of endogenous AA to cyclic endoperoxides, as well as to inhibit release of AA from platelet phospholipids, most likely accounts for its higher antiaggregatory potency when compared with CPIB or CCCA.

Table 2. Effects of CPIB, CCCA and PCCA on malondialdehyde (MDA) production in human platelets*

Inducer + inhibitor (mM)	Percent of MDA produced†
Diluent + diluent	21.92 ± 2.0
Thrombin + diluent	100.00 ± 0.2
Thrombin + CPIB (1.25)	47.95 ± 1.6
Thrombin + CPIB (2.50)	16.42 ± 0.7
Thrombin + CCCA (1.25)	32.71 ± 1.1
Thrombin + CCCA (2.50)	34.99 ± 1.1
Thrombin + PCCA (0.25)	31.88 ± 2.1
Thrombin + PCCA (0.50)	16.77 ± 0.6
Thrombin + aspirin (1.00)	25.42 ± 2.5
Na-Arachidonate + CPIB (2.50)	90.90 ± 1.2
Na-Arachidonate + CCCA (2.50)	88.30 ± 0.5
Na-Arachidonate + PCCA (0.25)	86.40 ± 0.2
Na-Arachidonate + PCCA (0.50)	62.90 ± 0.4
Na-Arachidonate + aspirin (1.00)	15.70 ± 0.2

* Inhibitors were added 1 min before addition of thrombin (2 units/ml) or AA (1 mM).

† Values are the means ± S.E.M. of four experiments. All values were significantly different ($P < 0.05$) from the thrombin-treated sample.

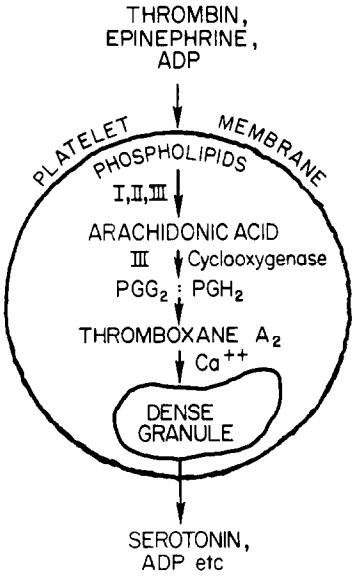


Fig. 5. Prostaglandin-mediated pathway of platelet activation: Possible sites of antiaggregatory action of CPIB (I), CCCA (II) and PCCA (III). Thrombin, ADP and epinephrine induce platelet secretion and aggregation by the release of arachidonic acid from platelet phospholipids, which is then converted into prostaglandin endoperoxides PGG_2 and PGH_2 by the enzyme cyclo-oxygenase. PGG_2 and PGH_2 are then converted to thromboxane A_2 (TXA_2) by thromboxane synthetase. PGG_2, PGH_2 and TXA_2 mediate platelet secretion and thus aggregation. CPIB (I), CCCA (II) and PCCA (III) all inhibit the release of arachidonic acid (site of action #1) from platelet phospholipids and thereby block postaglandin biosynthesis. This may be one mechanism of their inhibitory action. PCCA (III), in addition, inhibits conversion of arachidonic acid into prostaglandins (site of action #2).

Aspirin, a known inhibitor of cyclo-oxygenase [27], and PCCA have nearly superimposable inhibitory concentration–response curves for AA-induced [^{14}C]-5-HT secretion and inhibit platelet aggregation at similar concentrations. PCCA also did not block platelet aggregation induced by U46619 (2 μM, data not shown), a stable analog of prostaglandin endoperoxide (PGH_2) which has been postulated to induce platelet aggregation by mechanisms similar to that of thromboxane A_2 [28]. This finding suggests that PCCA inhibits platelet aggregation and secretion prior to thromboxane A_2 formation, but it does not preclude its possible action on thromboxane A_2 synthesis. PCCA most likely inhibits platelet secretion by interfering with both the release of AA from platelet phospholipids and, more specifically, the conversion of AA to prostaglandin endoperoxides (Fig. 5).

Clofibrate has been shown to inhibit the activity of a wide variety of enzyme systems, *in vitro*, among which are included adenylate cyclase and acetyl CoA carboxylase [10]. As reported in this paper, CPIB, CCCA and PCCA all inhibited the proaggregatory actions of ADP, epinephrine, collagen and thrombin in a concentration-dependent manner (Figs. 2 and 3). In earlier reports [4, 29], we also showed that CPIB actually increased the aggregatory and secretory responses to both AA and phospholipase C. In addition, our results have indicated that both CPIB and CCCA enhanced the onset of AA-induced aggregation. Subsequent studies have confirmed the suggestion [30] that the potentiating effect of CPIB is related to its ability to lower cyclic AMP in platelets [29]. In spite of potentiating platelet aggregation by AA, both CPIB and CCCA partially inhibited [^{14}C]-5-HT secretion at higher concentrations (2.5 mM). This latter phenomenon may reflect inhibition of the component of secretion that depends on the biosynthesis of prostaglandins from endogenous AA [31] and that is inhibitable by CPIB and CCCA owing to their abilities to block release of AA from platelet phospholipids (Table 1).

The two chloro analogs, CPIB and CCCA, are most closely related since they differ in molecular weight by only two hydrogen atoms (see Fig. 1) and have similar log P values of 2.57 and 2.50 respectively [11, 12]. As we have noted, the open-chain (CPIB) and cyclic analogs (CCCA) have similar antiaggregatory potencies and modes of action. The more lipophilic phenyl-substituted analog (PCCA) has a log P value of 3.86 [10] and is a more potent inhibitor when compared with these chloro analogs. An increase in the lipophilicity of these structurally related carboxylic acid analogs may also be correlated with a decrease in their critical micellar concentrations. However, we did not observe any modification of the surface tension by these agents until the concentrations were at least 5- to 10-fold greater than those used in these experiments.* This finding, coupled with the observed qualitative and quantitative differences in antiaggregatory activities of these compounds, suggests that the effects of PCCA, CCCA and CPIB are mediated by a specific interaction with enzyme systems rather than by a nonspecific detergent action. Thus, the increased lipophilicity of PCCA may account for its additional selective inhibitory action on prostaglandin biosynthesis in platelets. Although all these structures can be drawn in more than one way so that parts of these molecules fit a proposed template for the cyclooxygenase receptor site [32], it remains difficult to explain the marked differences in potency for the phenyl and chloro analogs on this basis. It does seem, however, that increased lipophilicity and, perhaps, hydrophobic binding of the phenyl group to the enzyme may play an important role in the process of platelet function inhibition.

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REFERENCES

1. R. J. Havel and J. P. Kane, *A. Rev. Pharmac.* **13**, 287 (1973).
2. B. M. Wolfe, J. P. Kane, R. J. Havel and H. P. Brewster, *J. clin. Invest.* **52**, 2146 (1973).
3. M. A. D'Costa and A. Angel *J. clin. Invest.* **55**, 138 (1975).
4. Huzoor-Akbar and N. G. Ardlie, *Thromb. Res.* **10**, 95 (1977).
5. A. C. Carvalho , R. W. Colman and R. S. Lees, *Circulation* **50**, 570 (1974).
6. C. Y. Lin and S. Smith, *Life Sci.* **18**, 563 (1976).
7. Committee of Principal Investigators, *Br. Heart J.* **40**, 1069 (1978).
8. D. J. Svoboda and D. L. Azarnoff, *Cancer Res.* **39**, 3419 (1979).
9. *F.D.A. Drug Bull.* **9**, (3), 14 (1979).
10. D. T. Witiak, H. A. I. Newman and D. R. Feller, in *Clofibrate and Related Analogs* (Ed. G. L. Grunewald), p. 54. Marcel Dekker, New York (1977).
11. D. T. Witiak, W. P. Heilman, S. K. Sankarappa, R. D. Cavestri and H. A. I. Newman, *J. med. Chem.* **18**, 934 (1975).
12. D. T. Witiak, E. S. Stratford, R. Nazareth, G. Wagner and D. R. Feller, *J. med. Chem.* **14**, 758 (1971).
13. T. S. Zimmerman, O. D. Ratnoff and A. E. Powell, *J. clin. Invest.* **50**, 244 (1971).
14. R. H. Aster and J. H. Jandl, *J. clin. Invest.* **43**, 843 (1964).
15. P. Han and N. G. Ardlie, *Br. J. Haemat.* **26**, 373 (1974).
16. N. G. Ardlie and P. Han, *Br. J. Haemat.* **26**, 331 (1974).
17. N. G. Ardlie, M. A. Packham and J. F. Mustard, *Br. J. Haemat.* **19**, 7 (1970).
18. G. Brecher and E. P. Cronkite, *J. appl. Physiol.* **3**, 365 (1950).
19. G. V. R. Born, *Nature, Lond.* **194**, 927 (1962).
20. J. F. Mustard, D. W. Perry, N. G. Ardlie and M. A. Packham, *Br. J. Haemat.* **22**, 193 (1972).
21. S. Rittenhouse-Simmons, F. A. Russell and D. Deykin, *Biochem. biophys. Res. Commun.* **70**, 295 (1976).
22. T. K. Bills, J. B. Smith and M. J. Silver, *Biochim. biophys. Acta* **424**, 303 (1976).
23. J. B. Smith, C. M. Ingerman and M. J. Silver, *J. Lab. clin. Med.* **88**, 167 (1976).
24. B. Samuelsson, M. Hamberg, C. Malmsten and J. Svensson, in *Advances in Prostaglandins and Thromboxane Research* (Eds. B. Samuelsson and R. Paoletti), p. 737. Raven Press, New York (1976).

25. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
26. R. L. Kinlough-Rathbone, M. A. Packham, H. J. Reimers, J. P. Cazenave and J. F. Mustard, *J. Lab. clin. Med.* **90**, 707 (1977).
27. G. J. Roth and P. W. Majerus, *J. clin. Invest.* **56**, 624 (1975).
28. R. A. Coleman, P. P. A. Humphrey, I, Kennedy, G. P. Levy and P. Lumley, *Br. J. Pharmac.* **68**, 127 (1980).
29. D. R. Feller, H. A. I. Newman, D. T. Witiak, R. H. Fertel and Huzoor-Akbar, *Clin. Res.* **28**, 310A (1980).
30. Huzoor-Akbar and N. G. Ardlie, *Br. J. Haemat.* **39**, 71 (1976).
31. W. E. M. Lands, in *Advances in Prostaglandins and Thromboxane Research* (Eds. B. Samuelsson and R. Paoletti), p. 7. Raven Press, New York (1976).
32. R. A. Appleton and K. Brown, *Prostaglandins* **18**, 29 (1979).