Pergamon

Biochemical Pharmacology, Vol. 48, No. 10, pp. 1909–1916, 1994.
Elsevier Science Ltd
Printed in Great Britain.
0006-2952/94 $7.00 + 0.00

0006-2952(94)00374-2

PLATELET-ACTIVATING FACTOR (PAF)-INDUCED PLATELET AGGREGATION

MODULATION BY PLASMA ADENOSINE AND METHYLXANTHINES

KAILASH C. AGARWAL,* ELLEN CLARKE,† SHARON ROUNDS,† ROBERT E. PARKS, Jr. and HUZOOR-AKBAR‡

Section of Molecular and Biochemical Pharmacology, Division of Biology and Medicine, Brown University, Providence, RI 02912; †Pulmonary Medicine Section, VA Medical Center, Providence, RI; and ‡Department of Biological Sciences, Ohio University, Athens, OH, U.S.A.

(*Received* 18 *March* 1994; *accepted* 12 *July* 1994)

Abstract—This study examined the role of plasma adenosine in the modulation of platelet-activating factor (PAF) activity on platelet aggregation and serotonin (5-HT) release in human platelet-rich plasma (PRP). In addition, the effects of methylxanthines (e.g. theophylline and caffeine) were studied on PAF-induced platelet aggregation in PRP isolated from blood samples from healthy subjects. Also, PAF-induced platelet aggregation was examined in PRP samples of patients receiving theophylline treatment. These studies demonstrate that plasma adenosine levels (0.1 to 0.3 μM) play a key role in negative modulation of PAF activity on platelet aggregation and 5-HT release. After depletion of plasma adenosine, the platelet-aggregating activity of PAF was increased greatly (>10-fold). PAF at concentrations of 0.1 to 12 μM caused no 5-HT release in PRP containing normal amounts of adenosine (blood collected in the presence of 2′-deoxycoformycin and dilazep), whereas PAF at 0.1 μM caused 5-HT release (45%) in adenosine-depleted PRP, demonstrating that plasma adenosine is much more inhibitory of 5-HT release than platelet aggregation. The adenosine antagonists theophylline (50 μM), caffeine (50 μM) and a xanthine derivative, 3,7-dimethyl-1-propargylxanthine (DMPX, 10 μM) (a more specific adenosine A_2 receptor antagonist), potentiated PAF activity on platelet aggregation in PRP samples containing adenosine. Also, patients receiving theophylline treatments showed significantly greater platelet aggregation induced by PAF in their PRP samples. PAF induced a rapid increase (80% in 15 sec) in intracellular Ca^{2+} mobilization, which was strongly inhibited by adenosine (IC_{50}, 0.3 μM). Our studies suggest that agents that can increase plasma adenosine levels (e.g. inhibitors of adenosine uptake and adenosine metabolism) or methylxanthines may be useful in altering (inhibiting or enhancing, respectively) PAF actions on platelets and other tissues.

Key words: human platelets; platelet-activating factor; plasma adenosine; theophylline; caffeine; dipyridamole; calcium

PAF§ (1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine), a potent lipid autacoid, exerts a wide variety of biological activities [1–5]. In human platelets, PAF induces shape change, aggregation and release of granule contents by stimulation of the phosphatidylinositol cycle, intracellular Ca^{2+} mobilization, and the release of arachidonic acid [1, 6–8]. Specific PAF receptor sites have been identified in rabbit [9] and human [10, 11] platelets. Platelet aggregation induced by PAF is only weakly inhibited by aspirin, whereas collagen- or arachidonic acid-induced platelet aggregation is strongly inhibited by aspirin [12]. Adenosine, a potent antiplatelet and vasodilatory agent [13], is produced continuously in the body by vascular endothelium [14], platelets [15], and several other tissues [16]. However, only low levels of adenosine (0.1 to 0.3 μM) [15, 17] are seen in human plasma due to its rapid metabolism by cellular adenosine deaminase and adenosine kinase [18, 19]. Our laboratory has demonstrated recently that plasma adenosine plays a key role in negative modulation of platelet activity [15]. The present studies examined the role of plasma adenosine and methylxanthines (theophylline and caffeine) on PAF-induced platelet aggregation. In addition, PAF effects on platelet aggregation were assessed in PRP samples of patients receiving theophylline treatment. Our data demonstrate that plasma adenosine and methylxanthines play key roles in the modulation of PAF actions on human platelets. Preliminary findings of these studies have been presented [20, 21].

*Corresponding author: Kailash C. Agarwal, Ph.D., Section of Molecular and Biochemical Pharmacology, Box G-B413, Brown University, Providence, RI 02912. Tel. (401) 863-1603; FAX (401) 863-1595.

§Abbreviations: PAF, platelet-activating factor; PRP, platelet-rich plasma; 5-HT, 5-hydroxytryptamine or serotonin; dCF, 2′-deoxycoformycin; ADA, adenosine deaminase; DMPX, 3,7-dimethyl-1-propargylxanthine; cAMP, cyclic 3′,5′-adenosine monophosphate; and EHNA, erythro-9-(2-hydroxy-3-nonyl) adenine.

MATERIALS AND METHODS

Chemicals. Adenosine, ADP, PAF, theophylline, caffeine, and ADA (EC 3.5.4.4, from calf intestinal mucosa, Type VI, sp. act. 193 U/mg protein) were

purchased from the Sigma Chemical Co. (St. Louis, MO). DMPX (a selective adenosine A_2 antagonist) [22] was purchased from RBI Research Biochemicals (Natick, MA). Dilazep, 1,4-bis-[3-(3,4,5-trimethoxybenzoyl - oxy)-propyl] - perhydro - 1,4 - diazepin (Cormelian®, a potent inhibitor of the nucleoside transport system) [23], was a gift of Hoffmann-LaRoche, Inc., Nutley, NJ. dCF (Penostatin®, a tight-binding inhibitor of ADA) [24] was obtained from the Drug Development Branch, Division of Cancer Treatment of the National Cancer Institute, Bethesda, MD.

Platelet aggregation. This study employed blood samples from healthy volunteers and from stable out-patients who were treated with theophylline for chronic obstructive lung disease. The control out-patients were not receiving theophylline. Studies using human blood from healthy volunteers and patients were approved by the Institutional Review Committee. Informed consent was obtained from each subject before the blood was obtained. These subjects consumed a regular American diet and denied taking any drug known to affect platelet functions for at least 8 days. Freshly drawn blood was collected in 0.1 vol. of trisodium citrate (3.8%) alone, or containing dilazep (20 μM) and dCF (50 μM). PRP was separated by centrifugation of whole blood at 277 *g* for 8 min. Platelet-poor plasma (PPP) was obtained by centrifuging the remaining blood at 1530 *g* for 5 min. The PRP obtained from the blood collected in sodium citrate alone was incubated at room temperature (22°) with ADA (2 U/mL) for 10 min to degrade plasma adenosine. Platelet aggregation was evaluated in PRP containing 3–4 × 10^8 platelets/mL at 37° by the turbidometric method of Born [25] using a dual channel Payton Aggregometer (Payton Associates, Inc., Buffalo, NY) or a Chrono-log Whole-blood Aggrometer (Chrono-log Corp., Havertown, PA) interfaced with a dual channel recorder. The extent of aggregation was estimated by the percent of increase in light transmission in 4 min after the addition of PAF to PRP. PPP was employed as the blank to represent 100% light transmission.

5-HT uptake and release by platelets. The PRP samples were incubated with [^{14}C]5-HT (sp. act. 57.4 mCi/mmol) (0.2 μCi/mL of PRP) at 37° for 20 min, resulting in an uptake of 5-HT (95–98%) by platelets. Platelet aggregation was induced by PAF and, after 4 min, the contents were transferred to a micro-tube and centrifuged at 12,000 *g* at 4° for 1 min. An aliquot (150 μL) of the supernatant plasma containing the released 5-HT was counted for radioactivity. Both PAF-induced platelet aggregation and 5-HT release were measured in PRP containing normal amounts of adenosine (blood collected in the presence of dCF and dilazep), or adenosine-depleted PRP (ADA-treated). The EC_{50} values for PAF to cause 50% platelet aggregation or 5-HT release (of the maximum platelet aggregation or 5-HT release seen) were estimated from the concentration–response plots.

Adenosine assay. Fresh human blood (0.9 mL) was collected in a micro-tube with 0.1 mL of trisodium citrate (3.8%) alone, or containing dilazep (20 μM) and dCF (50 μM). Cell-free plasma was obtained by centrifugation of whole blood at 12,000 *g* for 2 min. Cell-free plasma obtained from the whole blood (collected in sodium citrate alone) was incubated with ADA (2 U/mL) for about 10 min at room temperature (22°) to degrade plasma adenosine. One volume of each, $ZnSO_4$ (0.25 M) and $Ba(OH)_2$ (0.25 M), was added to the cell-free plasma, and the precipitated proteins were removed by centrifugation at 12,000 *g* for 2 min. The supernatant (300 μL) or saline (300 μL) was transferred to a micro-tube (1.5 mL). To each tube, 30 μL containing 30 pmol of 1,N^6-ethenoadenosine and 50 μL containing 84 nmol of chloroacetaldehyde were added. After securing the tubes with screw-caps, they were immersed in a boiling water-bath for 15 min. The fluorescent 1,N^6-ethenoadenosine was separated and quantitated by a HPLC method [26], using a μBondapak C_{18} steel column (0.39 × 30 cm) (Waters Associates, Milford, MA) with a 0–50% methanol gradient and a 2 mL/min flow rate. The fluorescence was detected using a Spectroflow Fluorometer (model 980) with an excitation monochromator set at 270 nm and a secondary filter with a cut-off below 418 nm. Samples containing saline and ethenoadenosine served as standards to quantitate ethenoadenosine in other samples.

Theophylline assay. Theophylline levels in cell-free plasma were measured using a fluorescence polarization immunoassay (assay kits purchased from Abbott Laboratories, Abbott Park, IL).

Platelet intracellular Ca^{2+}. PAF-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilization was measured in washed platelet suspensions using the Fura 2 dye method [27]. Acid-citrate dextrose (ACD: trisodium citrate, 2.2 g; anhydrous citric acid, 0.73 g; and dextrose, 2.45 g per 100 mL of distilled water) was added in 0.15 vol. to the fresh citrated-PRP, and, after mixing, the PRP was centrifuged at 1530 *g* for 10 min to isolate platelet pellets. Platelets were then washed twice by resuspension in Tyrode buffer (pH 6.5), and finally resuspended in the same buffer. Fura 2 (5 μL of 1 mM) was added to the platelet suspension (995 μL) to give a final concentration of 5 μM, and the platelet suspension was incubated at room temperature (22°) for 30 min. The excess of the dye was removed by washing the platelets with Tyrode buffer (pH 6.5). The platelets were resuspended in Tyrode buffer (pH 7.4) containing $MgCl_2$ (1 nM), $CaCl_2$ (2 mM) and no albumin. PAF (0.1 μM)-induced $[Ca^{2+}]_i$ mobilization in platelets was estimated (0–120 sec) by measuring the increase in the fluorescence intensity with excitation at 340 nm and emission at 500 nm.

RESULTS

Role of plasma adenosine in PAF actions on platelets. The role of plasma adenosine in PAF-induced platelet aggregation was examined in human PRP containing steady-state levels of adenosine (0.23 ± 0.09 μM; blood collected in the presence of dCF and dilazep) or minimal amounts of adenosine (0.032 ± 0.013 μM after ADA treatment) [15, 28]. Aggregation tracings of Fig. 1 represent one typical experiment out of four experiments employing blood samples from different donors. PAF (0.05 and

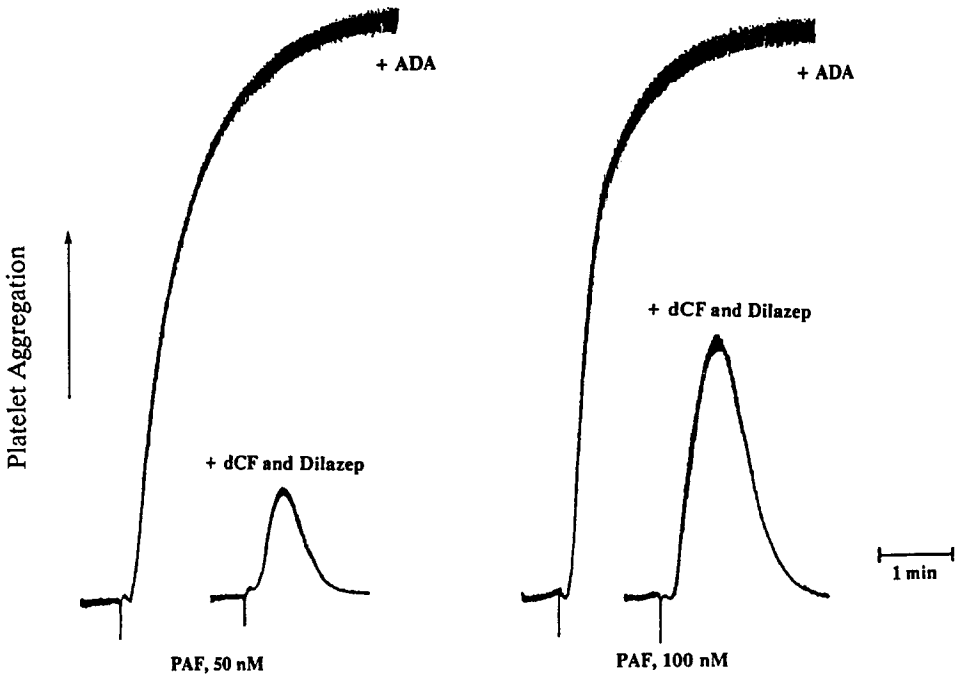

Fig. 1. PAF-induced platelet aggregation in PRP with physiological amounts of adenosine (blood collected in the presence of dCF and dilazep) and in adenosine-depleted PRP (ADA-treated). These PRP samples were prepared as described in Materials and Methods. Platelet aggregation was induced by PAF (50 and 100 nM or 0.05 and 0.1 μM). The aggregation tracings represent one typical experiment out of four separate experiments, using blood from different donors.

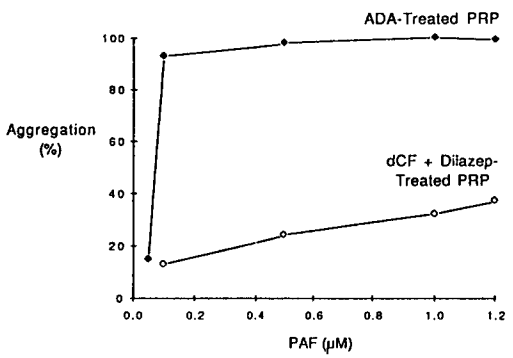

Fig. 2. Concentration–response effects of PAF on platelet aggregation. The experiments employed freshly prepared, dCF + dilazep-treated and ADA-treated (PRPs see Materials and Methods). Platelet aggregation was induced in PRP by PAF (0.05 to 1.2 μM).

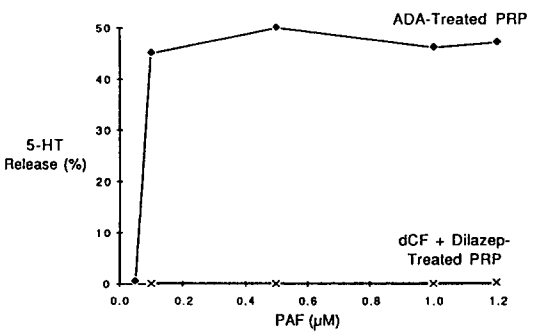

Fig. 3. Concentration–response effects of PAF on 5-HT release. Platelets were prelabled with [^{14}C]5-HT in the PRPs of the experiments of Fig. 2, and 5-HT release was measured in the plasma after 4 min of PAF-induced aggregation. The details for labeling platelets with [^{14}C]5-HT and for measurements of the released 5-HT are described in the Materials and Methods.

0.1 μM) produced only weak and reversible platelet aggregation in PRP with physiological amounts of adenosine. However, after depletion of plasma adenosine, PAF produced a much greater extent of platelet aggregation that was irreversible, demonstrating an important role of adenosine in modulation of PAF effect on platelet aggregation.

To examine further the role of plasma adenosine in PAF activity, concentration–response effects of PAF were measured on both platelet aggregation and the release of 5-HT from dense granules (Figs. 2 and 3). Again, these PAF effects were examined in PRP with steady-state levels of adenosine and in PRP with minimal amounts of adenosine (ADA-treated). As seen in Fig. 2, PAF (0.1 to

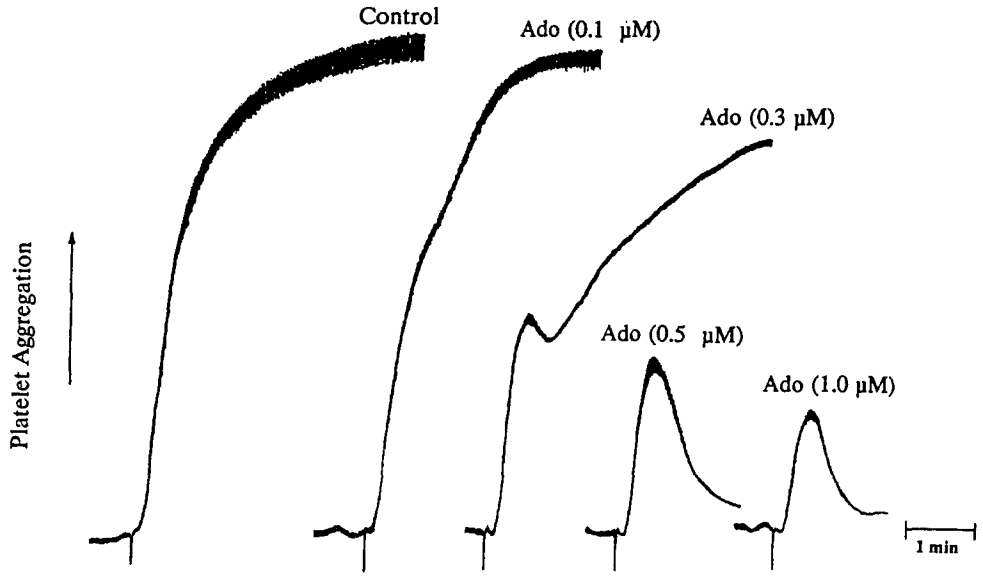

Fig. 4. Inhibition of PAF-induced platelet aggregation in PRP by replenished adenosine. Control: fresh PRP was treated with ADA (2 U/mL) for 10 min. The ADA-treated PRP was incubated with dCF (5 μM) and dilazep (2 μM) to inactive ADA and to block adenosine uptake by platelets, respectively; after about 2 min, the PRP was replenished with adenosine (Ado, 0.1, 0.3, 0.5 or 1.0 μM). After a 1-min incubation, platelet aggregation was induced by PAF (0.1 μM).

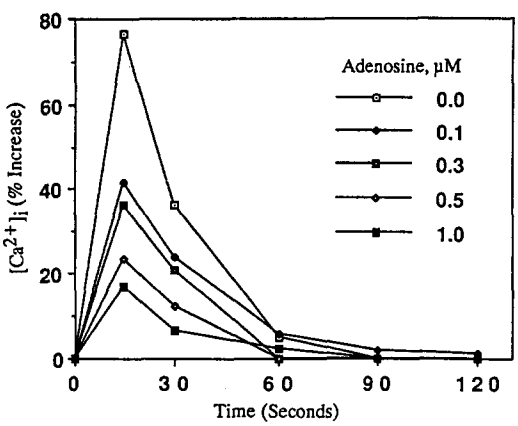

Fig. 5. Effect of adenosine on PAF-induced intracellular Ca^{2+} mobilization. The washed suspension of platelets containing Fura 2 was incubated with ADA (2 U/mL) for 5 min to degrade any adenosine that might be present. The ADA activity was then inhibited by dCF (5 μM, 5-min incubation), and adenosine uptake was inhibited by dilazep (2 μM) followed by addition of adenosine (0 to 1.0 μM). PAF (0.1 μM) was added immediately (within 30 sec) to induce $[Ca^{2+}]_i$ mobilization, which was measured during an incubation of 15–120 sec at 37°.

1.2 μM) produced only weak and reversible platelet aggregation (15–35%) in the PRP with adenosine, whereas PAF even at a low concentration (0.1 μM) caused >90% aggregation in adenosine-depleted PRP. The PAF EC_{50} value for platelet aggregation in adenosine-depleted PRP was >20-fold lower (0.07 μM) than the EC_{50} value (>1.2 μM) in PRP with adenosine. Similarly, PAF (0.1 to 1.2 μM), which had no effect on the release of 5-HT from the platelet granules in the PRP with normal adenosine levels, caused 45–48% of 5-HT release (EC_{50}, 0.07 μM) in adenosine-depleted PRP (Fig. 3). Similar effects of plasma adenosine on PAF-induced platelet aggregation and 5-HT release were seen in PRP samples of three other subjects. These findings demonstrate that plasma adenosine acts as a natural inhibitor of both platelet aggregation and the release of 5-HT from dense granules.

Effects of PAF in PRP replenished with adenosine. The experiments of Fig. 4 examined PAF effects on platelet aggregation in PRP replenished with known amounts of adenosine. The PRP was first treated with ADA (2 U/mL, 10 min) to degrade plasma adenosine. The ADA activity and adenosine uptake by platelets were then blocked by dCF (5 μM) and dilazep (2 μM), respectively. Incubation of the ADA-treated PRP for 2 min with dCF and dilazep did not affect PAF activity on platelet aggregation (data not shown). After dCF and dilazep treatment, the PRP was replenished with adenosine (0.1 to 1 μM) and after 1 min, platelet aggregation was induced by PAF (0.1 μM). As shown in Fig. 4, platelet aggregation was inhibited by adenosine in a concentration-dependent manner with an IC_{50} of about 0.4 μM, demonstrating further the significance of plasma adenosine in modulation of PAF actions on platelets. Similar adenosine inhibitory effects were seen in two other experiments using blood from different donors.

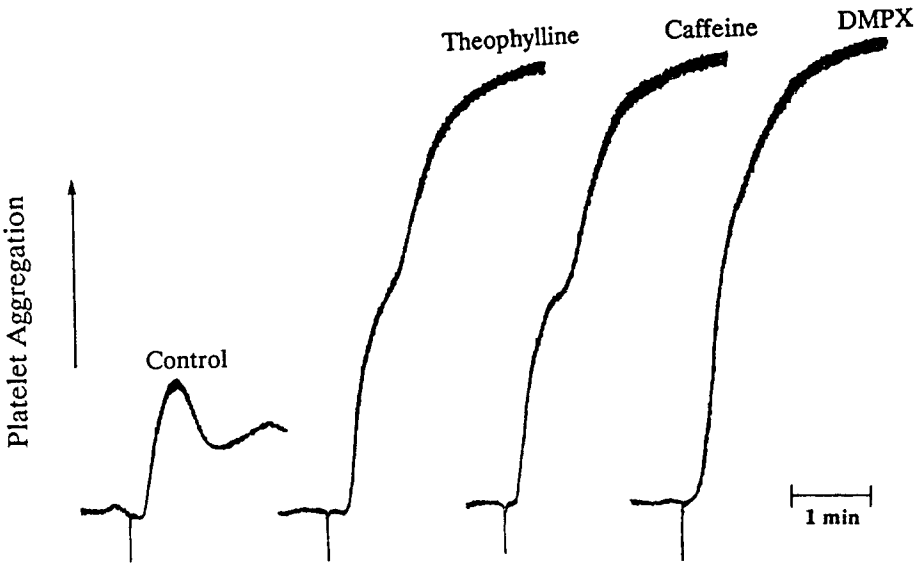

Fig. 6. Effects of theophylline, caffeine and DMPX on PAF-induced platelet aggregation in PRP with replenished adenosine. The ADA-treated PRP was incubated with dCF (5 μM) plus dilazep (2 μM) (as in Fig. 4). This dCF/dilazep-treated PRP was then incubated (37°) with saline (control), theophylline (50 μM), caffeine (50 μM) or DMPX (10 μM) for 1 min and then with adenosine (1 μM); after 4 min, platelet aggregation was induced by PAF (0.1 μM).

Effects of adenosine on PAF-induced intracellular Ca^{2+} *mobilization.* The experiments of Fig. 5 examined the effects of adenosine (0.1 to 1.0 μM) on PAF-induced $[Ca^{2+}]_i$ mobilization in washed platelet suspensions. PAF induced an increase of 80% in $[Ca^{2+}]_i$ mobilization in 15 sec followed by a rapid decrease to the initial $[Ca^{2+}]_i$ levels in about 1 min. These effects were measured in platelet suspensions that were treated with ADA (2 U/mL) to degrade any adenosine that might have been present. To examine the effects of adenosine, the ADA-treated platelet suspension was further treated with dCF (5 μM) and dilazep (2 μM) to block ADA activity and adenosine uptake by platelets, respectively. This dCF/dilazep-treated platelet suspension was then replenished with adenosine (0.1 to 1.0 μM), and after 1 min, PAF (0.1 μM) was added to induce platelet $[Ca^{2+}]_i$ mobilization. As seen in Fig. 5, adenosine blocked $[Ca^{2+}]_i$ mobilization in a concentration-dependent manner with an IC_{50} of about 0.3 μM, suggesting that adenosine acts as a key modulator of PAF-induced $[Ca^{2+}]_i$ mobilization, a process that is essential for platelet aggregation.

Effects of methylxanthines on PAF actions. Adenosine inhibits platelet aggregation by acting on its specific A_2 receptors that are coupled with adenylate cyclase [29, 30]. Several methylxanthines, which act as adenosine receptor antagonists, were examined for their effects on platelet aggregation in PRP containing adenosine. The data of Fig. 6 show that theophylline (50 μM), caffeine (50 μM) and an A_2 selective antagonist, DMPX (10 μM), potentiated PAF-induced platelet aggregation. Similar potentiating effects of these methylxanthines were seen in two other experiments employing blood from different donors. Theophylline (50 μM), caffeine (50 μM), and DMPX (10 μM) alone showed no aggregatory effects on platelets. Our findings indicate that these methylxanthines antagonize the inhibitory effect of plasma adenosine on platelet aggregation, thus potentiating the action of PAF.

Effect of PAF on platelets from patients receiving theophylline. This study employed 22 stable outpatients (Table 1), 12 of whom were receiving chronic treatment with theophylline (250–750 mg/day) for chronic obstructive pulmonary disease. Plasma theophylline levels were 9.2 ± 1.1 μg/mL (51 ± 6 μM). Concentration–response effects of PAF on platelet aggregation were measured in PRP samples, and the EC_{50} values for PAF were estimated from the concentration–response plots. As seen in Table 1, the PAF EC_{50} values were significantly lower in PRP samples of patients receiving the theophylline treatment than in the control subjects. The plasma adenosine levels were not significantly different between the two groups, although adenosine levels in these subjects were about 2-fold greater than our reported normal levels in healthy subjects [28]. Patients included in this study were mostly of an older age group, whereas, the subjects employed in the measurements of healthy subjects were mostly of a younger age group [28]. Furthermore, it is likely that these subjects may have received other treatments that may have effects on adenosine levels. These studies demonstrate that therapeutic levels of plasma theophylline enhanced PAF effects on platelet aggregation, similar to our findings with theophylline added to PRP (Fig. 6).

DISCUSSION

Adenosine was first identified as a potent inhibitor

Table 1. Base-line clinical characteristics, plasma adenosine and theophylline levels, and EC_{50} values for platelet aggregation in two study groups

	Theophylline group	Control group
No. of subjects (N)*	12	10
Age (years)	68 (59–73)	54 (30–67)
Sex	10 M, 2 F	8 M, 2 F
Platelet count ($\times 10^3/\mu L$)	165–320	185–350
FEV_1† (L)	1.5 (0.5–3.2)	2.3 (1.3–3.4)
Theophylline dose (mg/day)	250–750	
Theophylline ($\mu g/mL$)	9.2 ± 1.1 (2.3–15.4)	
Plasma adenosine (μM)	0.549 ± 0.092	0.517 ± 0.073
Coffee consumption (cups/day)	0.5	1.8
PAF, EC_{50}‡ (μM)	0.112 ± 0.018§ (0.015–0.20)	0.180 ± 0.037 (0.030–0.430)

Data are presented as means, or mean ± SEM. Values in parentheses represent ranges.

*No subject reported taking aspirin or other antiplatelet agents for 8 days prior to the study.

†FEV_1: forced expiratory volume in 1 sec.

‡EC_{50} values: concentrations of PAF causing 50% platelet aggregation. These values were determined from the concentration–response plots.

§$P < 0.05$, compared with the control, using Student's *t*-test.

of platelet aggregation in 1962 by Born [31]. Subsequently, many other investigators reported antiplatelet activity of adenosine and its derivatives [13, 29, 30, 32]. Most of these investigators examined adenosine effects on platelet aggregation by adding exogenous adenosine to PRP, and the inhibitory effects were seen with 2–40 μM adenosine. Compared with the normal physiological levels of adenosine (0.1 to 0.3 μM), the concentrations used in the previous antiplatelet studies were much greater. This laboratory has reported recently that these low levels of plasma adenosine play a key role in modulation of platelet function [15, 28]. Depletion of plasma adenosine causes a decrease in platelet cAMP of about 25%, producing a strong potentiation in the activity of ADP on both platelet aggregation and 5-HT release [15]. Similarly, our present studies demonstrate that after depletion of plasma adenosine, the PAF-aggregating activity was greatly potentiated; however, this potentiation of platelet aggregation was much greater than seen with ADP [15]. In addition, we found that plasma adenosine was much more inhibitory of PAF-induced platelet 5-HT release than platelet aggregation. For example, in adenosine-depleted PRP, a low dose of PAF (0.1 μM) caused a 45% release of 5-HT, whereas PAF with 12-fold higher levels (1.2 μM) caused no release of 5-HT in PRP with normal adenosine levels. This indicates that adenosine is an important modulator of platelet release of dense granule contents which include vasoactive agents, 5-HT, Ca^{2+}, ADP and ATP.

Furthermore, our studies demonstrated that addition of theophylline or caffeine to human PRP containing adenosine potentiates PAF-induced platelet aggregation, suggesting that these methylxanthines act primarily as adenosine receptor antagonists. Similar potentiation in the PAF aggregating activity was seen with a more specific adenosine A_2 receptor antagonist, DMPX. These findings are consistent with those showing that methylxanthines at physiological levels act primarily as adenosine receptor antagonists [29, 33–35]. In addition, both theophylline and caffeine act as specific competitive inhibitors to block the increase in platelet cAMP caused by adenosine [29, 33]. In contrast, theophylline at higher levels (500 μM) potentiate prostaglandin E_1-induced increases in platelet cAMP because of its weak inhibitory effect on cAMP phosphodiesterase [32]. Our studies further demonstrate that platelets from patients receiving chronic theophylline treatment show increased sensitivity to PAF. Although we have not yet investigated PAF effects on platelets in blood samples from humans who consume large amounts of coffee, tea or foods that contain methylxanthines, it is likely that their platelets will also show greater responsiveness to PAF. It would be interesting to examine such PAF effects in those subjects. In this regard, another laboratory has reported that chronic caffeine consumption by healthy subjects causes up-regulation of platelet adenosine receptors [34]. In addition, they have reported that platelets from these subjects after chronic caffeine consumption show decreased inhibitory effects of the adenosine analog, 5′-*N*-ethylcarboxamidoadenosine (NECA) on thrombin-induced platelet aggregation [34].

PAF actions in platelets are mediated by an increase in $[Ca]_i^{2+}$ mobilization [8, 27]. The increase in $[Ca^{2+}]_i$ caused by PAF in platelets is primarily from influx of extracellular Ca^{2+} [8]. Our studies demonstrate that the precence of extracellular adenosine produces strong inhibition (IC_{50}, 0.3 μM) of PAF-induced $[Ca^{2+}]_i$ mobilization in human platelets (Fig. 5). Recently we reported similar strong inhibition of vasopressin-induced $[Ca^{2+}]_i$ mobilization by adenosine in human platelets [36]. In addition, a recent study has shown an inhibitory effect of adenosine on thrombin-induced $[Ca^{2+}]_i$ mobilization in human platelets with an IC_{50} of about 0.5 μM [37], which is similar to our data for adenosine (IC_{50} 0.3 μM) to block PAF-induced $[Ca^{2+}]_i$ mobilization. Furthermore, they found that caffeine antagonizes the adenosine-induced inhibition of

platelet aggregation and $[Ca^{2+}]_i$ mobilization [37]. It is not clear whether adenosine inhibits $[Ca^{2+}]_i$ mobilization by elevating platelet cAMP levels or by acting as a calcium antagonist (calcium channel blocker) as has been proposed in atrial myocardium [38, 39]. These investigators reported that adenosine directly affects Ca^{2+} influx in guinea-pig atria by a direct action on the L-type calcium channels [38, 39].

Macrophages, neutrophils and platelets play a role in adult respiratory distress syndrome (ARDS), but their potential actions and interactions are not well understood. Several studies suggest that leukocyte release of PAF may cause platelet aggregation with the formation of thromboxane A_2 (TxA_2), which then produces pulmonary hypertension and edema [40–42]. Antiplatelet agents (imidazole, 13-aza-prostanic acid [42] and prostacyclin [43]) diminish pulmonary hypertension and prevent lung edema. Since adenosine strongly inhibits PAF-induced platelet aggregation, and thus blocks production of TxA_2, adenosine may play a role in preventing PAF-mediated pulmonary hypertension and edema. Agents such as nucleoside transport inhibitors (e.g. dipyridamole) or adenosine deaminase inhibitors (e.g. dCF or EHNA) may prove useful to block PAF actions. On the other hand, PAF activity can be stimulated by methylxanthines as we have demonstrated using platelets as a model of PAF–adenosine interactions. Such studies are important to further investigate the role of adenosine in the regulation of the biological activity of PAF.

Acknowledgements—The authors thank Dr. Alice A. Bonitati for assisting in the identification of patients, and Ms. Qi Zhao for her excellent technical assistance. This work was supported by grants from the National Institutes of Health (CA 07340 and HL 34009) and the Department of Veterans Affairs (Merit Review).

REFERENCES

1. Benveniste J, Le Couedic JP and Kamoun P, Aggregation of human platelets by platelet-activating factor. *Lancet* **i**: 344–345, 1977.
2. Demopoulos CA, Pinckard RN and Hanahan DJ, Platelet-activating factor. Evidence for 1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). *J Biol Chem* **254**: 9355–9358, 1979.
3. Vargaftig BB, Lefort J. Chignard M and Benvenite J, Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur J Pharmacol* **65**: 185–192, 1980.
4. Hanahan DJ, A biologically active phosphoglyceride. *Annu Rev Biochem* **55**: 483–509, 1987.
5. Fauler J, Sielhorst G and Frolich JC, Platelet-activating factor induces the production of leukotrienes by human monocytes. *Biochim Biophys Acta* **1013**: 80–85, 1989.
6. Marcus AJ, Safier LB, Ullman HL, Wong KT, Broekman MJ, Weksler BB and Kaplan KL, Effects of acetyl glyceryl ether phosphorylcholine on human platelet function *in vitro*. *Blood* **58**: 1027–1031, 1981.
7. Cattaneo M, Canciani MT and Mannucci PM, Human platelet aggregation and release reaction induced by platelet activating factor (PAF-acether)—Effects of acetylsalicylic acid and external ionized calcium. *Thromb Haemost* **53**: 221–224, 1985.
8. Hallam TJ, Sanchez A and Rink TJ, Stimulus–response coupling in human platelets: Changes evoked by platelet-activating factor in cytoplasmic free calcium monitored with the fluorescent calcium indicator quin2. *Biochem J* **218**: 819–827, 1984.
9. Hwang S-B, Lam M-H and Pong S-S, Ionic and GTP regulation of binding of platelet-activating factor to receptors and platelet-activating factor-induced activation of GTPase in rabbit platelet membrane. *J Biol Chem* **261**: 532–537, 1986.
10. Valone FH, Coles E, Reinhold VR and Goetzl EJ, Specific binding of phospholipid platelet-activating factor by human platelets. *J Immunol* **129**: 1637–1641, 1982.
11. Ukena D, Dent G, Birke FW, Robaut C, Sybrecht GW and Barnes PJ, Radioligand binding of antagonists of platelet-activating factor to intact human platelets. *FEBS Lett* **228**: 285–289, 1988.
12. Taylor ML, Misso NLA, Stewart GA and Thompson PJ, The effects of varying doses of aspirin on human platelet activation induced by PAF, collagen and arachidonic acid. *Br J Clin Pharmacol* **33**: 25–31, 1992.
13. Born GVR, Haslam RJ, Goldman M and Lowe RD, Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation and as vasodilators in man. *Nature* **205**: 678–680, 1965.
14. Nees S and Gerlach E, Adenine nucleotides and adenosine metabolism in cultured coronary endothelial cells: Formation and release of adenine compounds and possible functional implications. In: *Regulatory Functions of Adenosine* (Eds. Berne RM, Rall TW and Rubio R), pp. 347–360. Martinus Nijhoff, The Hague, 1983.
15. Agarwal KC, Modulation of platelet functions by plasma adenosine levels. In: *Role of Adenosine and Adenine Nucleotides in the Biological System* (Eds. Amai S and Nakazawa M), pp. 457–468. Elsevier Science BV, Amsterdam, 1991.
16. Berne RM, Winn HR, Knabb M, Ely SW and Rubio R, Blood flow regulation by adenosine in heart, brain, and skeletal muscle. In: *Regulatory Functions of Adenosine* (Eds. Berne RM, Rall TW and Rubio R), pp. 293–317. Martinus Nijhoff, The Hague, 1983.
17. Sollevi A, Torssel L, Owall A, Edlund A and Lagerkranser M, Levels and cardiovascular effects of adenosine in humans. In: *Topics and Perspectives in Adenosine Research* (Eds. Gerlach E and Becker BF), pp. 559–613. Springer, Heidelberg, 1987.
18. Dawicki DD, Agarwal KC and Parks RE Jr, Potentiation of the antiplatelet action of adenosine in whole blood by dipyridamole or dilazep and the cAMP phosphodiesterase inhibitor, RA233. *Thromb Res* **43**: 161–175, 1986.
19. Dawicki DD, Agarwal KC and Parks RE Jr, Adenosine metabolism in human whole blood: Effects of nucleoside transport inhibitors and phosphate concentration. *Biochem Pharacol* **37**: 621–626, 1988.
20. Agarwal KC, Zhao Q and Parks RE Jr, Modulation of platelet-activating factor (PAF) actions on human platelets by adenosine (Ado) and methylxanthines *FASEB J* **6**: A1313, 1992.
21. Clarke E, Agarwal KC, Bonitati A and Rounds S, Increased platelet sensitivity to platelet-activating factor (PAF) in patients receiving theophylline. *Am Rev Respir Dis* **147**: A62, 1993.
22. Ukena D, Shamim MT, Padgett W and Daly JW, Analogs of caffeine: Antagonists with selectivity for A_2 adenosine receptors. *Life Sci* **39**: 743–750, 1986.
23. Paterson ARP, Jakobs ES, Harley ER, Fu N-W, Robins MJ and Cass CE, Inhibition of nucleoside transport. In: *Regulatory Functions of Adenosine* (Eds. Berne RM, Rall RW and Rubio R), pp 203–220. Martinus Nijhoff, The Hague, 1983.
24. Agarwal RP, Spector T and Parks RE Jr, Tight-binding

inhibitors—IV. Inhibition of adenosine deaminases by various inhibitors. *Biochem Pharmacol* **26**: 359–367, 1977.
25. Born GVR, Quantitative investigations into the aggregation of blood platelets. *J Physiol (Lond)* **162**: 67P–68P, 1962.
26. Wojcik WJ and Neff NH, Adenosine measurement by a rapid HPLC-fluorometer method: Induced changes of adenosine content in regions of rat brain. *N. Neurochem* **39**: 280–282, 1982.
27. Bellinger OK, Kirchmaier CM, Schirmer A and Breddin HK, Influence of Quin 2, Fura 2 and Indo 1 on platelet function and on the measurement of cytosolic free calcium iron. *Platelets* **2**: 19–24, 1991.
28. Agarwal KC, Modulation of vasopressin actions on human platelets by plasma adenosine and theophylline: Gender differences. *J Cardiovasc Pharmacol* **21**: 1012–1018, 1993.
29. Haslam RJ and Cusack NH, Blood platelet receptors for ADP and adenosine. In: *Purinergic Receptors* (Ed. Burnstock G), pp. 223–285. Chapman & Hill, London, 1981.
30. Agarwal KC, Adenosine and platelet function. In: *Role of Adenosine in Cerebral Metabolism and Blood Flow* (Eds. Stefanovich V and Okyayuz-Baklouti I), pp. 107–124. VNU Science Press BV, New York, 1988.
31. Born GVR, Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* **194**: 927–929, 1962.
32. Mills DCB and Smith JB, The influence on platelet aggregation of drugs. *Biochem J* **121**: 185–196, 1971.
33. Haslam RJ and Rosson GM, Effects of adenosine on levels of adenosine cyclic 3′,5′-monophosphate in human blood platelets in relation to adenosine incorporation and platelet aggregation. *Mol Pharmacol* **11**: 528–544, 1975.
34. Biaggioni I, Paul S, Puckett A and Arzubiaga C, Caffeine and theophylline as adenosine receptor antagonists in humans. *J Pharmacol Exp Ther* **258**: 588–593, 1991.
35. Daly JW, Analogs of caffeine and theophylline: Activity as antagonists at adenosine receptors. In: *Role of Adenosine and Adenine Nucleotides in the Biological system* (Eds. Imai S and Nakazawa M), pp. 119–129. Elsevier Science BV, Amsterdam, 1991.
36. Agarwal KC, Huzoor-Akbar and Parks RE Jr, Vasopressin-induced intracellular calcium mobilization in human platelets: Blockade by adenosine. *Thromb Haemost* **59**: 1243, 1993.
37. Paul SI, Feoktistov I, Hollister AS, Robertson D and Biaggioni I, Adenosine inhibits the rise in intracellular calcium and platelet aggregation produced by thrombin: Evidence that both effects are coupled to adenylate cyclase. *Mol Pharmacol* **37**: 870–875, 1990.
38. Cerbai E, Klöckner U and Isenberg G, Ca-antagonistic effects of adenosine in guinea pig atrial cells. *Am J Physiol* **255**: H872–H878, 1988.
39. Fassina G, De Biasi M and Caparrotta L, Adenosine: A natural modulator of L-type calcium channels in atrial myocardium. *Pharmacol Res* **23**: 319–326, 1991.
40. Halonen M, Palmer JD, Lohman C, McManus LM and Pinckard RN, Differential effects of platelet depletion on the physiological alterations of IgE anaphylaxis and acetyl glyceryl ether phosphorylcholine infusion in the rabbit. *Am Rev Respir Dis* **124**: 416–421, 1981.
41. McManus LM, Fitzpatrick FA, Hanahan DJ and Pinckard RN, Thromboxane B_2 release following acetyl glyceryl ether phosphorylcholine (AGEPC) infusion in the rabbit. *Immunopharmacology* **5**: 197–207, 1983.
42. Heffner JE, Shoemaker SA, Canham EM, Patel M, McMurtry IF, Morris HG and Repine JE, Acetyl glyceryl ether phosphorylcholine (AGEPC)-stimulated human platelets cause pulmonary hypertension and edema in isolated rabbit lungs: Role of thromboxane A_2. *J Clin Invest* **71**: 351–357, 1983.
43. Halonen M, Lohman C, Dunn AM, McManus LM and Palmer JD, Participation of platelets in the physiologic alterations of the AGEPC response and of IgE anaphylaxis in the rabbit: Effects of PGI_2 inhibition of platelet function. *Am Rev Respir Dis* **131**: 11–17, 1985.