Reduced Platelet Thromboxane Formation in Uremia

EVIDENCE FOR A FUNCTIONAL CYCLOOXYGENASE DEFECT

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ABSTRACT A qualitative platelet abnormality and a bleeding tendency are frequently associated with renal failure and uremia. We demonstrated previously that uremic patients display an abnormal platelet aggregation to arachidonic acid and reduced malondialdehyde production in response to thrombin and arachidonic acid. The objectives of this investigation were: (*a*) to compare platelet prostaglandin (PG) and thromboxane (TX) production in whole blood and in platelet-rich plasma (PRP) of 21 uremic patients and 22 healthy subjects; (*b*) to evaluate the concentration and activity of platelet PG- and TX-forming enzymes; (*c*) to assess the functional responsiveness of the platelet TXA_2/PGH_2 receptor; (*d*) to explore the hemostatic consequences of partially reduced TXA_2 production.

Platelet immunoreactive TXB_2 production during whole blood clotting was significantly reduced, by ~60%, in uremic patients as compared to age- and sex-matched controls. Exogenous thrombin (5–30 IU/ml) failed to restore normal TXB_2 production in uremic platelets. Uremic PRP produced comparable or slightly higher amounts of TXB_2 than normal PRP at arachidonate concentrations 0.25–1 mM. However, when exposed to substrate concentrations >2 mM, uremic PRP produced significantly less TXB_2 than normal PRP. To discriminate between reduced arachidonic acid oxygenation and altered endoperoxide metabolism, the time course of immunoreactive TXB_2 and PGE_2 production was measured during whole blood clotting. The synthesis and release of both cyclooxygenase-derived products was slower and significantly reduced, at all time intervals considered. Furthermore, PGI_2 production in whole blood, as reflected by serum immunoreactive 6-keto-$PGF_{1\alpha}$ concentrations, was significantly reduced in uremic patients as compared with healthy subjects. PGH synthase levels, as determined by an immunoradiometric assay, were not significantly different in platelets from uremic patients as compared to control platelets. A single 40-mg dose of aspirin given to five healthy volunteers reduced their serum TXB_2 to levels found in uremic patients. This was associated with a significant increase of threshold aggregating concentrations of ADP and arachidonic acid and prolongation of bleeding time. Substantially similar threshold concentrations of U46619, a TXA_2 agonist, induced aggregation of normal and uremic platelets. Prostacyclin induced a significant elevation of uremic platelet cyclic AMP, which was suppressed by U46619, further suggesting normal responsiveness of the TXA_2/PGH_2 receptor.

We conclude that: (*a*) an abnormality of platelet arachidonic acid metabolism exists in uremia, leading to a reduced TXA_2 production; (*b*) the characteristics of this abnormality are consistent with a functional cyclooxygenase defect; (*c*) reduced TXA_2 production may partially explain the previously described abnormality of platelet function in uremia.

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INTRODUCTION

A bleeding tendency is frequently associated with renal failure and uremia (1). The existence of an ac-

quired platelet abnormality in these patients is widely accepted (2–5). However, the mechanism(s) underlying this abnormality has not been elucidated (see ref. 6 for a review). Previous studies from our laboratories have established an abnormal platelet aggregation to exogenous arachidonic acid and reduced malondialdehyde (MDA)[1] production in response to thrombin and arachidonic acid (7, 8). Both findings suggested that an abnormal platelet prostaglandin (PG) and thromboxane (TX) production might contribute to the observed defect in platelet function. The aims of the present study were: (*a*) to compare platelet PG and TX production in whole blood and in platelet-rich plasma (PRP), in response to endogenous and exogenous stimuli, in uremic patients and in healthy subjects; (*b*) to evaluate the concentration and activity of platelet PG- and TX-forming enzymes in an attempt to identify the site(s) of platelet dysfunction; (*c*) to investigate the functional responsiveness of platelets to TXA_2 and prostacyclin (PGI_2); (*d*) to explore the hemostatic consequences of partially reduced platelet TXA_2 production.

These studies demonstrate that an abnormality of platelet arachidonic acid metabolism exists in uremia, the characteristics of which are consistent with a functional cyclooxygenase defect.

METHODS

Subjects. 21 patients (12 male, M; 9 female, F; ages 22–74 yr; 48±15 yr, mean±SD) with chronic renal failure (due to chronic glomerulonephritis in 9, chronic pyelonephritis in 6 and polycystic kidney disease in 6) on maintenance hemodialysis and 22 normal volunteers (11 M, 11 F; ages 23–50 yr; 36±9 yr, mean±SD) were studied. The 21 patients had serum creatinine concentrations ranging from 9.4 to 17.4 mg/dl (mean±SD: 13.3±2.4), platelet counts ranging from 120,000 to 280,000/μl (mean±SD: 185,000±44,000) and serum albumin concentrations ranging from 3.4 to 4.8 g/dl (mean±SD: 4.2±0.3). Uremic patients were receiving 12 m^2 h of weekly hemodialysis in three sessions. The dialyzers used were: Coil kidney (Vita 2 Bellco, Mirandola, Italy) with cuprophan membrane in 11 patients and parallel flow (Gambro, 11.5 μM, Lund, Sweden) in 10 patients. All patients were studied immediately before a routine dialysis, i.e., 68 h after the end of the previous session.

A careful interview was entertained with each subject in order to exclude the intake of aspirin-containing medications during the 2 wk preceding the study. Peripheral blood samples were drawn by venipuncture, in the fasting state.

Materials. Sodium arachidonate was prepared by dissolving arachidonic acid (Sigma Chemical Co., St. Louis, MO) in 0.1 M sodium carbonate, pH 10.0. U46619 [(15S)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z,13E dienoic acid] (kindly supplied by Upjohn Co., Kalamazoo, MI) was dissolved in ethanol. PGI_2 Na salt (kindly supplied by Carlo Erba-Farmitalia, Milano, Italy) was dissolved in 0.05 M Tris buffer, pH 9.4.

Whole blood clotting. Platelet TXA_2, PGE_2, and leukocyte PGI_2 production in response to endogenous thrombin was studied by letting multiple 1-ml aliquots of whole blood to clot at 37°C for 5–60 min, as previously described (9). Additional experiments were performed by letting 1-ml aliquots of whole blood to clot at 37°C for 60 min, in the presence of increasing concentrations of added thrombin (Topostasin, Roche, Milano, Italy) i.e., 5–30 IU/ml. The separated sera were frozen and kept at −20°C until assayed.

Treatments of platelet-rich plasma (PRP). 9 vol of venous blood were mixed with 1 vol of 3.8% sodium citrate. PRP was prepared by centrifugation at 190 *g* for 15 min at room temperature. 1-ml aliquots of PRP were incubated at 37°C with increasing concentrations of added sodium arachidonate, i.e., 0.25–5 mM. Two min after addition of the stimulus, a 100-μl sample was removed, immediately added to 400 μl of buffer containing 2 μg/ml indomethacin (Merck & Co., Inc., Rahway, NJ), vortexed, and frozen in liquid nitrogen at −80°C. Samples were subsequently thawed, spun at 12,500 *g* for 3 min and the supernates stored at −20°C until assayed. In 11 uremic patients and 13 normal volunteers the threshold aggregating concentration of the endoperoxide-analog U46619, a relatively stable TXA_2 agonist, was determined. Platelet aggregation was performed as previously described (10). Threshold was defined as the lowest concentration that caused irreversible aggregation within 3 min of addition to PRP. Moreover, in nine uremic patients and four healthy subjects, the functional responsiveness of platelets to TXA_2 was further characterized by examining the ability of U46619 to inhibit PGI_2-stimulated cyclic (c)AMP accumulation, as recently described by Wu et al. (11). PRP was divided into four 4-ml aliquots. In aliquot 1, PRP was incubated with 3 μM U46619 for 90 s at room temperature. 3 nM freshly prepared PGI_2 was added and incubated for an additional 90 s. In aliquot 2, PRP was incubated with ethanol for 90 s followed by PGI_2 for an additional 90 s. In aliquot 3, PRP was incubated with U46619 for 90 s followed by Tris buffer for an additional 90 s. In aliquot 4, PRP was incubated with ethanol for 90 s followed by Tris buffer for an additional 90 s. The mixtures were quickly frozen in liquid nitrogen and stored at −70°C until assayed.

Aspirin study in healthy subjects. To explore the possible hemostatic consequences of a reduced platelet TXA_2 production, five healthy subjects (three F, two M; age 31.4±7.2 yr; range 20–41) received a single 40-mg dose (0.68±0.09 mg/kg) of aspirin (Bayer AG, Leverkusen, West Germany) i.e., a dose of the drug reducing serum TXB_2 concentrations by ~65%, 24 h after oral administration in healthy subjects (12). Bleeding time, platelet aggregation in response to sodium arachidonate and ADP, and serum TXB_2 concentrations were measured prior to and 24 h after oral aspirin administration.

Bleeding time was measured in duplicate by an automatic template device (Simplate II, General Diagnostic, Warner Lambert Co., Morris Plains, NJ).

Immunoradiometric assay for quantitating platelet PGH synthase. Platelet samples from healthy subjects and uremic patients were analyzed for PGH synthase protein concentrations. PGH synthase was solubilized by sonication and homogenization of the platelet sample in 1 ml of 0.1 M Tris-chloride, pH 8.0 containing 1% Tween 20 (Sigma Chemical

[1] *Abbreviations used in this paper:* MDA, malondialdehyde; PF_3, platelet factor 3; PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e., PGE_2 or PGI_2); PRP, platelet-rich plasma; RIA, radioimmunoassay; TLC, thin-layer chromatography; TX, thromboxane.

Co.). Three different aliquots of each sample (10-100 μl) were used in triplicate in the standard assay (13). The characteristics of monoclonal antibodies against PGH synthase, preparation of ^{125}I-IgG$_1$ (*cyo*-3) and IgG$_{2b}$-(*cyo*-5)-*Staphylococcus aureus* complexes are described in detail elsewhere (13). Purified sheep vesicular gland PGH synthase (14) was used as the standard. Protein content was determined by the Lowry method (15).

cAMP determinations. 1 ml of 5% TCA was added to 1 ml of PRP and immediately frozen in liquid nitrogen. After thawing at room temperature, platelets were shaken at 4°C for 30 min. The protein precipitate was removed by centrifugation at 2,500 *g* for 30 min. The supernates were extracted with water saturated ether, and the residual ether of the aqueous extract was evaporated under nitrogen at 50°C. Recovery of cAMP was 90±11% (mean±SD) as determined by [^{3}H]cAMP (New England Nuclear, Boston, MA: 33 Ci/mM), and concentrations were corrected accordingly. cAMP was determined by radioimmunoassay (RIA), according to Steiner et al. (16), using a commercially available kit (Becton Dickinson, Novate Milanese, Milano, Italy).

RIA for TXB$_2$, PGE$_2$, and 6-keto-PGF$_{1\alpha}$. Serum and plasma TXB$_2$ (the stable breakdown product of TXA$_2$), PGE$_2$, and 6-keto-PGF$_{1\alpha}$ (the stable breakdown product of PGI$_2$) concentrations were determined by previously described RIA techniques (17–19). Unextracted samples were diluted in the standard diluent of the assay (0.02 M PO_4 buffer pH 7.4) and assayed in a volume of 1.5 ml, at a final dilution of 1:500–1:15,000 for TXB$_2$, 1:50–1:1,000 for PGE$_2$ and 1:150 for 6-keto-PGF$_{1\alpha}$. 5,000 dpm of [^{3}H]TXB$_2$ (New England Nuclear: 150 Ci/mmol), [^{3}H]PGE$_2$ (Amersham International Limited, Buckinghamshire, England: 180 Ci/mmol) or [^{3}H]6-keto-PGF$_{1\alpha}$ (Amersham International Limited, 150 Ci/mmol) and appropriately diluted anti-TXB$_2$ (final dilution 1:1,000,000), anti-PGE$_2$ (final dilution 1:50,000) or anti-6-keto-PGF$_{1\alpha}$ serum (final dilution 1:350,000) were mixed and added in a volume of 1.25 ml to each assay tube. The least detectable concentration that could be measured with 95% confidence (i.e., 2 SD at zero) was 2 pg/ml for TXB$_2$ and PGE$_2$ assays, and 1 pg/ml for 6-keto-PGF$_{1\alpha}$. Consequently, the detection limit was 1 ng of TXB$_2$, 100 pg of PGE$_2$, and 150 pg of 6-keto-PGF$_{1\alpha}$ per milliliter of serum or plasma, respectively. Validation of TXB$_2$, PGE$_2$, and 6-keto-PGF$_{1\alpha}$ measurements was obtained by dilution and recovery studies and by characterization of the chromatographic pattern of distribution of extracted TXB$_2$-, PGE$_2$-, and 6-keto-PGF$_{1\alpha}$-like immunoreactivities on thin-layer chromatography (TLC), as described in detail elsewhere (9, 18, 19). The relative cross-reactions of anti-PGE$_2$ serum GP 356-23/5 and of anti-6-keto-PGF$_{1\alpha}$ serum 1 with TXB$_2$, i.e., 0.015 (18), and 0.003% (19), respectively, allow reliable determinations of PGE$_2$ and 6-keto-PGF$_{1\alpha}$ even in the presence of 10^3 higher TXB$_2$ concentrations in the same sample.

Statistical analysis. Results were analyzed using parametric analysis of variance and Student's *t* test.

RESULTS

Platelet TXB$_2$ production during whole blood clotting was significantly ($P < 0.0005$) reduced in uremic patients as compared to age- and sex-matched controls. Serum TXB$_2$ concentrations averaged 120±67 ng/ml (mean±SD, $n = 17$; range 29–255) in uremic patients vs. 317±76 ng/ml (mean±SD, $n = 17$; range 180–450) in healthy subjects. When calculated as TXB$_2$ produced/10^6 platelets, mean (±SD) values were 0.65±0.36 and 1.44±0.35 ng, respectively. 13 of the 17 uremics had serum TXB$_2$ concentrations <2 SD of the normal mean. TXB$_2$-like immunoreactivity of uremic and control sera met classical criteria of specificity for RIA measurements: i.e., it was recovered quantitatively when added to serum, and its concentrations decreased in a linear fashion upon dilution. Furthermore, when subjected to extraction and TLC, the vast majority (>90%) of the recovered immunoreactivity (50–60%) cochromatographed with authentic TXB$_2$.

Because thrombin is a major stimulus to platelet aggregation and release during whole blood clotting (20), the possibility of a defective thrombin formation as the cause of reduced TXB$_2$ production was examined by letting multiple 1-ml aliquots of whole blood to clot in the presence of increasing concentrations of exogenous thrombin. As shown in Fig. 1, uremic platelets produced significantly ($P < 0.0005$) lower TXB$_2$ than normal platelets, throughout the whole range of thrombin concentrations tested. This finding suggested a more likely abnormality of the enzymes of the arachidonic acid cascade. Defective phospholipase activity would result in reduced arachidonic acid release in response to endogenous as well as exogenous stimuli. Therefore, we studied the effects of a wide range of added substrate concentrations on TXB$_2$ production in PRP. As shown in Fig. 2, uremic PRP produced comparable or slightly higher amounts of TXB$_2$ than normal PRP at arachidonate concentrations 0.25–1 mM. However, when exposed to substrate concentrations >2 mM, uremic PRP produced significantly ($P < 0.01$) less TXB$_2$ than normal PRP. These results were suggestive of either PGH synthase or TX synthase deficiency. In order to discriminate between reduced arachidonic acid oxygenation and altered endoperox-

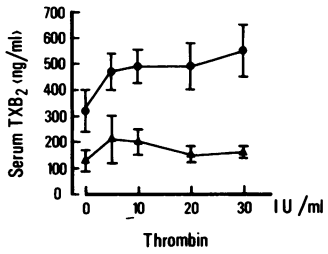

FIGURE 1 TXB$_2$ production during whole blood clotting in the presence of varying concentrations of thrombin. Nine normal subjects (●) and six uremic patients (▲) were studied and each bar represents mean±SD. Because no statistically significant differences were found in platelet counts, TXB$_2$ production is expressed as actually measured serum TXB$_2$ concentrations. TXB$_2$ levels were significantly ($P < 0.0005$) lower in uremic than in normal sera throughout the whole range of thrombin concentrations.

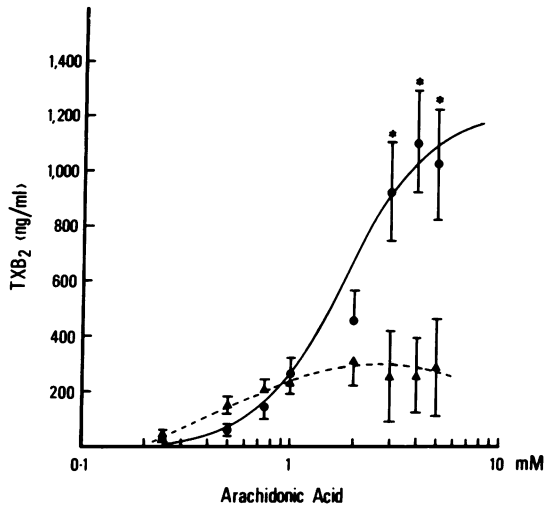

FIGURE 2 TXB_2 production in PRP in response to arachidonic acid. 15 normal subjects (the solid line, ●) and 15 uremic patients (the broken line, ▲) were studied and each bar represents mean±SD. *$P < 0.01$: uremics vs. normals.

ide metabolism, we measured the time course of TXB_2 and PGE_2 production during whole blood clotting in 6 uremic patients and 15 healthy subjects. As shown in Fig. 3, the synthesis and release of both cyclooxygenase-derived products was slower and significantly

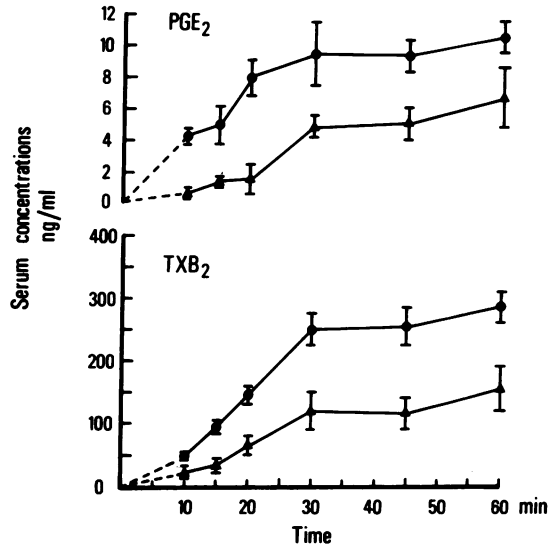

FIGURE 3 Time course of PGE_2 (upper panel) and TXB_2 (lower panel) production during whole blood clotting. 15 normal subjects (●) and six uremic patients (▲) were studied and each bar represents mean±SD. PGE_2 and TXB_2 levels were significantly ($P < 0.005$) lower in uremic than in normal sera at all time intervals considered.

($P < 0.005$) reduced at all time intervals considered, thus suggesting a more likely PGH synthase defect.

In order to discriminate between reduced enzyme activity and decreased enzyme protein levels, we used an immunoradiometric assay for quantitating PGH synthase protein concentrations in uremic and control platelet samples. PGH synthase levels averaged 1.82±1.63 U/mg protein in platelets from uremic patients as compared to 2.28±1.47 (mean±SD, $n = 10$, P = NS) in control platelets. Only 3 of 10 uremic platelet samples analyzed had PGH synthase levels <1 SD of the normal mean. In 10 paired samples, serum TXB_2 determinations were simultaneously obtained. The results of these analyses are detailed in Table I. Uremic platelets produced significantly ($P < 0.001$) lower amounts of TXB_2 than control platelets in whole blood, though having comparable enzyme protein levels in all but one sample. TXB_2 production did not correlate with PGH synthase concentrations to any statistically significant extent. PGI_2 production in whole blood, as reflected by serum 6-keto-$PGF_{1\alpha}$ concentrations, was significantly ($P < 0.01$) reduced in uremic patients (0.59±0.48 ng/ml, mean±SD, $n = 5$) as compared with healthy subjects (1.18±0.13 ng/ml).

A single 40-mg dose of aspirin given to five healthy volunteers significantly ($P < 0.005$) reduced their serum TXB_2 to levels found in uremic patients, i.e., from 385±86 to 122±36 ng/ml (mean±SD). Such a partially reduced platelet TXA_2 production was associated with a statistically significant increase of threshold aggregating concentrations of ADP (1.3±0.7 → 4.4±2.2 μM) and arachidonic acid (0.3±0.1 → 0.7±0.5) and prolongation of bleeding time (246±83 → 336±116 s).

TABLE I
Whole Blood TXB_2 Production and Platelet PGH Synthase Levels in Paired Samples from Five Healthy Subjects (HS) and Five Uremic Patients (UP)

Subject	Serum TXB_2	PGH synthase
	ng/10⁸ platelets	*U/10⁸ platelets*
HS 1	1.22	4.50
HS 2	1.22	—
HS 3	1.13	9.99
HS 4	1.83	7.04
HS 5	0.94	11.25
Mean±SD	1.27±0.33	8.2±3.0
UP 1	0.28	12.21
UP 2	0.72	0.70
UP 3	0.39	9.30
UP 4	0.08	6.21
UP 5	0.21	7.32
Mean±SD	0.34±0.24*	7.1±4.3

* $P < 0.001$, UP vs. HS.

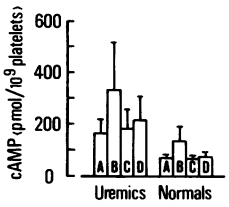

FIGURE 4 Changes in platelet cAMP content in response to PGI_2 and U46619. Nine uremic patients and four healthy subjects were studied and each bar represents mean±SD. A, control; B, PGI_2; C, U46619; D, PGI_2 + U46619. B vs. A, $P < 0.01$ for both uremics and normals.

In addition, we have investigated the possibility that TXA_2/PGH_2 receptor abnormalities might contribute to the observed defect of uremic platelet function. Substantially similar threshold concentrations of the TXA_2 agonist U46619 induced aggregation of normal and uremic platelets. Threshold aggregating concentration of U46619 averaged 0.25±0.11 μM (mean±SD, $n = 11$) for uremic platelets as compared to 0.17±0.09 μM (mean±SD, $n = 13$, P = NS) for normal platelets. To further substantiate the normal responsiveness of the platelet TXA_2 receptor, the effects of PGI_2 and U46619 on platelet cAMP were investigated in nine uremic patients and four healthy subjects. Basal cAMP levels in uremic platelets (165±54 pM/10^9 platelets; mean±SD) were significantly ($P < 0.005$) higher than in normal platelets (68±14 pM/10^9 platelets). PGI_2 induced a statistically significant ($P < 0.01$) twofold increase of uremic platelet cAMP, which was suppressed by U46619 (Fig. 4), as seen in normal platelets.

DISCUSSION

Uremic bleeding is associated with abnormalities of platelet function tests. These include prolonged bleeding time, decreased platelet adhesiveness to foreign surfaces, reduced platelet aggregation in response to various agents and abnormal PF_3 availability (4, 5). Because arachidonic acid metabolism via the cyclooxygenase pathway plays an important role in modulating platelet function (21), we investigated this metabolic pathway in uremia. This study demonstrates that platelet PG and TX production is reduced in uremic patients, in response to exogenous as well as endogenous stimuli. Recently, Smith and Dunn (22) have reported a substantial reduction of ADP-induced platelet aggregation and TXB_2 production in seven undialyzed or inadequately dialyzed uremic patients when compared to three adequately dialyzed patients or three healthy subjects.

Since a different pattern of response can be obtained depending upon the concentration of the exogenous stimulus (8), we have investigated platelet PG and TX production in response to endogenous thrombin (9). Whole blood clotting is a model that perhaps more closely mimics the in vivo situation. Under these circumstances thrombin is a major stimulus to platelet aggregation and release (20). Both the absolute amount of TXB_2 produced after whole blood clotting and the time course of its formation were significantly altered in uremic patients.

Since TXA_2 reacts with plasma proteins to form a large proportion of covalently bound derivatives, obviously unavailable to further hydrolysis to TXB_2, this compound is more likely to reflect the biologically active fraction of released TXA_2 (9). The high degree of reproducibility of serum TXB_2 levels within a group of 45 healthy subjects (9) indicates that a relatively constant fraction of platelet TXA_2 normally escapes such covalent linkage to human serum albumin. Although changes in serum TXB_2 concentrations might reflect alterations of these binding phenomena, this seems unlikely to account for reduced serum TXB_2 levels in uremia in view of substantially unchanged albumin levels and comparably reduced serum PGE_2 and 6-keto-$PGF_{1\alpha}$ concentrations.

Since these patients are known to have a defective prothrombin consumption, as a consequence of abnormal PF_3 availability (5), we determined whether reduced serum TXB_2 concentrations merely reflected defective thrombin formation. Failure of exogenously added thrombin to restore normal TXB_2 production suggested an abnormality of PG- and TX-forming enzymes.

Thrombin-induced platelet activation is associated with the release of arachidonate from membrane phospholipids, most likely through the sequential activation of a phosphatidylinositol-specific phospholipase C and a diglyceride lipase (23). A defect of these enzyme activities seems unlikely in view of the failure of exogenous arachidonic acid to restore normal TXB_2 production in uremic PRP or whole blood.

Given a normal substrate availability, either a defect of PGH synthase or TX synthase might account for the reduced TXA_2 synthesis. A reduced conversion of arachidonic acid because of a PGH synthase defect would result in reduced formation of the PG-endoperoxide intermediates and therefore similarly reduced synthesis of TXA_2 and PG. On the other hand, a reduced conversion of the PG endoperoxides because of a TX synthase defect would result in decreased TXA_2 and normal or increased PG synthesis. In fact, pharmacologically induced inhibition of platelet TXA_2 synthesis by a selective TX synthase inhibitor in man is associated with markedly enhanced PGE_2 formation in whole blood (24). Thus, the finding of similarly impaired PGE_2 and TXB_2 production during whole blood

clotting can be interpreted as reflecting defective formation of platelet PG endoperoxides in uremia. The results of the immunoradiometric assay indicate substantially unaltered levels of PGH synthase reactivity in the vast majority of uremic platelet samples examined. Thus the integrated information derived from these experiments is consistent with a functional defect of platelet cyclooxygenase activity being the main cause of reduced TXA_2 formation in uremia. The possibility that elevated cAMP levels found in uremic platelets may contribute to the reduced TXA_2 production has been considered, in view of previous studies demonstrating that cAMP may regulate arachidonic acid oxygenation (25, 26). However, it should be noted that inhibition of TXB_2 (27) or MDA (28) formation and oxygen consumption (28) by cAMP was found at low, but not at high, substrate concentrations, a finding not consistent with the results of the present study. The results of the aspirin study further indicate that partial acetylation of platelet cyclooxygenase by low-dose aspirin (29) can reproduce the abnormality of TXA_2 production described in uremics. Since PGH synthase reactivity is not influenced by inactivation of the enzyme with aspirin (13), our results are compatible with the presence of an endogenous inhibitor sharing with aspirin the same reaction site(s) on the platelet enzyme. Furthermore, the finding of significantly reduced concentrations of 6-keto-$PGF_{1\alpha}$ in uremic sera suggests that PGH synthase of PGI_2-producing leukocytes (30) is inhibited to a similar extent as the platelet enzyme. An alternative explanation consistent with this finding is that reduced PGI_2 production in whole blood simply reflects reduced availability of platelet-derived PG endoperoxides feeding PGI_2-synthase. The hypothesis of an endogenous inhibitor of cyclooxygenase activity is consistent with preliminary findings of Smith and Dunn (22) that the defect in platelet aggregation and TXB_2 production can be induced in normal platelets incubated with uremic platelet-poor plasma.

As for the relevance of a partially reduced platelet TXA_2 production to the bleeding tendency frequently associated with renal failure and uremia, the effects of low-dose aspirin in healthy subjects suggest that this abnormality may have clinically relevant hemostatic consequences.

Finally, we have investigated whether an abnormal platelet response to TXA_2 might contribute to the uremic defect in platelet function. Our finding that similar threshold concentrations of U46619 (an endoperoxide analog that mimics TXA_2 activity) aggregate uremic and normal PRP argues against such a disorder. Further evidence for intact TXA_2/PGH_2 receptor responsiveness in uremic platelets was obtained by demonstrating that the same analog was capable of suppressing cAMP elevation induced by PGI_2, similarly to its effect in normal platelets (11).

We conclude that: (*a*) an abnormality of platelet arachidonic acid metabolism exists in uremia, leading to a reduced TXA_2 production; (*b*) the characteristics of this abnormality are consistent with a functional defect at the cyclooxygenase level; (*c*) defective TXA_2 production may partially explain the previously described abnormality of platelet function in uremic patients.

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