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Biochemistry

Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides

(platelet aggregation/rabbit aorta contracting substance/guinea pig lung)

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ABSTRACT **An unstable [$t_{1/2}$ at 37° = 32 ± 2 (SD) sec] intermediate, thromboxane A_2, was detected in the conversion of prostaglandin G_2 into 8-(1-hydroxy-3-oxopropyl)-9,12*L*-dihydroxy-5,10-heptadecadienoic acid (thromboxane B_2) in platelets. The intermediate was trapped by addition of methanol, ethanol, or sodium azide to suspensions of washed human platelets incubated for 30 sec with arachidonic acid or prostaglandin G_2. The structures of the resulting derivatives demonstrated that the intermediate possessed an oxane ring as in thromboxane B_2 but lacked its hemiacetal hydroxyl group. Additional experiments using $^{18}O_2$ or [$^2H_8$]arachidonic acid in the formation of thromboxane B_2 and CH_3O^2H for the trapping of thromboxane A_2, together with information on the $t_{1/2}$ of the intermediate, indicated the presence of an oxetane structure in thromboxane A_2.**

Incubation of arachidonic acid or prostaglandin G_2 with washed platelets led to formation of an unstable factor that induced irreversible platelet aggregation and caused release of [^{14}C]serotonin from platelets that had been incubated with [^{14}C]serotonin. The properties and the mode of formation of this factor indicated that it was identical with thromboxane A_2. Furthermore, evidence is presented that the more unstable and major component of rabbit aorta contracting substance (RCS) formed in platelets and guinea pig lung is also thromboxane A_2.

Recently the hemiacetal derivative of 8-(1-hydroxy-3-oxopropyl)-9,12*L*-dihydroxy-5,10-heptadecadienoic acid (PHD) and 12*L*-hydroxy-5,8,10-heptadecatrienoic acid (HHT) were found to be the major metabolites of prostaglandin G_2 (PGG_2) in suspensions of human platelets (1, 2). Conversion of PGG_2 into PHD was suggested to occur by rearrangement of the endoperoxide structure followed by incorporation of one molecule of H_2O (1). The results of the present study demonstrate the formation of an unstable, biologically active oxane intermediate between PGG_2 and PHD in human platelets. The name thromboxanes is introduced for this new group of compounds of which the unstable intermediate is thromboxane A_2 and the previously recognized compound, provisionally called PHD, is thromboxane B_2.

MATERIALS AND METHODS

[*1*-^{14}C]Arachidonic acid (The Radiochemical Centre, Amersham) was diluted with unlabeled arachidonic acid (Nu Chek Prep, Inc., Elysian, Minn.) and purified by silicic acid chromatography. The specific radioactivities of two samples prepared in this way were 0.6 and 2 Ci/mol. PGG_2 was prepared from arachidonic acid as described (3) and kept in acetone solution at −20°. [*1*-^{14}C]PGG_2 (specific radioactivity, 2 Ci/mol) was analogously prepared starting with [*1*-^{14}C]arachidonic acid.

Washed human platelets were isolated from blood collected from donors who had not taken aspirin for at least 1 week (3). The platelets were suspended in modified Krebs-Henseleit bicarbonate medium (4) (not containing calcium, supplemented with 0.2% glucose), pH 7.4 (10^6 platelets/μl). Platelet rich plasma was obtained by centrifugation (200 × *g* for 15 min) of blood collected with 0.13 volume of 0.1 M trisodium citrate. Platelet aggregation was monitored with a Chronolog aggregometer model 300. The aggregometer tube contained 0.5 ml of suspension of platelets or platelet rich plasma preincubated at 37° for 2 min with 14 μM indomethacin. This concentration of indomethacin was sufficient to give complete inhibition of the aggregating effect of arachidonic acid (tested in concentrations up to 13 μg/ml). Release of serotonin in platelet rich plasma was measured as described in Table 1.

Quantitative determination of prostaglandin endoperoxides (sum of PGG_2 and PGH_2) was performed by a method recently described in detail (3, 5).

RESULTS

Experiments with $^{18}O_2$

A siliconized two-necked flask containing 30 ml of platelet suspension was evacuated and flushed with argon six times. Subsequently, 100 ml of $^{18}O_2$ (98 atom %, Miles Laboratories) were introduced followed by 1.6 mg of [*1*-^{14}C]arachidonic acid in 0.2 ml of ethanol. The mixture was stirred at 37° for 10 min. The three major products formed from arachidonic acid by washed platelets, i.e., 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), 12*L*-hydroxy-5,8,10-heptadecatrienoic acid (HHT), and thromboxane B_2, were isolated as their methyl esters. The trimethylsilyl (Me_3Si) ether derivatives were analyzed by mass spectrometry. The Me_3Si derivatives of the compounds formed upon treatment of the methyl ester of thromboxane B_2 with sodium borohydride and with methoxyamine hydrochloride were also analyzed (1).

HETE and HHT were both found to be labeled with ^{18}O in the hydroxyl group (about 60% molecules lacking ^{18}O and about 40% molecules containing one ^{18}O). This was in agreement with their postulated formation from arachidonic acid by action of a lipoxygenase (HETE) and by fatty acid cyclooxygenase (HHT) (1).

Abbreviations: PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; HETE, 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid; HHT, 12*L*-hydroxy-5,8,10-heptadecatrienoic acid; PHD, thromboxane B_2 [8-(1-hydroxy-3-oxopropyl)-9,12*L*-dihydroxy-5,10-heptadecadienoic acid]; Me_3Si, trimethylsilyl; RCS, rabbit aorta contracting substance.

Table 1. Release of [^{14}C]serotonin (% of incorporated radioactivity) by incubation mixtures of arachidonic acid plus platelet suspensions

Exp.	Time of incubation, arachidonic acid + platelet susp. (sec)	Release of [^{14}C]serotonin (%)
I	30	72
I	30	75
II	30	48
II	60	30
II	180	2

Platelet rich plasma pretreated with [^{14}C]serotonin (1.5 ml) was stirred at 37° for 2 min with 5.3 mM $CaCl_2$ and 14 μM indomethacin and subsequently treated with 0.3 ml (Exp. I) or 0.2 ml (Exp. II) of platelet suspension incubated for various times with 5 μg/ml of arachidonic acid. After 15 sec, 0.2 ml of ice-cold 0.1 M EDTA was added and the radioactivity present in the supernatant obtained by centrifugation was determined.

Thromboxane B_2 was found to be labeled with three atoms of ^{18}O. These were present in the nonhemiacetal hydroxyl group of the oxane ring ([Me_3SiO—CH=CH—CH_2—CH=CH—$(CH_2)_3$—$COOCH_3$]$^+$; 58% 0 ^{18}O and 42% 1 ^{18}O), in the hydroxyl group at C-12 (Me_3SiO^+=CH—C_5H_{11}; 60% 0 ^{18}O and 40% 1 ^{18}O), and as the ether oxygen of the oxane ring [Me_3SiO^+=CH—CH=CH—CH(OSiMe_3)—C_5H_{11} in the mass spectra of *O*-methyloxime and reduced derivatives; 33% 0 ^{18}O, 48% 1 ^{18}O, and 19% 2 ^{18}O]. The absence of ^{18}O in the hemiacetal hydroxyl group was shown by, e.g., the ion Me_3SiO^+=CH_2 in the mass spectrum of the reduced derivative (99% 0 ^{18}O and 1% ^{18}O).

Trapping experiments

A number of nucleophiles added to platelets incubated for a short time with arachidonic acid and PGG_2 partly inhibited formation of thromboxane B_2 and resulted in formation of derivatives of thromboxane B_2. Experiments with methanol, ethanol, and sodium azide are described below.

Methanol. [*1*-^{14}C]Arachidonic acid was incubated with washed platelets for either 30 sec or 5 min. The reaction was terminated by addition of 25 volumes of methanol. Thin-layer radiochromatographic analysis of the esterified products from the two incubations showed formation of the previously recognized compounds, i.e., the methyl esters of thromboxane B_2, HHT, and HETE. The product isolated after incubation for 30 sec in addition contained two derivatives which were less polar than the methyl ester of thromboxane B_2 (R_F = 0.20 and 0.26; Fig. 1). Part of these derivatives was treated with $NaBH_4$ in methanol. No conversion could be detected by thin-layer chromatography. The remaining part was converted into Me_3Si derivatives and subjected to gas-liquid chromatography (1% SE 30 ultraphase on GasChrom Q; column temp, 249°). The equivalent chain lengths were C-24.1 (derivative having R_F = 0.20) and C-23.8 (derivative having R_F = 0.26). The mass spectra of the two derivatives were similar except for certain minor differences in intensity (Fig. 2). Ions were present at m/e 542 (M), 510 (M-32; loss of CH_3OH), 439 [M-(32+71); loss of CH_3OH plus ·C_5H_{11}],420 [M-(32+90); loss of CH_3OH plus Me_3SiOH], 381 [M-(71+90); loss of ·C_5H_{11} plus Me_3SiOH], 256 ([Me_3SiO—CH=CH—CH_2—CH=CH—$(CH_2)_3$—$COOCH_3$]$^{+\cdot}$), 225 ([(CH=CH)$_2$—CH($OSiMe_3$)—C_5H_{11}]$^+$), 199 ([CH=CH—CH($OSiMe_3$)—C_5H_{11}]$^+$), 173 (Me_3SiO^+=CH—C_5H_{11}), and 159 (Me_3SiO^+=CH—CH=CH—OCH_3). A number of these ions appeared in the mass spectrum of the Me_3Si derivative of the methyl ester thromboxane B_2 (1), i.e., m/e 510, 439, 420, 256, 225, 199, and 173. The other ions were shifted by 58 units to lower m/e values, i.e., 542, 381, and 159. Since a shift by 58 units corresponded to the difference between the molecular weights of Me_3SiO· (89) and CH_3O· (31), it seemed likely that the two derivatives isolated after addition of CH_3OH to platelets incubated for 30 sec with arachidonic acid were epimers of a mono-*O*-methylated derivative of thromboxane B_2. That these derivatives contained only two hydroxyl groups was demonstrated by the mass spectra of the corresponding [$^2H_9$]Me_3Si ethers (molecular ion at m/e 560). The methoxy group was not attached to C-12, as shown by the structures of the ions at m/e 225, 199, and 173, or to C-1 of the propyl side chain (m/e 256). Its location in the remaining position, i.e., at C-3 of the propyl chain, was strongly suggested by the ion at m/e 159, which was formed by expulsion of three carbons from the oxane ring. The fact that treatment with $NaBH_4$ did not result in reduction also supported the location of the methoxy group in the position indicated since the acetal should be stable towards $NaBH_4$.

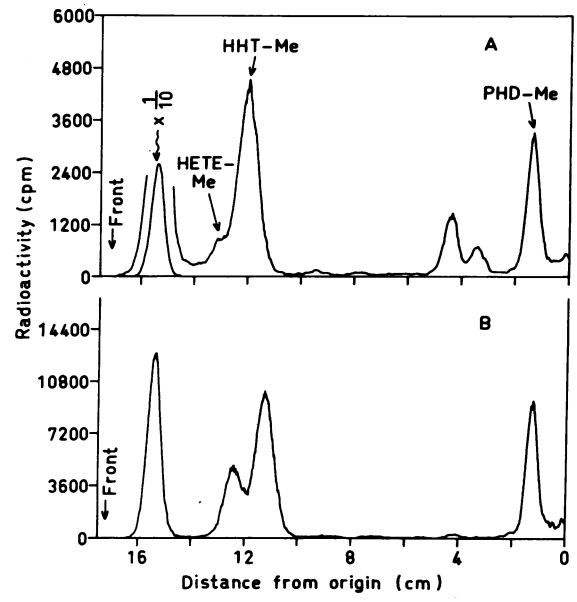

FIG. 1. Thin-layer radiochromatograms of products isolated after incubation of [*1*-^{14}C]arachidonic acid (10 μg) with 1 ml of platelet suspension (10^6 platelets/μl) for 30 sec (*upper*) and 5 min (*lower*). The reactions were terminated by addition of 25 ml of methanol and the esterified product was subjected to thin-layer chromatography (solvent system: organic layer of ethyl acetate/2,2,4-trimethylpentane/water, 75:75:100, v/v/v).

In another experiment 25 volumes of CH_3O^2H (99 atom %; E. Merck, Darmstadt) were added to platelets incubated for 30 sec with arachidonic acid. Mono-*O*-methyl-thromboxane B_2 was isolated and analyzed by mass spectrometry. No incorporation of ^2H could be detected.

Incubation of 2.8 μg of [*1*-^{14}C]PGG_2 with 3 ml of platelet suspension at 37° for 5 min afforded a mixture of labeled HHT and thromboxane B_2. When 25 volumes of methanol were added after 30 sec of incubation, two additional derivatives appeared, i.e., the mono-*O*-methyl-thromboxane B_2 epimers described above.

Ethanol and Sodium Azide. Addition of 36 volumes of ethanol to platelets incubated with arachidonic acid for 30 sec resulted in formation of two derivatives. The methyl es-

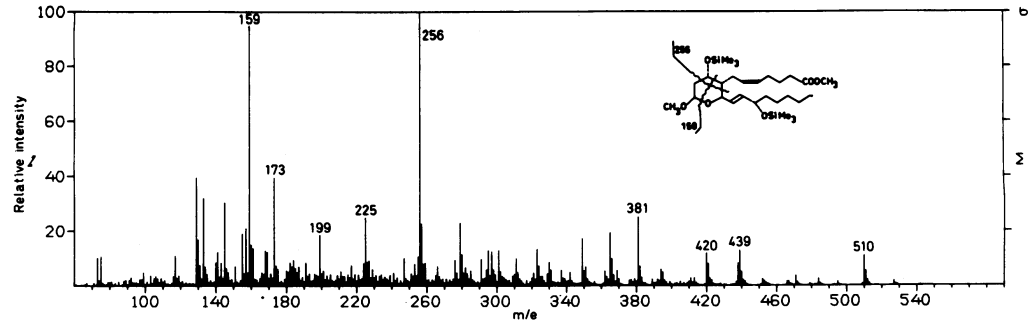

FIG. 2. Mass spectrum recorded on the Me_3Si derivative of the major of the two compounds (C-23.8) formed upon addition of methanol to platelets incubated with arachidonic acid.

ters of these were less polar on thin-layer chromatography than the methyl ester of thromboxane B_2 (R_F = 0.28 and 0.36). These derivatives were not formed when the time of incubation was 5 min. The equivalent chain lengths of the Me_3Si derivatives found on gas-liquid chromatography were C-24.4 (R_F = 0.28) and C-24.1 (R_F = 0.36). The mass spectra of the two derivatives (molecular ion at m/e 556) were similar and were analogous to those of the methyl ester—Me_3Si derivatives of thromboxane B_2 and mono-*O*-methyl-thromboxane B_2. It could be deduced from the mass spectra that the two compounds were probably epimers of mono-*O*-ethyl-thromboxane B_2 in which the ethoxy group was part of an acetal structure.

Five volumes of 5 M sodium azide added to platelets incubated with arachidonic acid for 30 sec also resulted in formation of a derivative, the methyl ester of which was less polar on thin-layer chromatography than the methyl ester of thromboxane B_2 (R_F = 0.53). When the time of incubation was 5 min the derivative could not be detected. The Me_3Si ether was subjected to gas-liquid chromatography (C-24.2). The mass spectrum showed prominent ions at m/e 525 (M-28; loss of N_2), 510 [M-(28+15); loss of N_2 plus $\cdot CH_3$], 454 [M-(28+71); loss of N_2 plus $\cdot C_5H_{11}$], 255 [Me_3SiO—$CH{=}CH$—CH_2—$CH{=}CH$—$(CH_2)_3$—$COOCH_3$ minus 1 H], and 173 ($Me_3SiO^+{=}CH$—C_5H_{11}) in agreement with a derivative of thromboxane B_2 in which the hemiacetal hydroxyl group had been replaced by an azido group.

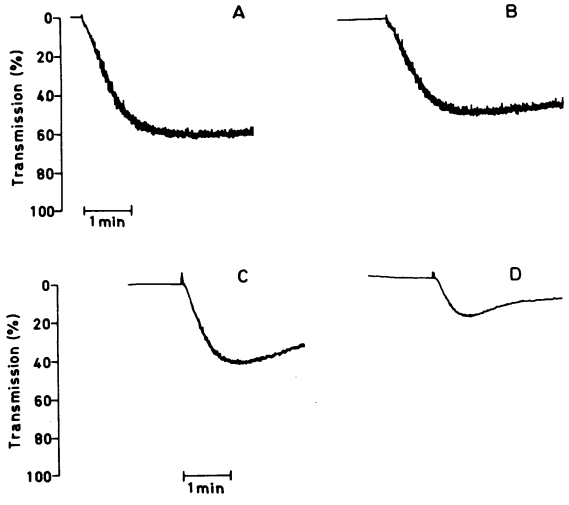

FIG. 3. Platelet aggregation induced by (A) 0.1 ml of platelet suspension incubated for 30 sec with 250 ng of arachidonic acid (containing 1.2 ng of PGG_2) and (B) 0.1 ml of platelet suspension incubated for 30 sec with 100 ng of arachidonic acid (containing 0.4 ng of PGG_2). Reference curves of platelet aggregation induced by direct addition of (C) 46 ng of PGG_2 and (D) 23 ng of PGG_2 are also shown. The aggregometer tube contained 0.5 ml of platelet suspension that had been preincubated for 2 min at 37° with 14 μM indomethacin.

Stability in Aqueous Medium. In order to determine the half-life in aqueous medium at 37° of the intermediate detected as described above, 20 ml of platelet suspension were incubated with 70 μg of [1-^{14}C]arachidonic acid for 45 sec. The mixture was filtered under a slight vacuum (45 sec) and the clear filtrate was kept at 37°. Aliquots of 1 ml were removed after different times (15 sec–5 min) and immediately added to 25 ml of methanol containing 45,000 cpm of tritium-labeled mono-*O*-methyl-thromboxane B_2. This compound was prepared by incubation of [5,6,8,9,11,12,14,15-$^3H_8$]arachidonic acid with platelets for 30 sec, followed by addition of 25 ml of methanol. The samples containing ^{14}C- and tritium-labeled mono-*O*-methyl-thromboxane B_2 were extracted with diethyl ether and subjected to reversed phase partition chromatography and thin-layer chromatography. By plotting the $^{14}C/^3H$ ratios of the isolated samples of mono-*O*-methyl-thromboxane B_2 (logarithmic scale) against time (linear scale) it was found that the decay of the unstable intermediate followed first-order kinetics. The half-life at 37° was 32 ± 2 (SD) sec (three experiments).

Platelet aggregation

Aggregating Factor from Arachidonic Acid. Arachidonic acid (0.25–5.0 μg) was added to 1 ml of suspension of platelets. After stirring at 37° for 30 sec (during which time the platelets partly aggregated), 0.1 ml was rapidly transferred to the aggregometer tube which contained 0.5 ml of platelet suspension preincubated with 14 μM indomethacin. As seen in Fig. 3, aggregation took place. A linear *logarithmic* dose-response relationship was obtained in the concentration range used. The amounts of prostaglandin endoperoxides (sum of PGG_2 and PGH_2) present in 1 ml of suspension incubated for 30 sec with 1.0 and 2.5 μg of arachidonic acid were 4 and 12 ng, respectively. The amounts present in the sample transferred (0.1 ml) were thus 0.4 and 1.2 ng. Since PGG_2 and PGH_2 in these concentrations produced insignificant aggregation (ref. 3 and Fig. 3), it was clear that a factor different from the endoperoxides must be responsible for the aggregation observed after addition of the incubation mixture of arachidonic acid and platelets. This factor could not be arachidonic acid since the platelets in the recipient tube were made totally unresponsive towards the aggregating effect of arachidonic acid by preincubation with indomethacin. ADP (20 μM) and serotonin (50 μM) were also tested and found to produce no aggregation, in agreement with previous work (6, 7).

In another experiment, 3 ml of platelet suspension were incubated with 15 μg of arachidonic acid for 40 sec, and the mixture was subsequently filtered into a flask under a slight vacuum. This operation required 45 sec. The clear filtrate was kept at 37° for different times, and an aliquot (0.1 ml) was added to the aggregometer tube for assay of aggregating activity. Another aliquot (0.7 ml) was added at the same moment to ethanol-$SnCl_2$ for quantitative analysis of prostaglandin endoperoxides. It was apparent that the filtrate contained an aggregating factor since the aggregating activity of the filtrate could not be explained by its content of endoperoxides. In this experiment it was also noted that the aggregating factor was very unstable. Its disappearance followed first-order kinetics and had $t_{1/2}$ = 41 ± 7 (SD) sec (three experiments).

Appearance of PGG_2 + PGH_2 and the aggregating factor upon incubation of arachidonic acid with platelets for different times was also studied. The amount of endoperoxides was found to be highest in the very early phase of the incubation period (maximum around 20 sec or earlier), whereas the amount of the aggregating factor attained maximum values somewhat later (40–60 sec).

Addition of 0.1 ml of incubation mixture of arachidonic acid and washed platelets to platelet rich plasma also resulted in aggregation. The extent of aggregation was considerably greater with 30 sec of incubation than with 3 min. The aggregation caused by mixtures incubated for 3 min was not due to the unstable aggregating factor because of its short-lasting existence (see above) but probably to ADP.

Aggregating Factor from PGG_2. Fig. 4 shows platelet aggregation induced by 0.1 ml of platelet suspensions that had been incubated at 37° with 75–19 ng of PGG_2 for 30 sec. In this experiment platelet suspensions in incubation and aggregometer tubes were both preincubated with 14 μM indomethacin. The amounts of PGG_2 + PGH_2 present in the samples (0.1 ml) transferred to the aggregometer tube were 6.4 ng (Fig. 4A) and 2.8 ng (Fig. 4B). These amounts were much smaller than those needed to produce an equal response in the aggregometer. Incubation of PGG_2 apparently resulted in formation of the same aggregating factor as that formed from arachidonic acid. This was supported by the fact that indomethacin, which inhibits PGG_2 formation from arachidonic acid in platelets (1), also blocked formation of the aggregating factor from arachidonic acid—thus indicating that PGG_2 was a necessary precursor of the aggregating factor—and by the finding that the arachidonic acid and PGG_2 derived factors were both very unstable and had comparable half-lives.

Release of Serotonin. Arachidonic acid incubated for 30 sec with washed platelets induced release of [^{14}C]serotonin when added to platelet rich plasma (Table 1). In this case the platelet rich plasma was pretreated with [^{14}C]serotonin and with 5.3 mM $CaCl_2$ and 14 μM indomethacin. Virtually no release was detected when the time of incubation of arachidonic acid with the washed platelets was 3 min.

Rabbit Aorta Contracting Factor. Arachidonic acid (1–5 μg) was added to 1 ml of suspension of platelets. After stirring at 37° for 30 sec the mixture was rapidly (45 sec) filtered and an aliquot of the filtrate was immediately tested on the isolated rabbit aorta strip. This resulted in a strong contraction. The response was virtually absent when the filtrate was incubated for 3 min or more at 37° prior to test on the aorta strip. PGG_2 (0.6–1 μg) was incubated with 1 ml of indomethacin-treated (14 μM) platelet suspension for 30 sec and subsequently tested on the aorta strip. Again a strong contraction was elicited. The half-life at 37° of the PGG_2-derived aorta contracting factor was 34 ± 6 (SD) sec (five experiments).

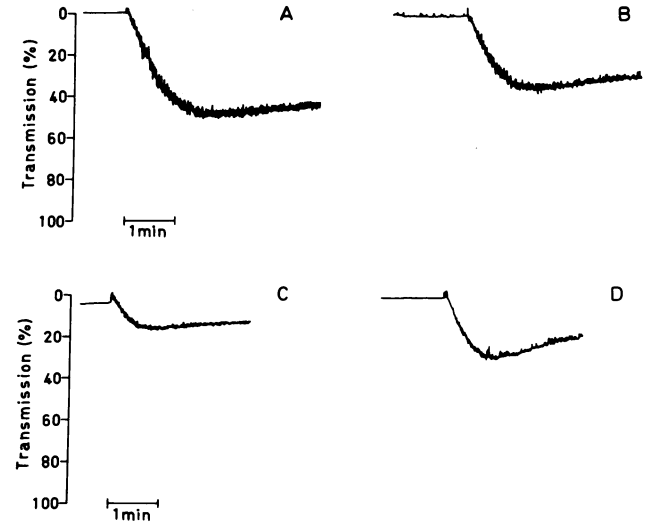

FIG. 4. Platelet aggregation induced by (A) 0.1 ml of platelet suspension incubated for 30 sec with 75 ng of PGG_2 (containing 6.4 ng of PGG_2), (B) 0.1 ml of suspension incubated for 30 sec with 38 ng of PGG_2 (containing 2.8 ng of PGG_2), and (C) 0.1 ml of suspension incubated for 30 sec with 19 ng of PGG_2. Reference curve of platelet aggregation induced by direct addition of 19 ng of PGG_2 is also shown (D). The platelets in the incubation and aggregometer tubes were both preincubated for 2 min at 37° with 14 μM indomethacin.

DISCUSSION

The present paper is concerned with an unstable, biologically active compound named thromboxane A_2. It was detected as an intermediate in the conversion of PGG_2 to thromboxane B_2 [8-(1-hydroxy-3-oxopropyl)-9,12*L*-dihydroxy-5,10-heptadecadienoic acid (PHD)] in human platelets. Incubation of arachidonic acid with platelets under $^{18}O_2$ led to formation of thromboxane B_2 labeled with ^{18}O in the hydroxyl group at C-12 as well as in the nonhemiacetal hydroxyl group and the ether oxygen of the oxane ring. It thus appeared that the two oxygens of the peroxide bridge of PGG_2 were retained in the oxane ring of thromboxane B_2. The origin of the oxygen of the hemiacetal hydroxyl group could not be determined by this experiment since the hemiacetal structure should be in equilibrium with the acyclic aldehyde-alcohol in which rapid exchange of the aldehyde oxygen with oxygen of H_2O will take place. That the hemiacetal hydroxyl group originated in H_2O was strongly indicated by the trapping experiments in which CH_3OH, C_2H_5OH, and N_3^- were able to compete with H_2O and to afford derivatives of thromboxane B_2 in which the hemiacetal hydroxyl group was replaced with methoxy, ethoxy, and azido groups, respectively (Fig. 5). These experiments showed that the conversion of PGG_2 into thromboxane B_2 proceeded with formation of an unstable intermediate ($t_{1/2}$ at 37°, 32 sec) which was at least partly released to the surrounding medium. Because of its origin and structure, the intermediate is named thromboxane A_2. Fig. 5 shows a structure of the unstable intermediate which is in agreement with the experimental data. The acetal carbon atom binding two oxygens in the very strained bicyclic structure should be susceptible to attack by nucleophiles such as H_2O (affording thromboxane B_2) and, e.g., methanol, ethanol, and azide (affording

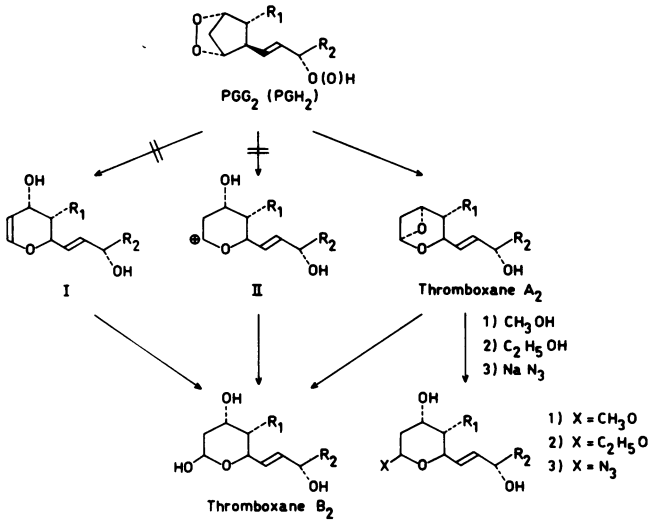

FIG. 5. Scheme of transformations of endoperoxides into thromboxane derivatives.

derivatives of thromboxane B_2 isolated in the trapping experiments). This structure was also in agreement with the previous finding that the hydrogens at carbons 5, 6, 8, 9, 11, 12, 14, and 15 in arachidonic acid and PGG_2 were all retained in the conversion into thromboxane B_2 (1). Addition of CH_3O^2H to platelets incubated with arachidonic acid led to formation of mono-*O*-methyl-thromboxane B_2 lacking carbon-bound ^2H. This finding excluded an alternate structure of the unstable intermediate, i.e., an unsaturated oxane (I in Fig. 5). Furthermore, the $t_{1/2}$ of thromboxane A_2 seemed to exclude a carbonium ion structure (II in Fig. 5) which, in aqueous medium, should be considerably less stable.

It was also found that incubation of arachidonic acid or PGG_2 with washed platelets led to formation of an unstable factor that induced irreversible platelet aggregation and caused release of [^{14}C]serotonin from platelets preincubated with [^{14}C]serotonin. This factor was distinguished from arachidonic acid, PGG_2, ADP, and serotonin and had the same $t_{1/2}$ as thromboxane A_2, indicating that the aggregating factor and thromboxane A_2 are identical.

Piper and Vane (8) in 1969 found that unstable material contracting the isolated rabbit aorta (rabbit aorta contracting substance, RCS) was released from sensitized guinea pig lungs after perfusion with antigen. Later, SRS-C and arachidonic acid were also found to cause release of RCS (9). In addition, platelets released RCS during aggregation (10). Formation of RCS was blocked by aspirin and indomethacin, inhibitors of the fatty acid cyclo-oxygenase involved in the conversion of arachidonic acid into PGG_2 (8–10). It was therefore suggested that RCS was an endoperoxide intermediate in prostaglandin biosynthesis (11). RCS from guinea pig lung and platelets was recently characterized (5) and found to contain at least two factors that contracted the rabbit aorta, i.e., PGG_2 and/or PGH_2, and an unstable factor. The half-life of the endoperoxides at 37° was about 5 min, whereas that of the unstable factor, which was responsible for the major part of the smooth muscle stimulating activity of RCS, was about 30 sec.

As described here, the unstable component of RCS was also formed in platelets from PGG_2 and had a $t_{1/2}$ of 34 sec. The identical mode of formation and half-life of the aggregating factor discovered in this study and of the unstable component of RCS indicate that they are due to the same compound, i.e., thromboxane A_2.

It is suggested that the thromboxanes, in addition to the endoperoxides and the stable prostaglandins, constitute important mediators of the actions of polyunsaturated fatty acids transformed via the cyclooxygenase pathway.

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