ENDOGENOUS FORMATION OF THE PROSTAGLANDIN ENDOPEROXIDE METABOLITE, THROMBOXANE B_2, BY BRAIN TISSUE

Leonhard S. Wolfe, Klara Rostworowski and Jean Marion

Donner Laboratory of Experimental Neurochemistry, Montreal Neurological Institute, McGill University, Montreal H3A 2B4 Canada.

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SUMMARY

Thromboxane B_2 was formed from endogenous precursors during short incubations of guinea pig and rat cerebral cortex. The amount formed by guinea pig brain tissue was 5-6 times the formation of prostaglandin $F_{2\alpha}$ and E_2. Noradrenalin stimulated and indomethacin and mercaptoethanol inhibited thromboxane B_2 formation. The mass spectrum of the brain compound was identical to thromboxane B_2 formed from arachidonic acid by guinea pig lung and human platelets.

Recent studies by Swedish investigators have shown that human platelets and guinea pig lung rapidly convert added arachidonic acid into the hemiacetal derivative of 8-(1-hydroxy-3-oxopropyl)-9,12L-hydroxy-5,10-heptadecadienoic acid (PHD) recently renamed thromboxane B_2 (1,2). This stable compound is formed by enzymatic transformation of the prostaglandin endoperoxides (PGG_2 and PGH_2) first to a very unstable, biologically highly active bicyclic intermediate, thromboxane A_2, followed by the incorporation of water (2,3). Much recent evidence implicates the formation of PGG_2 and thromboxane A_2 as the important metabolites of arachidonic acid which cause platelet aggregation and the platelet release reaction induced by collagen or thrombin (2-5). Furthermore, thromboxane A_2 is almost certainly identical to rabbit aorta contracting substance, RCS, released from sensitized guinea pig lung challenged with antigen (6,7).

Brain tissue slices and homogenates are very active in forming prostaglandin E_2 and $F_{2\alpha}$ from an endogenous pool of arachidonic acid (8). The present study was undertaken to see if the prostaglandin endoperoxide to thromboxane pathway was also present in brain tissue as found for human

platelets and guinea pig lung. With some surprise it was found that considerably more thromboxane B_2 was formed during short incubations of guinea pig cerebral cortex homogenates or slices than PGE_2 and $PGF_{2\alpha}$.

Materials and Methods

[5,6,8,9,11,12,14,15-$^2H_8$] arachidonic acid was prepared from eicosatetraynoic acid (gift from Dr. D. Garmaise, Abbott Laboratories, Montreal) by catalytic reduction with deuterium gas and subsequent purification (9). [1-^{14}C]; [5,6,8,9,11,12,14,15-$^2H_8$] thromboxane B_2 was prepared by incubation of freshly collected human platelets for 5 min at 37° with 800 µg of deuterium labelled and 7 µCi of [1-^{14}C] arachidonic acid (Amersham, England, 58 mCi/mmol). Before incubation the platelets were washed once with 0.15 M NaCl-0.15 M Tris-HCl buffer pH 7.4-77 mM EDTA (90:8:2 by vol) and suspended in Krebs-Henseleit medium without calcium for the incubations (see 4). The deuterated thromboxane B_2 containing a small amount of radioactivity was purified by silicic acid and preparative thin layer chromatography by methods developed by Hamberg and Samuelsson (3). The trimethylsilyl ether derivative of the methyl ester had a C-value of 24.4 (1% SE-30, 220°) and the mass spectrum showed a base peak of m/e 260. The yield of deuterated thromboxane B_2 was quantitated by GLC with 20-ethyl prostaglandin $F_{2\alpha}$ as internal standard (C-value, 25.7). The deuterated thromboxane was used as the internal standard for multiple-ion analysis of thromboxane formation by incubated tissue homogenates (see 1,10). Ions of m/e 256 and 260 were monitored using a 16-inch 1% OV-101 column, temperature 170°, retention time 5.5 min.

Tissues were homogenized in 0.1 M phosphate buffer, pH 7.4 with or without addition of arachidonic acid depending on the particular experiment and incubated at 37° for 5 min. Cerebral cortex slices were incubated in the phosphate buffer. The reaction was stopped by the addition of 10 volumes of ethanol, internal standard added (1.5-2 µg) and thromboxane B_2 purified first by silicic acid column chromatography and then as the methyl ester by preparative thin layer chromatography as previously described (3). High purity arachidonic acid was obtained from NuChek Prep. Inc., Elysian, Minnesota.

Results and Discussion

When a washed suspension of human platelets prepared from 10 ml blood was incubated at 37° for 5 min with [1-^{14}C] arachidonic acid (20 µg) and the radioactive products separated on thin layers of Silica gel G as the methyl esters, a radiochromatogram almost identical to that obtained by Hamberg and Samuelsson (3) was obtained (Figure 1A). Incubations of guinea pig lung homogenized in the presence of the same amount of [1-^{14}C] arachidonic acid showed even greater conversion into two more polar products, 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and thromboxane B_2 (Figure 1B). The identity of each of the products after purification by methods already described (1,3) was confirmed by gas-liquid chromatography-mass spectrometry of the methyl ester trimethylsilyl ether derivatives and the mass spectra were found to be identical to

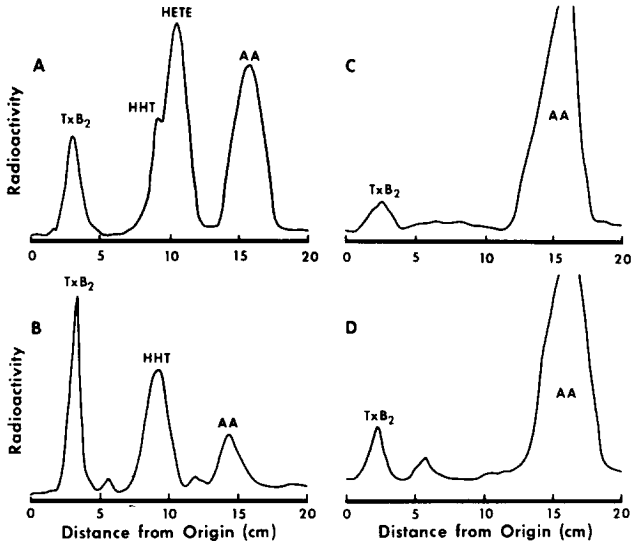

Figure 1. Thin layer radiochromatograms of the methyl esters of products formed by 5 min incubations at 37° of various tissues with [1-^{14}C] arachidonic acid (10^6 dpm). A. washed human platelets from 10 ml blood; B. guinea pig lung homogenate (1 g); C. guinea pig cerebral cortex homogenate (1 g); D. rat cerebral cortex homogenate (1 g). AA, arachidonic acid; HETE, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid; HHT, 12L-hydroxy-5,8,10-heptadecatrienoic acid; TxB_2, thromboxane B_2.

those previously reported (3). Homogenates of cerebral hemispheres from guinea pig and rat brain incubated under similar conditions showed only very small amounts of radioactivity in the region of the radiochromatogram where thromboxane B_2 runs (Figure 1C and D). However, brain tissue homogenates and slices incubated with labelled arachidonic acid also show negligible radioactivity in prostaglandin $F_{2\alpha}$ or E_2, yet significant amounts of prostaglandins are formed (8,11). In brain, almost all prostaglandin synthesis is derived from the considerable pool of endogenous free arachidonic acid (100-150 μg/g tissue) which appears 5-10 minutes after death of the animal (8,12, and unpublished work). Thus radiochromatography alone would not give a true indication of the capacity of brain tissue to synthesize thromboxane B_2 from the prostaglandin endoperoxides.

Quantitative measurement of thromboxane B_2 formation during 5 min incuba-

Table 1. Thromboxane B_2 and prostaglandin formation by various tissues

Tissue	AA added μg	TxB_2	$PGF_{2\alpha}$	PGE_2
		μg/g/5 min incubation		
Guinea pig				
lung homogenate	10	5.729 (2)	-	-
kidney homogenate	10	0.026 (2)	-	-
cerebral cortex homogenate	10	1.705 (2)	-	-
cerebral cortex homogenate	0	1.551±0.203 (4)	0.286	0.074
cerebral cortex slices	0	0.555 (2)	0.170	0.056
cerebral cortex + 1 mM NE	0	2.633 (2)	-	-
cerebral cortex + 0.1 mM INDO	0	0.034 (2)	-	-
cerebral cortex + 0.5 mM mercaptoethanol	0	0.019	-	-
Rat				
cerebral cortex homogenate	0	0.361 (2)	0.301	0.089

Abbreviations: AA, arachidonic acid; NE, noradrenalin; INDO, indomethacin; TxB_2, thromboxane B_2.
Analyses were done by multiple ion monitoring with a LKB-9000 gas chromatograph-mass spectrometer (13). Deuterated TxB_2, $PGF_{2\alpha}$ and PGE_2 were used as internal standards and carriers. Ions monitored for the methyl ester-trimethylsilyl ether derivatives were m/e 256, 260 for TxB_2, 423, 427 for $PGF_{2\alpha}$ and 425, 429 for PGE_2 after conversion to PGB_2. Results are expressed as mean of duplicate determinations or mean ± S.D.

tions of cerebral cortex showed considerable formation of thromboxane B_2 (Table 1). In guinea pig cerebral homogenates, thromboxane B_2 synthesis was 5-6 times greater than the combined formation of $PGF_{2\alpha}$ and PGE_2. Norepinephrine stimulated and indomethacin and 2-mercaptoethanol almost completely inhibited thromboxane B_2 formation. Incubation (5 min) of slices of guinea pig cerebral cortex also synthesized considerable thromboxane B_2 from endogenous arachidonic acid. The capacity of rat cerebral cortex homogenates to synthesize thromboxanes from endogenous precursors was less than the guinea pig but equivalent to the amount of PGE_2 and $PGF_{2\alpha}$ formed in the same period.

Additions of arachidonic acid only slightly increased the amount of thromboxane B_2 formed by brain tissue (Table 1). Guinea pig lung homogenates were exceedingly active in thromboxane B_2 formation but kidney homogenates by comparison showed little activity.

The large amounts of endogenously formed thromboxane B_2 in short period incubations of guinea pig brain tissue measured by the multiple ion monitoring technique utilizing [$^2H_8$] thromboxane B_2 as internal standard was surprising. To exclude the possibility that in brain we were monitoring an unknown compound with a fragment ion of m/e 256, co-eluting with thromboxane B_2, parallel incubations of 3-4 g of guinea pig lung homogenates and brain tissue were carried out. [1-^{14}C] arachidonic acid was added to the lung tissue homogenates but not to the brain. The thromboxane B_2 formed by both tissues was purified as the free acid by silicic acid column chromatography by the methods outlined by Hamberg and Samuelsson (3). The ethyl acetate fraction for the lung tissue contained 20-25 percent of the total radioactivity. The fractions from both lung and brain tissue were evaporated to dryness, methylated with diazomethane and further purified by preparative thin layer chromatography in the system ethyl acetate: 2,2,4 trimethylpentane: water (50:50:100 by vol). The radioactive zone and corresponding zone in the brain sample were eluted from the silica gel with acetone and chromatographed again in the system ethyl acetate saturated with water. The zones corresponding in R_F to PGE_2 methyl ester in this system (3) and for the lung tissue containing the radioactivity were removed, eluted with acetone, evaporated to dryness and transferred to capillary tubes, silylated with Tri-Sil Z and the mass spectra obtained in the gas chromatograph-mass spectrometer. The mass spectrum of the endogenously formed compound in brain which had exactly the same retention time as the lung thromboxane B_2 (C-value 24.4, 1% OV-101) is shown in Figure 2. The ions at m/e 585, 529, 510, 439, 420, 366, 323, 301, 295, 256 (base peak), 225 and 217 corresponded exactly to those reported in the literature for thromboxane B_2 (3) and were identical to the mass spectrum of the thromboxane B_2 isolated from guinea pig lung.

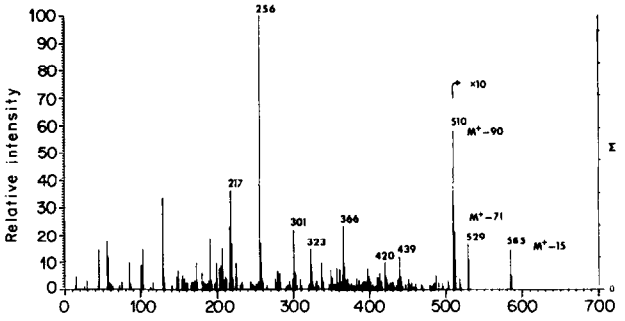

Figure 2. Mass spectrum of the methyl ester trimethylsilyl ether derivative of thromboxane B_2 formed during 5 min incubations of guinea pig brain homogenates from endogenously released arachidonic acid.

The formation of the prostaglandin endoperoxide metabolite thromboxane B_2 has so far been demonstrated in human platelets (2), guinea pig lung (1) and human umbilical artery (13). The present work shows unequivocally that this compound is also formed from endogenous arachidonic acid by guinea pig and rat brain tissue and in amounts equal to or greater than the prostaglandins. The prostaglandin endoperoxides and the unstable thromboxane A_2 have recently been shown to be important mediators in platelet aggregation and to induce the release of serotonin from the platelet α-granules (2-4). Furthermore, the activity of fatty acid cyclooxygenase which transforms arachidonic acid into the endoperoxides and consequently thromboxane formation is controlled by the platelet concentration of cAMP (14). The finding of thromboxane formation in brain tissue suggests new possibilities for investigation of the functional role of prostaglandins, prostaglandin endoperoxides and their metabolites in neurotransmission in the central nervous system.

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