Synthesis of [5,6-^{3}H]Arachidonic Acid and Its Use in Development of a Sensitive Assay for Prostacyclin Synthetase†

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ABSTRACT: [5,6-^{3}H]Arachidonic acid has been prepared by catalytic reduction of eicosa-*cis*-8,11,14-trien-5-ynoic acid (IV) over Lindlar catalyst. When either [5,6-^{3}H]arachidonic acid or [5,6-^{3}H]PGH_2 is converted into PGI_2 by swine aortic microsomes, the tritium at C-6 is lost to the medium. Thus, the progress of this enzymic rearrangement may be monitored by following the rate of tritium release. As swine aortic microsomes contain only low levels of cyclooxygenase, it is necessary to fortify the system with ram seminal vesicular microsomes (rich in cyclooxygenase) when [5,6-^{3}H]arachidonic acid is used as the indirect substrate.

Moncada et al. (1976) first described the formation by blood vessels of a novel prostaglandin with potent antiaggregatory and vasodilating properties. This substance was found to be derived from arachidonic acid via the prostaglandin endoperoxides (PGG_2[1] and PGH_2) and was subsequently identified as (5*Z*)-9-deoxy-6(9α)-oxido-11α,15(*S*)-dihydroxyprosta-5,13-dienoic acid and named PGI_2 (Johnson et al., 1976). PGI_2 is readily hydrolyzed to 6-keto-$PGF_{1\alpha}$ at physiological pH (see Scheme I). The discovery of PGI_2 complements the earlier finding of thromboxane A_2 (TXA_2) which elicits opposing physiological actions (Hamberg et al., 1975).

Prostacyclin synthetase, the enzyme which catalyzes the transformation of prostaglandin endoperoxide to PGI_2, has been demonstrated to be present in the microsomal fraction of a number of tissues in addition to the blood vessels (Sun et al., 1977; Cottee et al., 1977; Pace-Asciak, 1976; Isakson et al., 1977; Wallach, 1978). A number of methods have been devised to detect the enzyme activity (Moncada et al., 1976; Salmon et al., 1978; Salmon, 1978; Tai & Tai, 1978). However, they either lack the desired specificity or require tedious extraction and separation procedures. To facilitate studies on the nature and physiological role of this enzyme, it is necessary to develop a sensitive and specific enzymic assay.

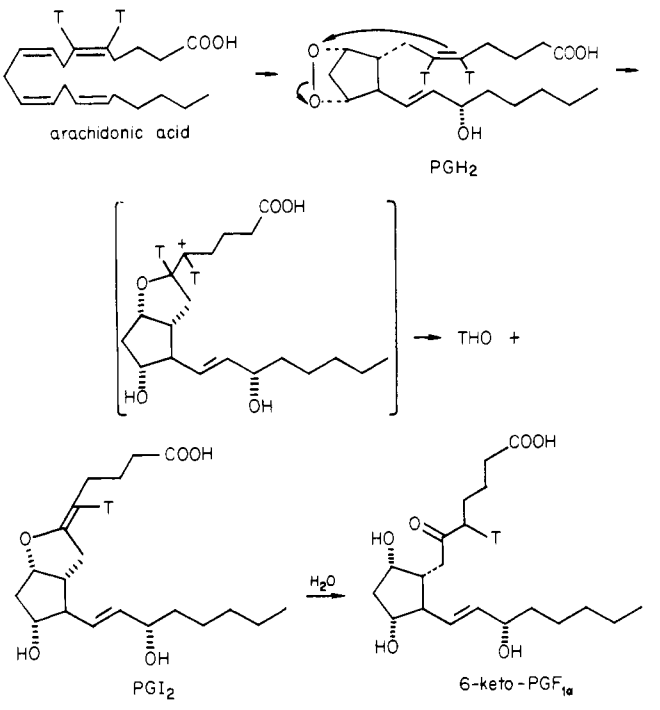
During the enzymatic rearrangement of prostaglandin endoperoxides (PGG_2 and PGH_2) into PGI_2, the hydrogen at C-6 is lost to the medium. Thus, prostacyclin synthetase activity may be quantitatively measured by following the release of tritium from C-6 labeled prostaglandin endoperoxide, PGH_2.

This paper describes the preparation of [5,6-^{3}H]arachidonic acid and its use in the development of a rapid and specific assay for prostacyclin synthetase.

Experimental Section

Arachidonic acid, DL-isoproterenol, and human hemoglobin were obtained from Sigma Chemical Co. Norit A (neutral) was purchased from Amend Drug and Chemical Co. Prostaglandin standards were kindly supplied by the Upjohn Co. Analytical precoated layers of silica gel F-254 glass plates for thin-layer chromatography (TLC) and silica gel (MN-Kiesegel, 70–270 mesh) were products of Brinkman. Silicic acid

Scheme I



powder (Mallinckrodt 2847, 100 mesh) mixed with 15% Celite was used for column chromatography. PGH_2 was biosynthesized according to Hamberg & Samuelsson (1973). 15-Hydroperoxy-5,8,11,13-eicosatetraenoic acid was prepared by the method of Hamberg & Samuelsson (1967). All solvents were of reagent grade and were redistilled.

Mass spectra were obtained with a Model 1015 Finnigan quadrupole mass spectrometer by using direct-probe introduction with an ion source temperature of 30 °C, an electron potential of 70 eV, and an ionizing current of 250 μA. Proton magnetic resonance spectra were determined on a Varian EM-390 spectrometer at 90 MHz in deuterated chloroform with Me_4Si as an internal standard.

cis-Tetradeca-2,5,8-trien-1-ol (II). Tetradeca-2,5,8-triyn-1-ol (I) (Osbond et al., 1961) (1.067 g, 5.28 mmol) in absolute

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[1] Abbreviations used: PGG_2, 15-hydroperoxy-9α,11α-peroxidoprosta-5,13-dienoic acid; PGH_2, 15-hydroxy-9α,11α-peroxidoprosta-5,13-dienoic acid; PGI_2, (5*Z*)-9-deoxy-6(9α)-oxido-11α,15(*S*)-dihydroxyprosta-5,13-dienoic acid; HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; 6-keto-$PGF_{1\alpha}$, 6-oxo-9α,11α,15(*S*)-trihydroxy-13-*trans*-prostenoic acid; TXA_2, thromboxane A_2.

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ethanol (30 mL) containing a 5% solution of quinoline in 0.2 mL of absolute ethanol was partially hydrogenated with Lindlar catalyst (0.8 g). After 30 min, 390 mL of H_2 was taken up (theoretical, 387 mL), the catalyst was removed by filtration through a cone of Celite, and the solvent was evaporated on a rotary evaporator. The only product was chromatographed over a silica gel column (1.5 × 25 cm). Elution of the column with ethyl acetate–hexane (4:6) afforded 0.96 g of II (colorless oil) with a strong odor: NMR ($CDCl_3$) δ 0.96 (3 H, t, CH_3), 1.30 (6 H, m, CH_2), 2.0 (3 H, m, C=CHCH_2 and OH), 2.82 (4 H, q, C=CHCH_2CH=C), 4.20 (2 H, d, C=CHCH_2OH), 5.06–5.74 (6 H, m, vinylic H). The mass spectrum gave *m/e* 208 as the molecular ion.

cis-1-Bromotetradeca-2,5,8-triene (III). Into a 200-mL three-necked round-bottom flask was placed a solution of triphenylphosphine (2.42 g, 9.2 mmol) in dry acetonitrile (45 mL) under N_2 in a ice–water bath. Bromine (0.6 mL, 9.3 mmol) was added dropwise from a syringe. After addition, a slight excess of Br_2 persisted (light yellow) and was removed by adding a small amount of triphenylphosphine. The adduct, triphenylphosphine dibromide, appeared as a white precipitate.

After the ice–water bath was removed, a solution of II (1.9 g, 9.1 mmol) in dry CH_3CN (20 mL) was added dropwise from a dropping funnel. When the addition was complete, the white precipitate disappeared. After the reaction mixture was stirred at room temperature for 30 min, the contents were transferred to a 250-mL round-bottom flask and the CH_3CN was evaporated on a rotary evaporator (bath temperature 45 °C). The residue was dissolved in ether, washed with saturated Na_2CO_3 solution (2 × 50 mL), and dried over $MgSO_4$. After evaporation, the residue (2.5 g) was triturated with hexane to remove the insoluble byproduct, triphenylphosphine oxide. The pure product was obtained by chromatography of the hexane fraction over a silica gel column (1.5 × 25 cm). Elution of the column with 2% ethyl acetate in hexane afforded 0.9 g of pure III: NMR ($CDCl_3$) δ 0.9 (3 H, t, CH_3), 1.32 (6 H, m, CH_2), 2.09 (2 H, m, C=CHCH_2), 2.75–3.04 (4 H, C=CHCH_2CH=C), 4.02 (2 H, d, BrCH_2CH=C), 5.06–5.90 (6 H, m, vinylic H). The rest of the column fractions (1.2 g) was further purified by preparative TLC (developed in 3% ethyl acetate in hexane) to yield another 1.02 g (total 1.92 g, 78%).

Eicosa-cis-8,11,14-trien-5-ynoic Acid (IV). A solution of 5-hexynoic acid (2 g, 17.5 mmol) in dry tetrahydrofuran (20 mL) was added dropwise to a solution of ethylmagnesium bromide, prepared from magnesium turnings (0.86 g, 35.4 mol) and ethyl bromide (4.4 g, 40 mmol) in dry tetrahydrofuran (30 mL) at 0 °C under N_2. After the reaction mixture was refluxed for 2 h, the contents were cooled to 0 °C and cuprous chloride (125 mg) was added. After 15 min, *cis*-1-bromotetradeca-2,5,8-triene (III) (1.9 g, 7 mmol) in dry tetrahydrofuran (15 mL) was added over 5 min. After the reaction mixture was refluxed for 4 h, another portion of cuprous chloride (100 mg) was added and the contents were refluxed for an additional 14 h.

The cooled reaction mixture was carefully acidified with 2 N H_2SO_4 and extracted with diethyl ether. The ethereal layer was washed with a saturated NH_4Cl solution until free from Cu^{2+} and SO_4^{2-} and then extracted with three portions of 2 N ammonia to which 5% NaCl had been added. The combined aqueous layers were washed once with ether, acidified, and extracted three times with ether. The combined ethereal layers were washed successively with water and saturated NaCl solution and dried over Na_2SO_4. After evaporation of the solvent, the excess 5-hexynoic acid was recovered by Kugelrohr distillation. The product was purified by preparative TLC by using 50% ethyl acetate in hexane as the developing solvent and yielded 558 mg (42.5%) of IV as a colorless oil: NMR ($CDCl_3$) δ 0.9 (3 H, t, CH_3), 1.30 (6 H, m, CH_2), 1.80 (2 H, t, C≡CCH_2), 2.01 (2 H, m, C=CHCH_2C), 2.26 (2 H, m, CH_2CO_2H), 2.46 (2 H, t, CH_2CO_2H), 2.63–3.06 (6 H, m, C=CHCH_2CH=C and C=CHCH_2C≡C), 5.18–5.60 (6 H, m, vinyl H), 10.82 (1 H, br s, COOH). The mass spectrum for the methyl ester of the product gave *m/e* 316 (M^+) and 301 ($M^+ - CH_3$).

Catalytic Reduction of Eicosa-8,11,14-trien-5-ynoic Acid with Tritium Gas. To 45 mg of IV in 3.5 mL of dry petroleum ether (bp 65–67 °C) was added Lindlar's catalyst (16 mg) and 5% quinoline solution in petroleum ether (0.09 mL). The mixture was stirred under $^3H_2$ (1 atm) at 25 °C for 20 min. (Due to solvent evaporation, one cannot measure the amount of $^3H_2$ gas taken up. However, 20-min reaction time should be sufficient.) After 20 min, the contents was filtered through Celite and the catalyst was washed with 1 N HCl, water, and saturated NaCl solution and then dried over Na_2SO_4. After filtration, the solvent was evaporated and the desired [5,6-^{3}H]arachidonic acid was purified on a silicic acid–Celite column (1 × 30 cm) by using a 7% diethyl ether–Skelly B elution system (Sih & Takeguchi, 1973).

The purified [5,6-^{3}H]arachidonic acid has a specific activity of 120 mCi/mmol.

Preparation of Swine Aortic Microsomes. Swine aorta was chopped into pieces and homogenized in 5 volumes of 0.05 M Tris-HCl, pH 7.5, in a Waring blender for 2 min. The suspension was filtered through two layers of cheesecloth and the filtrate was centrifuged at 8000*g* for 10 min. The supernatant was further centrifuged at 105000*g* for 60 min. The pellet was homogenized in one-tenth the volume of the original aorta weight and stored in small aliquots at −76 °C.

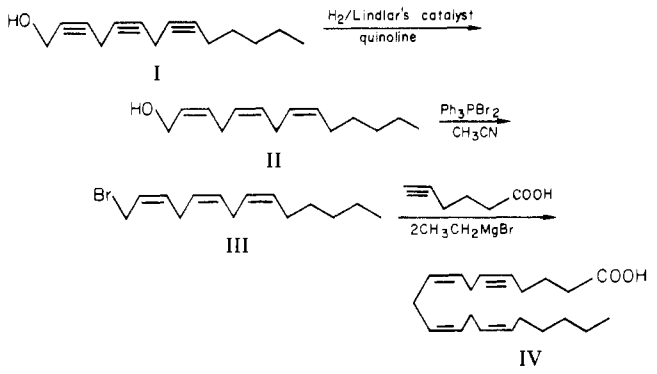
Prostacyclin Synthetase Assay. The reaction mixture contained [5,6-^{3}H]PGH_2 (2.8 nmol, 9400 cpm) and swine aortic microsomes in a final volume of 0.5 mL of 0.05 M Tris-HCl, pH 7.5. The reaction was initiated by the addition of PGH_2 and incubated at 22 °C for 1 min. The reaction was then terminated by the addition of 0.25 mL of cold 15% trichloroacetic acid. After the mixture was allowed to stand at 0 °C for 10 min, 0.2 mL of 10% charcoal suspension in water was added. After standing for 5 min at 22 °C, the mixture was centrifugated at 1000*g* for 5 min. The supernatant was decanted and the radioactivity was determined by liquid scintillation counting. If [5,6-^{3}H]arachidonic acid was used as the indirect substrate, the reaction mixture contained [5,6-^{3}H]arachidonic acid (20 nmol, 60000 cpm), DL-isoproterenol (0.5 μmol), hemoglobin (0.5 nmol), and microsomes in a final volume of 0.5 mL of 0.05 M Tris-HCl, pH 7.5. The reaction was initiated by the addition of microsomes. After the reaction mixture was incubated at 37 °C for 5 min, the reaction was terminated and the radioactivity was quantitatively assayed in the same manner as that described with PGH_2 as the substrate.

Results

Eicosa-*cis*-8,11,14-trien-5-ynoic acid (IV) was synthesized from the readily available tetradeca-2,5,8-triyn-1-ol (I) (Osbond et al., 1961) according to Scheme II.

Hydrogenation of I in the presence of Lindlar's catalyst afforded *cis*-tetradeca-2,5,8-trien-1-ol (II) in 95% yield. Treatment of II with triphenylphosphine perbromide yielded the allylic bromide III (78% yield), which was purified by column chromatography. Condensation of III with the Grignard salt of 5-hexynoic acid gave IV in 42% yield. After

Scheme II



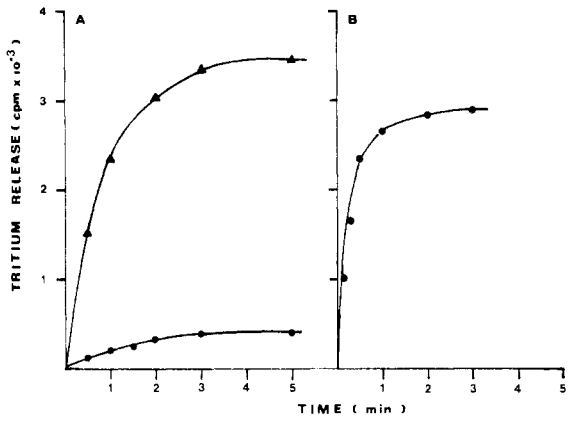


FIGURE 1: Release of tritium from (A) [5,6-^{3}H]arachidonic acid and (B) [5,6-^{3}H]PGH_2 as a function of time. The incubations were carried out as described under Experimental Section except that the reaction was terminated at the indicated time. Swine aortic microsomes (312 μg of protein) (●). Swine aortic microsomes (312 μg of protein) and sheep seminal vesicular microsomes (41 μg of protein) (▲). Sheep seminal vesicular microsomes alone released about the same amount of tritium as did swine aortic microsomes alone during 5 min of incubation.

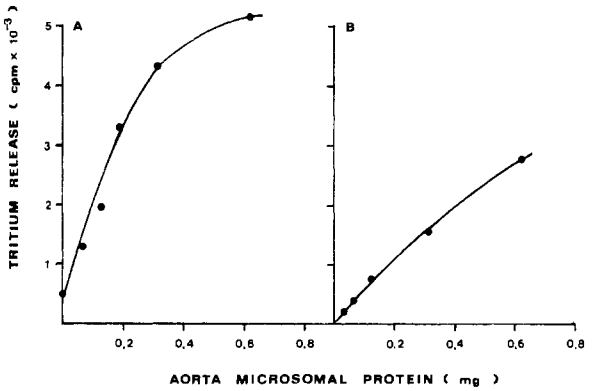


FIGURE 2: Release of tritium from (A) [5,6-^{3}H]arachidonic acid and (B) [5,6-^{3}H]PGH_2 as a function of swine aortic microsomal protein. The incubations were carried out as described under Experimental Section except that the amount of swine aortic microsomal protein was varied as indicated. Sheep seminal vesicular microsomes (41 μg of protein) were added as an enriched source of cyclooxygenase to generate PGH_2 when [5,6-^{3}H]arachidonic acid was used as an indirect substrate.

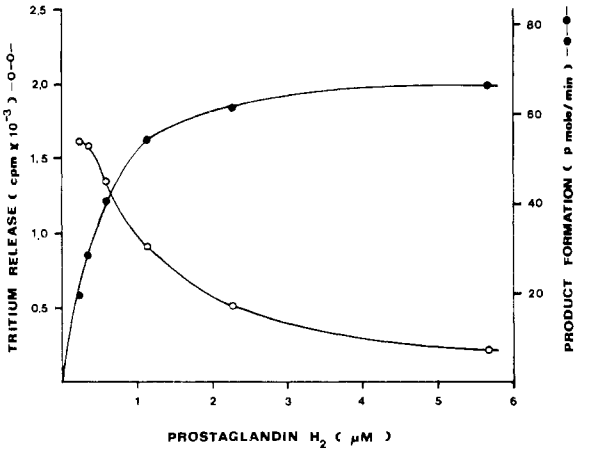


FIGURE 3: Effect of unlabeled PGH_2 concentrations on the rate of tritium release from [5,6-^{3}H]PGH_2. The incubations were carried out as described under Experimental Section except that unlabeled PGH_2 concentrations were varied as indicated and a smaller incubation volume (0.1 mL) was used. Reaction was terminated with 1 mL of 5% trichloroacetic acid. The amount of swine aortic microsomal protein used was 31.2 μg. Calculation of product formation was based on the assumption that no kinetic isotope effect was observed in the removal of C-6 tritium from [5,6-^{3}H]PGH_2.

purification by preparative thin-layer chromatography, IV was sent to New England Nuclear Corp. for catalytic reduction with tritium gas over Lindlar's catalyst. The resulting [5,6-^{3}H]arachidonic acid was purified by column chromatography by using a 7% diethyl ether–Skelly B elution system (Sih & Takeguchi, 1973) to give pure [5,6-^{3}H]arachidonic acid with a specific activity of 120 mCi/mmol which was used for all our subsequent studies.

Figure 1 shows the kinetics of tritium release from C-6 after incubation of [5,6-^{3}H]PGH_2 or [5,6-^{3}H]arachidonic acid with swine aortic microsomes. When PGH_2 was used as the substrate for prostacyclin synthetase, the reaction had a fast onset and reached a plateau within 1–2 min. Although the time course did not appear to be entirely linear for any given time period, we arbitrarily selected a 1-min incubation time for our subsequent assays. When PGH_2 was generated in situ by using arachidonic acid as the indirect substrate, the rate of tritium release was considerably slower. This suggests that the rate-limiting step in the overall biosynthesis of prostacyclin from arachidonic acid is the formation of the prostaglandin endoperoxides (PGG_2 and PGH_2). As expected, when pig aortic microsomes were fortified with ram seminal vesicle microsomes, a rich source of fatty acid cycloxygenase, the rate of tritium release was greatly enhanced.

The effect of aortic microsomal protein concentration on the rate of tritium release is shown in Figure 2. A linear relationship between the rate of tritium release and swine aortic microsomal protein up to 300 μg of protein was observed by using either PGH_2 generated in situ from arachidonic acid with ram seminal vesicular microsomes or PGH_2 directly as substrate. At higher protein concentration, substrate became rate limiting and deviation from linearity was observed.

Addition of increasing concentrations of unlabeled PGH_2 decreased the amount of radioactivity released from a constant quantity of labeled PGH_2 by dilution (Figure 3). The rate of conversion of PGH_2 into product when plotted against the substrate concentration exhibited a typical hyperbolic substrate saturation curve. The K_m value estimated from the double reciprocal plot gave a value of 1 μM.

Two tritium atoms are present in [5,6-^{3}H]arachidonic acid or [5,6-^{3}H]PGH_2. The C-6 tritium is released into the medium during prostacyclin formation whereas the C-5 tritium is retained in PGI_2 and its hydrolyzed product 6-keto-$PGF_{1\alpha}$. Accordingly, the quantity of tritium released into the medium should equal the corresponding amount of [5-^{3}H]-6-keto-$PGF_{1\alpha}$ formed. Figure 4 clearly shows that the quantity of tritium released from [5,6-^{3}H]arachidonic acid catalyzed by a combination of ram seminal vesicular and swine aortic

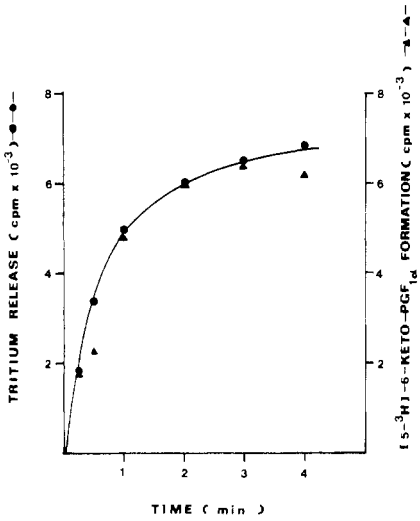


FIGURE 4: Correlation of C-6 tritium release and [5-^{3}H]-6-keto-$PGF_{1\alpha}$ formation from [5,6-^{3}H]arachidonic acid. [5,6-^{3}H]Arachidonic acid (20 nmol, 200 000 cpm), DL-isoproterenol (0.5 μmol), hemoglobin (0.5 nmol), swine aortic microsomes (312 μg of protein), and sheep seminal vesicular microsomes (41 μg of protein) in 0.5 mL of 0.05 M Tris-HCl, pH 7.5, were incubated at 37 °C and the reaction was terminated at the indicated time by acidification with 0.02 mL of 1 N HCl. The reaction mixture was extracted with two 2-mL portions of ethyl acetate. The dried extract was mixed with 50 μg of 6-keto-$PGF_{1\alpha}$ and chromatographed on a silica gel G plate (2 × 20 cm) developed in ethyl acetate–acetic acid–isooctane–water (11:2:5:10). The 6-keto-$PGF_{1\alpha}$ region was localized by iodine vapor and scraped off for radioactivity measurement. The aqueous layer was treated with trichloroacetic acid and charcoal suspension as described under Experimental Section. The supernatant after centrifugation was decanted and counted for radioactivity. Corrections for quenching and extraction were made before plotting the results.

microsomes coincided nicely with the amount of [5-^{3}H]-6-keto-$PGF_{1\alpha}$ produced in the time course studies of cyclooxygenase and prostacyclin synthetase catalyzed reactions. The determination of the amount of tritium release can therefore represent the quantity of radioactive prostacyclin biosynthesized.

Further confirmation of the validity of this enzymatic assay method was achieved by using inhibitors. Addition of 15-hydroperoxyarachidonic acid or tranylcypromine, inhibitors of prostacyclin synthetase, inhibited the release of tritium from [5,6-^{3}H]PGH_2 in a dose-dependent fashion as illustrated in Figure 5. The concentrations of 15-hydroperoxyarachidonic acid and tranylcypromine which gave 50% inhibition were estimated to be 0.7 μM and 1.35 mM, respectively.

Discussion

The published methods of assaying prostacyclin synthetase activity include the bioassay of the appearance of vasodilating or antiaggregatory substances (Moncada et al., 1976), immunochemical detection of the stable hydrolysis product, 6-keto-$PGF_{1\alpha}$ (Salmon, 1978; Tai & Tai, 1978), and the use of labeled substrate combined with thin-layer chromatography (Salomon et al., 1978). The bioassay requires tedious setup of tissue strips and lacks the desired specificity. Although the immunochemical method provides greater sensitivity and specificity, it requires the generation of antisera of high specificity and tedious manipulations of assay procedures. The labeled substrate assay requires cumbersome chromatographic separation procedures, and it has been difficult to completely separate 6-keto-$PGF_{1\alpha}$ from $PGF_{2\alpha}$. The radioactive assay method described herein offers both simplicity and specificity. The released tritium could be easily separated from unchanged labeled substrate by charcoal adsorption and precipitation of

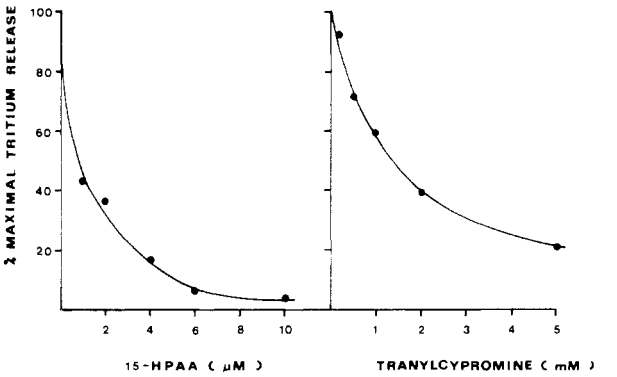


FIGURE 5: Effect of 15-hydroperoxyarachidonic acid (15-HPAA) and tranylcypromine on swine aortic prostacyclin synthetase activity. Enzyme was assayed as described under Experimental Section except that (A) 15-HPAA or (B) tranylcypromine was also included in the assay at the indicated concentrations. The amount of swine aortic microsomal protein used was 312 μg.

the latter. Enzymic or nonenzymic transformations of the substrate PGH_2 to the classical prostaglandins, thromboxanes, and HHT will not interfere with this assay, for the latter compounds are also adsorbed by charcoal. Furthermore, the substrate is only tritiated at C-5 and C-6 and there is no tritium loss at either position during any of the above transformations. The removal of tritium at C-6 is unique to prostacyclin synthetase and thus confers the desired specificity to this assay procedure.

The validity of this assay method was confirmed by the observations that the release of tritium at C-6 is both time dependent and enzyme protein dependent; the amount of radioactivity released into the medium from C-6 of the substrate agreed well with the amount of [5-^{3}H]-6-keto-$PGF_{1\alpha}$ formed. Also, the K_m for PGH_2 and I_{50} for 15-hydroperoxyarachidonic acid, a specific inhibitor of prostacyclin synthetase, agreed well with the values obtained by other assay methods using [1-^{14}C]PGH_2 combined with thin-layer chromatography as reported by Salmon et al. (1978).

This assay provides a rapid, sensitive, and specific means of measuring prostacyclin synthetase activity in crude enzyme preparations and should be quite suitable for monitoring enzyme activity during solubilization and purification of the enzyme. Also, this method may be useful in studies dealing with possible alteration of enzyme levels in various diseased states and during developmental changes, enzyme turnover, and regulation induced by various hormonal and pharmacological agents. However, the assay method may not be suitable for determination of enzyme acitivity in crude homogenates using arachidonic acid as an indirect substrate since the specific activity of substrate may vary significantly in different preparations because of the dilution of the isotope by endogenous pools of arachidonic acid. Therefore, successful application of the assay using arachidonic acid as an indirect substrate may require preparation of the microsomal fraction, whereas the assay using PGH_2 as substrate may use crude homogenates since any endogenous PGH_2 must have been degraded because of its unstable nature. Attempts to use tritiated arachidonic acid to monitor enzyme activity in prefused organs or in intact cells also require precautions. Not only isotope dilution may occur under these conditions but also catabolism of product prostaglandins via β-oxidation may cause release of tritium. Apparently, the assay method can be applied only to broken-cell preparation.

Although there may be a kinetic isotope effect associated with the removal of C-6 tritium in [5,6-^{3}H]PGH_2, and thus the actual amount of substrate converted into product could

be higher than that was observed, this method should still give a reliable indication of relative enzyme activity.

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Cold-Stable Microtubules from Brain†

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ABSTRACT: When microtubules are polymerized in crude brain extracts, a significant percentage of the microtubules formed do not depolymerize at 0 °C. These cold-stable microtubules are discarded during usual warm-assembly, cold-disassembly microtubule purification schemes and have received little attention. The yield of cold-stable microtubules formed in crude extracts is a function of the $CaCl_2$ concentration present during initial homogenization. Homogenization of mouse brains in buffer containing 1 mM EGTA resulted in a low yield of cold-stable microtubules (less than 10% of the total microtubule assembly, as determined by viscometry), while initial homogenization in 2 mM $CaCl_2$ increased the yield to 47% or higher. Cold-stable microtubules were purified by two cycles of polymerization and depolymerization using Ca^{2+}, instead of low temperatures, to depolymerize the cold-stable microtubules. The protein composition of the purified cold-stable microtubules was not distinguishable from that of cold-labile microtubules on the basis of gel electrophoresis and isoelectric focusing. The two types of microtubules were also not distinguishable by negative-stain electron microscopy. The cold stabilization of brain microtubules may be determined, at least in part, by a low molecular weight substance whose binding to microtubule protein is sensitive to Ca^{2+}. Our results indicate that cold stability is conferred upon incorporation of a cold-stabilizing factor (CSF) into microtubules during the polymerization process, and the ratio of the cold-stabilizing factor to total tubulin determines the proportion of cold-stable microtubules which form. The cold-stabilizing factor may serve an important role in the control of neuronal microtubule assembly or function.

Microtubules exhibit considerable variation in stability when exposed to low temperatures, high hydrostatic pressures, or drugs that inhibit microtubule polymerization. For example, outer doublet microtubules of sea urchin flagella are very stable and do not depolymerize at 0 °C or in the presence of colchicine, whereas many microtubules found in the cytoplasm of animal cells depolymerize rapidly at 0 °C or in low concentrations of colchicine (Behnke & Forer, 1967; see Snyder & McIntosh, 1976, for a review). Microtubules with different stabilities also coexist within the same cytoplasm. For example, kinetochore microtubules of PtK_1 rat kangaroo fibroblast spindles are stable at 0 °C, whereas interpolar microtubules of the same spindles depolymerize at this temperature (Brinkley & Cartwright, 1975).

Stable outer doublet microtubules from sea urchin sperm tails do not assemble or disassemble during flagellar beating and apparently function by a doublet-doublet sliding mechanism mediated by dynein cross bridges (Satir, 1968; Summers & Gibbons, 1971). By contrast, spindle microtubules are in a "dynamic equilibrium" with tubulin subunit pools (Inoué & Sato, 1967), and the functions of these microtubules appear to be integrally linked to their assembly and disassembly during the mitotic process (Margolis et al., 1978). Thus, an improved understanding of the chemical bases which underly the stability differences among microtubules might reveal important aspects of microtubule biochemistry which are directly related to their cellular functions.

Variations in the stabilities of microtubules could be due to chemical differences in tubulins. Such differences might be due to distinct tubulin genes responsible for the biosynthesis of unique tubulin molecules or to different forms of post-translational modification of a single tubulin molecule. Also, regulation of microtubule stability could be brought about through interaction of specific molecules with the surface or ends of microtubules. Such interactions could include the simple binding of cofactors to previously assembled microtubules, incorporation of cofactors into microtubules during assembly, or the association of complex enzyme systems with microtubules.

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