SHORT COMMUNICATIONS

BBA 53296

Biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ from tritium-labelled arachidonic acid by rat stomach homogenates

The isolation of prostaglandins in sheep vesicular glands and their biosynthesis in this tissue from essential fatty acids has been well documented[1,2]. Prostaglandin-like substances have been shown to occur in the rat stomach. These substances were also shown to be released in superfusates of the isolated rat stomach and the release was found to be related to the parasympathetic nerve stimulation[3-5]. Substances with properties of prostaglandins were shown to be formed in small amounts when rat stomach homogenates were incubated with a labelled precursor[6]. However, until now these substances have been characterized in this tissue only by (1) their chromatographic properties in comparison with standards, and (2) by their marked biological activity on the fundus of the rat[7]. In the present communication, mass spectral evidence is provided to demonstrate that prostaglandins E_2 and $F_{2\alpha}$ are biosynthesized from tritium-labelled arachidonic acid. Another compound with chromatographic properties similar to prostaglandin E_2, but not destroyed by alkali, was also obtained; preliminary results indicate that it is a prostanoic acid derivative[8].

5,6,8,9,11,12,14,15-[^{3}H]arachidonic acid was synthesized by selective reduction of 5,8,11,14-eicosatetraynoic acid (gift of Dr. J. E. PIKE, The Upjohn Co.) with tritium gas (New England Nuclear) and Lindlar catalyst. The product was purified to a constant specific activity of 0.45 mC/mg (95% radiochemical purity) on columns of silicic acid containing $AgNO_3$ (C. Pace-Asciak and L. S. Wolfe, unpublished results). The stomachs of 21 adult rats (Wistar strain) were rapidly excised, washed with ice-cold buffer (50 mM KH_2PO_4–NaOH, pH 7.4) and homogenized in the presence of substrate tritium-labelled arachidonic acid (210 μg) in 5 volumes ice-cold buffer con-

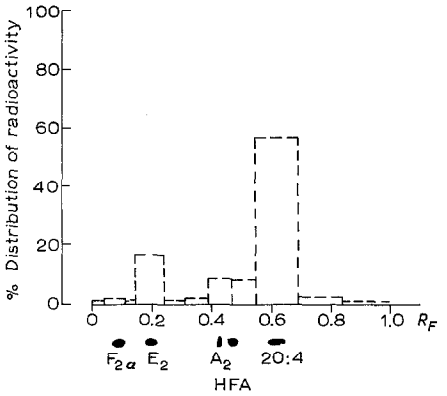

Fig. 1. Thin layer chromatogram of the chloroform–methanol extract of rat stomach homogenate (20 mM EDTA) incubated with [^{3}H]arachidonic acid. Standards of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin A_2 (PGA_2), 12-hydroxy stearic acid (HFA) and arachidonic acid (20:4) are shown.

taining reduced glutathione (56 μg/ml) and hydroquinone (0.57 μg/ml). The homogenate was then incubated at 37° for 30 min in an oxygen atmosphere. Twenty volumes of chloroform–methanol (2:1, by vol.) were added and after filtration, the clear solution was extracted once with one-tenth its volume of 0.03 M HCl, then once with water. Evaporation of the chloroform–methanol phase gave an extract which contained approximately 12% of the added radioactivity migrating on thin layer chromatography as prostaglandin-like compounds (Fig. 1). The extract was then fractionated on a column of silicic acid into three main classes by elution with chloroform, ethyl acetate and methanol. The chloroform eluate contained 80% of the radioactivity as unreacted arachidonic and neutral lipids; ethyl acetate eluted about 18% of the radioactivity migrating on thin layer chromatography as prostaglandin-like material, and methanol eluted negligible radioactive compounds with phospholipids. The ethyl acetate fraction was then purified again on another column of silicic acid using increasing amounts of ethyl acetate in chloroform. In other experiments when biological activity of various fractions was assayed, the major quantity of biological activity was eluted with chloroform–ethyl acetate (1:4, by vol.). Therefore, this fraction was taken and purified further by preparative thin layer chromatography on silica gel G plates into two zones corresponding to the prostaglandin E and F compounds (solvent system, chloroform–methanol–acetic acid–water; 90:9:1:0.65, by vol.). Standards of prostaglandin E_2 (R_F 0.37) and prostaglandin $F_{2\alpha}$ (R_F 0.20) were used. After purification the prostaglandin E-like fraction was found to contain biological activity equivalent to 24 μg prostaglandin E_2 by bioassay on the rat stomach fundus strip. The total sample was treated with a solution of ethanol–1 M KOH (1:1, by vol.) for 90 min at 23° to convert any prostaglandin E_2 present in the sample into the prostaglandin B_2 derivative by the well known procedure of BERGSTRÖM *et al.*[1]. Development of an absorption band at 278 nm was observed equivalent in intensity to the absorption which results when 23 μg of prostaglandin E_2 is treated with alkali. Purification by thin layer chromatography of the alkaline-treated prostaglandin E-like compounds after acidification and extraction gave 17% of the radioactivity spotted migrating as prostaglandin B compounds (R_F 0.59) and 73% as a novel prostanoic acid derivative migrating in the prostaglandin E region. The structure of this compound was shown to be 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid[8]. The specific activity of the prostaglandin B compounds was measured to be 0.040 mC which meant a 10-fold dilution of the original specific activity by endogenous arachidonic acid (after correcting for loss of tritium in converting prostaglandin E_2 into prostaglandin B_2). The prostaglandin B-like material was converted to its methyl ester with ethereal diazomethane. Gas chromatography on a column of 3% SE-30 on gas chrom Q (Applied Science) gave a product with a retention time of 24.2 *C*-value[9] which was identical with the methyl ester of standard prostaglandin B_2.

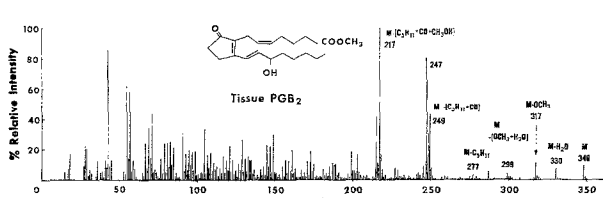

Fig. 2. Mass spectrum of the methyl ester derivative of stomach prostaglandin B_2 (PGB_2).

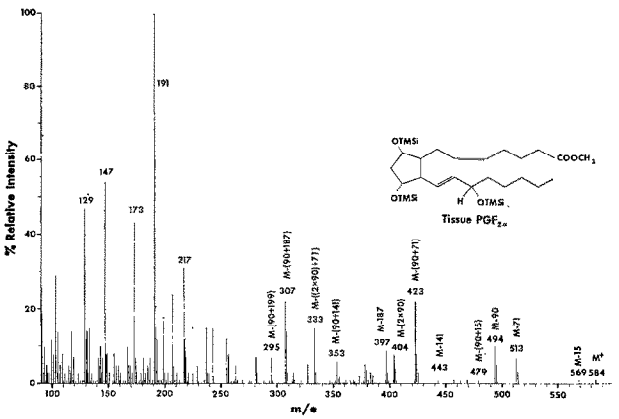

Fig. 3. Mass spectrum of the methyl ester and trimethylsilyl ether derivative of stomach prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

Its mass spectrum (Fig. 2) was identical to that of authentic prostaglandin B_2, proving therefore that prostaglandin E_2 had been formed in the biosynthesis experiment.

The prostaglandin F-like material was converted to its methyl ester derivative and then to the trimethylsilyl ether derivative with Tri-Sil Z. After 5 min at 60°, gas chromatography showed a single peak with a *C*-value of 24.2 as expected of prostaglandin $F_{2\alpha}$ (trimethylsilyl ether derivative of the methyl ester of prostaglandin $F_{1\alpha} = 24.5C$). The mass spectrum (Fig. 3) was identical to that of authentic prostaglandin $F_{2\alpha}$, establishing the isolated product as prostaglandin $F_{2\alpha}$.

The incorporation of radioactivity into prostaglandins E_2 and $F_{2\alpha}$ was negligible ($< 1.0\%$) unless EDTA was added to the buffer (Table I). An even larger amount of radioactivity was obtained in the prostaglandins if the radioactive fatty acid precursor was added to the buffer before the tissue was homogenized indicating a very rapid release of endogenous arachidonic acid into the medium and also indicating the rapidity of the biosynthesis of prostaglandins.

TABLE I

EFFECT OF EDTA (20 mM) ON CONVERSION (%)* OF [^{3}H]ARACHIDONIC ACID INTO PROSTAGLANDINS BY RAT STOMACH HOMOGENATES

Conditions	*Arachidonic acid*	*Hydroxy fatty acids and*		
		Prostaglandin A	*Prostaglandin E*	*Prostaglandin F*
Precursor added to homogenate:				
—	87.3	6.6	0.4	0.2
EDTA	75.8	10.7	3.8	1.2
Precursor added before homogenization:				
—	81.9	10.2	2.4	1.1
EDTA	61.8	13.9	12.8	1.9

* Percent conversion refers to quantity of radioactivity found by thin layer chromatography (silica gel G/chloroform–methanol–acetic acid–water (90:9:1:0.65, by vol.) migrating like standards indicated. It should be noted that prostaglandin E band consists of approx. 17% prostaglandin E_2 with the rest composed of a prostanoic acid derivative (See text).

The formation of prostaglandins E_2 and $F_{2\alpha}$ demonstrates the presence of the prostaglandin synthetase activity in rat stomach. Furthermore, another compound is formed from arachidonic acid, in greater quantity than either prostaglandin E_2 or $F_{2\alpha}$. We have recently shown this compound to be a novel prostanoic acid derivative. The specific activity of the prostaglandin E_2 that is formed demonstrates that a 10-fold dilution in specific activity of added arachidonic acid has taken place by endogenous arachidonic acid and that prostaglandin E_2 is formed from both substrates. Previous experiments with tissue immediately frozen in liquid nitrogen have shown that the level of free arachidonic acid is far too low to account for a 10-fold dilution in the substrate used, therefore the dilution must take place during homogenization and incubation of the tissue[6]. This suggests that an active lipase is present to release polyunsaturated fatty acids, which then act as substrates for prostaglandin synthesis. It has been suggested previously that a specific phospholipase A type hydrolysis of membrane phospholipids might be associated with endogenous prostaglandin formation[4]. It has also been shown that certain natural unsaturated fatty acids that are not precursors of prostaglandins can act as inhibitors to the conversion of arachidonic acid into prostaglandins[10]. It is possible therefore that prostaglandin synthesis might be regulated by the action of a phospholipase which releases from membrane phospholipids certain fatty acids for synthesis and others for control. Hydrolysis of phospholipids as a first step in the synthesis of prostaglandins could be a highly specific mechanism in which only certain membrane phospholipids are involved which contain high proportions of the precursor polyunsaturated fatty acids.

This work was supported by grants to L.S.W. from the Multiple Sclerosis Society of Canada, the Medical Research Council of Canada and the Donner Canadian Foundation. We wish to thank Dr. O. Mamer for recording the mass spectra on an LKB 9000 GC-MS mass spectrometer at the Royal Victoria Hospital, and Dr. J. E. Pike, The Upjohn Co., for supplying us with prostaglandin standards.

Donner Laboratory of Experimental Neurochemistry,
Montreal Neurological Institute,
Montreal (Canada)

C. PACE-ASCIAK
L. S. WOLFE

1 S. BERGSTRØM, R. RYHAGE, B. SAMUELSSON AND J. SJOVALL, *J. Biol. Chem.*, 238 (1963) 3555.
2 B. SAMUELSSON, E. GRANSTRØM AND M. HAMBERG, *Nobel Symposium 2, Prostaglandins*, Almqvist and Wiksell, Stockholm, 1967, p. 31.
3 F. COCEANI, C. PACE-ASCIAK, F. VOLTA AND L. S. WOLFE, *Am. J. Physiol.*, 213 (1967) 1056.
4 F. COCEANI, C. PACE-ASCIAK AND L. S. WOLFE, *Prostaglandin Symposium of the Worcester Foundation for Experimental Biology*, Interscience, 1967, p. 39.
5 A. BENNETT, C. A. FRIEDMAN AND J. R. VANE, *Nature*, 216 (1967) 873.
6 C. PACE-ASCIAK, K. MORAWSKA, F. COCEANI AND L. S. WOLFE, *Prostaglandin Symposium of the Worcester Foundation for Experimental Biology*, Interscience, 1967, p. 371.
7 F. COCEANI AND L. S. WOLFE, *Can. J. Physiol. Pharmacol.*, 43 (1965) 445.
8 C. PACE-ASCIAK AND L. S. WOLFE, *Chem. Commun.*, (1970), 1234.
9 A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 63 (1956) 144.
10 C. PACE-ASCIAK AND L. S. WOLFE, *Biochim. Biophys. Acta*, 152 (1968) 784.

Received September 7th, 1970