Effects of Essential Fatty Acid Deficiency on Prostaglandin Synthesis and Fatty Acid Composition in Rat Renal Medulla

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ABSTRACT

Studies are reported on the capacity of isolated rat renal papilla (inner medulla) to synthesize and release prostaglandin (PG) E from endogenous and exogenous precursor(s) during development of an essential fatty acid (EFA) deficiency in the rat. Weanling (21-day-old) male Sprague-Dawley rats were fed a fat-free diet supplemented with either 5% hydrogenated coconut oil (HCO) or 5% safflower oil (SO). At approximately 3, 6 and 7 weeks (6, 9 and 10 weeks of age), groups of animals fed each diet were killed for studies of PGE synthesis in the renal papillae. Differences in the fatty acid composition of the papillae lipids of the animals of each group were also determined. The in vitro production of PGE from endogenous precursor(s) was significantly reduced in the papillae from the 6-week-old rats fed the HCO diet compared to the control (SO) rats, and appeared to be near maximally depressed in the 10-week-old animals compared to that of animals fed an EFA deficient diet for over a year in an accessory experiment. Analyses of the fatty acids of the papillae lipids of the HCO groups showed that the levels of 18:2 and 20:4 were markedly reduced, and those of 16:1, 18:1 and 20:3 were elevated compared to the controls even in the 6-week-old animals, typical of an EFA deficiency. The papillae lipids of the animals fed the HCO diet were also depleted of their stores of 22:4ω6. A fatty acid believed to be derived by chain elongation of 20:3ω9, 22:3, was found in large concentrations in the papillae triglycerides of the EFA deficient rats. Incubations of exogenous arachidonic acid (20:4) in homogenates and tissue slices of the papillae of the HCO dietary groups showed that the PG synthetase was not impaired by an EFA deficiency. The rate of PGE synthesis in the papillae of the EFA deficient animals was generally enhanced when exogenous 20:4 was added, indicating that the concentration of available precursor(s) is a primary factor in the control of PGE synthesis in the papilla of the rat.

INTRODUCTION

Since the discovery that the kidney has a relatively high capacity to synthesize prostaglandins (PGs), there has been considerable interest in the role of renal PGs in kidney function and their possible involvement in blood pressure regulation (1). Several investigators have attempted to alter the course of experimental hypertension in rats (2,3) by chronic administration of nonsteroidal anti-inflammatory drugs such as indomethacin which block PG synthesis (4). However, due to the diverse actions (1) of these compounds and their toxicity to the gastrointestinal tract of rats, the conclusions of such studies might be open to question. Alternatively, essential fatty acid (EFA) deficiency, which has been shown to reduce PG synthesis in rabbit kidney (5), might serve as a useful model for studying potential roles of PGs in renal function. Rosenthal and coworkers (6) noted that hypertension was induced by high salt intake in rats fed an EFA deficient diet, but the degree of EFA deficiency and its effect on renal PG synthesis was not reported.

As recently summarized (7, and refs. therein), EFA deficiency has variable effects on the degree of depletion of PG precursors and PG synthesis depending on the tissue examined. For example, brain levels of arachidonic acid (20:4) and synthesis of PGF_{2α} by brain slices were only marginally affected by EFA deficiency in rats (7). Rat lung, small intestine (8) and kidney medulla (9) of rats fed an EFA deficient diet have been reported to give enhanced synthesis of PGs when supplied with exogenous 20:4; however, the decrease in endogenous PG synthesis relative to fatty acid composition of the tissues was not reported.

Although previous studies have shown that renal medullary lipid droplets (10,11) and medullary tissue (12) contain large amounts of triglycerides which are rich in polyunsaturated fatty acid PG precursors, the temporal relationship of the expected depletion of these fatty acids by EFA deficiency to renal PG synthesis has not been reported. In the present study, the synthesis of PGE in the renal papillae of rats fed a semisynthetic diet devoid of EFA was examined relative to changes in fatty acid composition.

MATERIALS AND METHODS

Animals

Littermate male Sprague-Dawley rats were
obtained (BioLab Corp., St. Paul, MN) at 21 days of age, divided into two groups and fed a basal fat-free diet supplemented with either 5% by weight safflower oil (SO) or 5% hydrogenated coconut oil (HCO). The composition of the basal fat-free diet was based on that described previously (13) with the exception that Williams-Briggs modified mineral mix (Teklad, Madison, WI) was employed rather than Wesson mineral mix. The complete diet was prepared in small fresh lots by adding HCO or SO (Teklad), vitamins A and E (ICN Pharmaceutical, Inc., Cleveland, OH) and vitamin D (Nutritional Biochemicals Corp. Cleveland, OH) in ether to the dry basal diet (Teklad test diet 76244). After placing the rats on the test diets, animals were killed at weekly intervals to determine the capacity for renal PGE synthesis from both endogenous and exogenous substrate. The kidneys were removed from anesthetized (sodium pentobarbital, 50 mg/kg) rats and rapidly chilled in ice-cold saline. The inner medulla (papilla only) was excised, weighed and incubated under different conditions as described below. In all experiments the papillae were preincubated in physiologic buffered saline medium (PBS) at 4 C for 5 min, and this medium discarded prior to 37 C incubations.

For comparison with the above experiments, animals fed similar diets for 51-63 weeks in another experiment were also used in this study.

Incubation of Renal Papillae

Intact papillae. Each papilla was halved lengthwise and incubated with gentle vortexing (Buchler Evapo-Mix) in 1 ml of preoxygenated PBS (Tyrodes) at 37 C for 15 min after which the PBS was removed and acidified (ca. pH 3) with formic acid.

Tissue homogenates. Upon completion of the above incubation, the tissue was homogenized in a glass microhomogenizer for 1 min with cold preoxygenated PBS (1 ml), and an aliquot incubated as such at 37 C for 15 min as above. Another aliquot was similarly incubated following addition of sodium arachidonate (Nu-Chek Prep, Elysian, MN, > 99% pure). These incubations were terminated by addition of 7 vol. of chloroform-methanol (1:1).

Tissue slices. Papillae were sliced (1 mm) with a H. Mickle chopper, preincubated as described above, followed by 5 successive 30 min incubations in 2 ml PBS (Krebs) medium in an atmosphere of 95% O2-5% CO2 with gentle shaking in a metabolic shaker. In these incubations, the medium was removed after each 30 min period and replaced with fresh prewarmed and oxygenated PBS. Sodium arachidonate was added at the beginning of the fifth incubation.

Extraction and Measurement of PGE

The acidified PBS from the intact papilla incubations was extracted with petroleum ether (3 x 2 vol, 30-60 C bp) after which acidic lipids were extracted into ethyl acetate. The chloroform-methanol extract of homogenates was evaporated to dryness, dissolved in PBS (pH 7.5) and extracted with petroleum ether. The aqueous phase was then acidified with formic acid and extracted with petroleum ether and ethyl acetate as above. Prostaglandin E in the extracted lipid was measured following its base-catalyzed conversion to PGB (14) by a high performance liquid chromatographic (LC) method. The development of the LC method and its verification by independent measurements of PGE in renal tissue has been described previously (15). Briefly, PGB is separated from other PGs and lipids on a silicic acid LC column and simultaneously quantitated by UV absorbance at 280 nm employing a LDC model 1280 UV photometer with an 8 µl flow cell. In the present study, PGs were separated with a 25 cm, 10 µ Varian SI60 column eluted at 0.4 ml/min (ISCO model 384 pump) with chloroform-acetonitrile-formic acid (87.3:12:0.7; v/v/v). Peak heights were measured for quantitation of the extracted PGB relative to a PGB standard prepared from authentic PGE2 (Upjohn, Kalamazoo, MI) as previously described (15). Inasmuch as this method does not distinguish between PGE1 and PGE2, the PG measured is referred to as PGE. Prostaglandin A, if present in the extracts, would also be converted to PGB and be measured by the LC method; however, this PG was not found in amounts exceeding 10% of the PGE synthesized by rat papillae (15).

Fatty Acid Analysis

The fatty acid composition of the triglyceride and polar lipid fractions isolated by thin layer chromatography (TLC) (16) from papillae lipid extracted with chloroform-methanol (2:1, v/v) was determined by gas liquid chromatography (GLC) of methyl esters prepared by transesterification with methanol using HCl as a catalyst (17). GLC was carried out with a Barber Coleman gas chromatograph equipped with a flame ionization detector and a 6' x 1/4” glass column packed with 15% FGS on gas chrom P, 100-200 mesh (Applied Science Lab., Inc., State College, PA), at 180 C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min and the percentage composition was calculated from the proportions of the peak areas by an automatic digital integrator.