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SYNTHESIS OF PROSTAGLANDIN E_2 AND PROSTAGLANDIN $F_{2\alpha}$ BY TOADFISH RED BLOOD CELLS

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SUMMARY: Saline washed red blood cells of the toadfish convert [1-^{14}C] arachidonic acid to products that cochromatograph with prostaglandin E_2 and prostaglandin $F_{2\alpha}$. This synthesis is inhibited by indomethacin (10 µg/ml). Conversion of arachidonic acid to prostaglandin E_2 was confirmed by mass spectrometry. When saline washed toadfish red blood cells were incubated with a mixture of [1-^{14}C]-arachidonic acid and [5,6,8,9,11,12,14,15,-^{3}H]-arachidonic acid, comparison of the isotope ratios of the radioactive products indicated that prostaglandin $F_{2\alpha}$ was produced by reduction of prostaglandin E_2. The capacity of toadfish red blood cells to reduce prostaglandin E_2 to prostaglandin $F_{2\alpha}$ was confirmed by incubation of the cells with [1-^{14}C] prostaglandin E_2.

The capacity to convert arachidonic acid to prostaglandins is widely distributed in mammalian tissues. An exception is the mammalian erythrocyte which appears to be devoid of cyclooxygenase activity (1). Recent evidence indicates the presence of cyclooxygenase in tissues of teleosts and other vertebrate classes (2,3). We now report that, in contrast to mammalian erythrocytes, saline washed red cells of the toad fish (Opsanus tau) have a high capacity to convert exogenous radiolabeled arachidonic acid to prostaglandin E_2 and to prostaglandin $F_{2\alpha}$.

METHODS

[1-^{14}C]-arachidonic acid (55 mCi/mmol), [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (78 Ci/mmol), and [1-^{14}C]-prostaglandin E_2 (40 mCi/mmol) were obtained from New England Nuclear (Boston, MA). Unlabeled arachidonic acid was purchased from Nuchek Prep (Elysian, MN). Authentic prostaglandin standards were the generous gift of Dr. John Pike of the Upjohn Co. (Kalamazoo, MI). Adult toadfish were purchased from the Marine Biological Laboratory (Woods Hole, MA).
methanesulfonate (Ayerst) and blood was collected from the caudal artery into a heparanized syringe. Plasma was removed after centrifugation, and the blood cells were washed three times with 0.01M Tris buffer, pH 7.4, containing 0.9% NaCl and resuspended in the same buffer. In some experiments, a more complete separation of erythrocytes from cocentrifuging leukocytes was achieved by layering the blood over an equal volume of Ficoll-Paque (Pharmacia). When

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about half of the blood cells had settled, the supernatant cells and buffer were removed, the bottom layer washed three times with Tris-saline buffer, and the cells were resuspended in the same buffer.

Aliquots of saline washed toadfish red cells were incubated in Tris-saline buffer with [^{14}C]-arachidonic acid, a mixture of [^{14}C]- and [^{3}H]-arachidonic acid, or [^{14}C]-prostaglandin E_2 at 37°C with shaking. At the end of the incubation, cells were centrifuged, and the supernatant medium was drawn off, acidified with formic acid (final concentration 1%), and extracted three times with an equal volume of ethyl acetate. After evaporation of the combined organic layers under a stream of nitrogen, the residue was redissolved in 50 µl methanol and applied to silica gel G/hy plates (0.25 mm; Brinkmann), which were developed with the solvent system: ethyl acetate: iso-octane: acetic acid: water (110/55/20/100; upper phase). Radioactivity was localized by radiochromatogram scanning (Packard 7220). In double isotope experiments, radioactive zones were scraped from the plate, suspended in 10 ml of Scintiverse (Fisher), and radioactivity was determined by scintillation counting using a double isotope program (Beckmann LS7500).

For determination of mass spectra, toadfish red cells were incubated with unlabeled arachidonic acid (50 µg/ml). After removal of the cells by centrifugation, the supernatent medium was desalted with a column of octadecylsilane (6 ml; Baker) as described by Powell (4). Prostaglandins were eluted with methyl formate. After evaporation of the solvent under a stream of nitrogen, the residue was treated successively with ethereal diazomethane, (15 min room temperature) methylhydroxylamine hydrochloride in pyridine (18 hr at 55°C), and N,O-bis (trimethylsilyl) trifluoroacetamide (3 hr at 55° C) and analyzed by gas chromatography-chemical ionization mass spectrometry using a column of 3% OV-1 (235°C) fitted to the inlet of a Finnegan 3200 Mass Spectrometer.

RESULTS AND DISCUSSION

When [1-^{14}C]-arachidonic acid was incubated with a Tris-saline suspension of human red blood cells, no detectable conversion to radioactive products occurs (Fig 1A), consistant with the reported inability of mammalian erythrocytes to generate prostaglandins (1). In contrast, when [1-^{14}C] arachidonic acid is incubated with saline washed toadfish red cells, the radioactive fatty acid is cleared from the medium by the cell suspension and radioactive products appear that cochromatograph with prostaglandin E_2 and prostaglandin $F_{2\alpha}$ (Fig 1B). Indomethacin (10 µg/ml) prevents formation of radioactive products, but uptake of [^{14}C]-arachidonic acid by the cells (data not shown).

To determine whether prostaglandin $F_{2\alpha}$ was derived by reduction of prostaglandin E_2 or by direct reduction of the endoperoxide precursor, prostaglandin H_2, aliquots of toadfish red cells were incubated with a mixture of [1-^{14}C]-arachidonic acid and [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (5). Since ^{3}H is lost in synthesis of prostaglandin E_2, this product will be

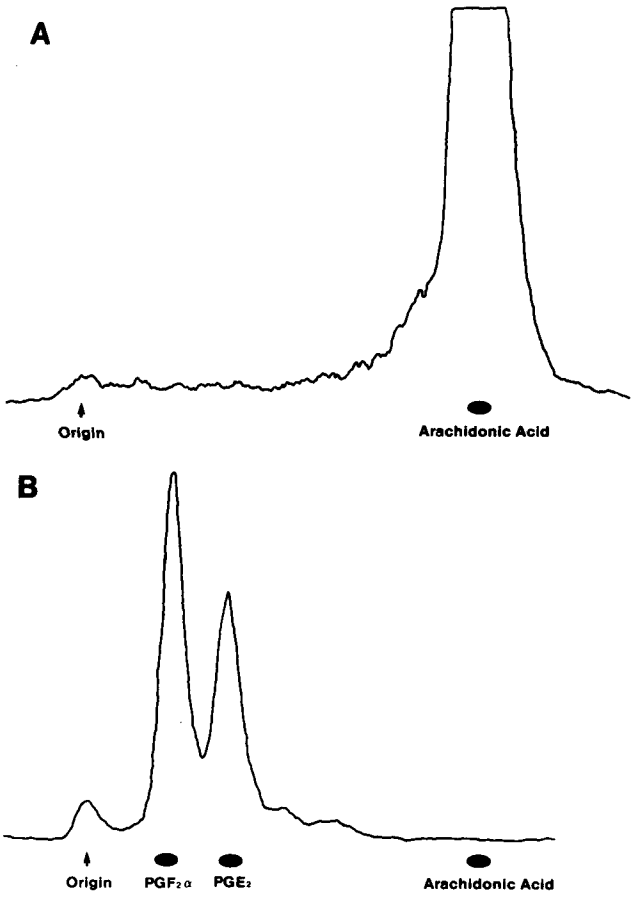

Figure 1. Radiochromatogram scans of products recovered from the medium after incubation of [1-^{14}C]-arachidonic acid (1μCi) with Tris-saline washed human (A) or toadfish (B) red blood cells for 1 hr at 37°C. Aliquots of red blood cells represented the red cell content of 5 ml (A) or 1 ml (B) or original blood volume.

enriched in ^{14}C relative to the starting material (by 1.14, or greater if there is an isotope effect in removal of ^{3}H). If prostaglandin $F_{2\alpha}$ is produced by reduction of prostaglandin E_2, it will also be enriched in ^{14}C. Since no carbon-tritium bonds are broken in the synthesis of prostaglandin H_2 or reduction of prostaglandin H_2 to prostaglandin $F_{2\alpha}$, the latter process will yield prostaglandin $F_{2\alpha}$ with the same isotope ratio as the substrate mixture, or enriched in tritium if there is an isotope effect in the synthesis of prostaglandin E_2, favoring withdrawal of ^{14}C from the endoperoxide pool (reference 5). Since both prostaglandin E_2 and prostaglandin $F_{2\alpha}$ produced

Table 1
$^{14}C/^{3}H$ Ratio, Relative to Starting Material, of Products of Incubation of Arachidonic Acid with Toadfish Red Blood Cells (N=4)

	Prostaglandin E_2	Prostaglandin $F_{2\alpha}$
Found	1.19±0.04	1.12±0.01
Expected	$\geq$ 1.14	

by the toadfish red cells were enriched in ^{14}C (Table I), it appears that the latter product is primarily a metabolite of prostaglandin E_2 in these cells.

Consistant with this finding, intact toad fish red cells are able to progressively reduce a pool of exogenous [$1^{4}C$]-prostaglandin E_2 to prostaglandin $F_{2\alpha}$ (Fig 2). Human erythrocytes contain an NADPH-linked reductase that converts prostaglandin E_2 to prostaglandin $F_{2\alpha}$ (6), but the intact cells are unable to effect reduction of exogenous prostaglandin E_2 (7), perhaps because of the inability of the latter to gain access to the cytoplasmic enzyme (8). Evidently the same permeability barrier does not prevent metabolism of exogenous prostaglandin E_2 by the intact toadfish red cell.

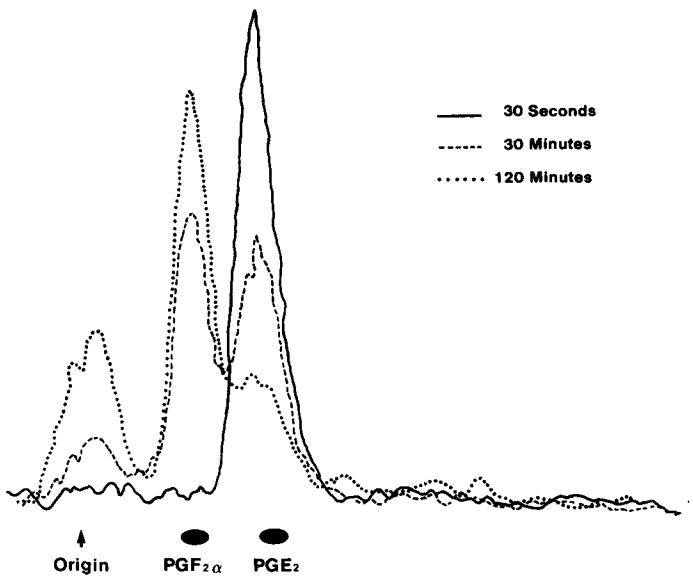

Figure 2. Radiochromatogram scans of products recovered from the medium after incubation of [1-^{14}C]-prostaglandin E_2 (0.1 μCi) with aliquots of toadfish red blood cells for 30 sec (-), 30 min (---), or 120 min (···) at 37°C. Aliquots of red blood cells corresponded to the red cell content of 1 ml of original blood volume.

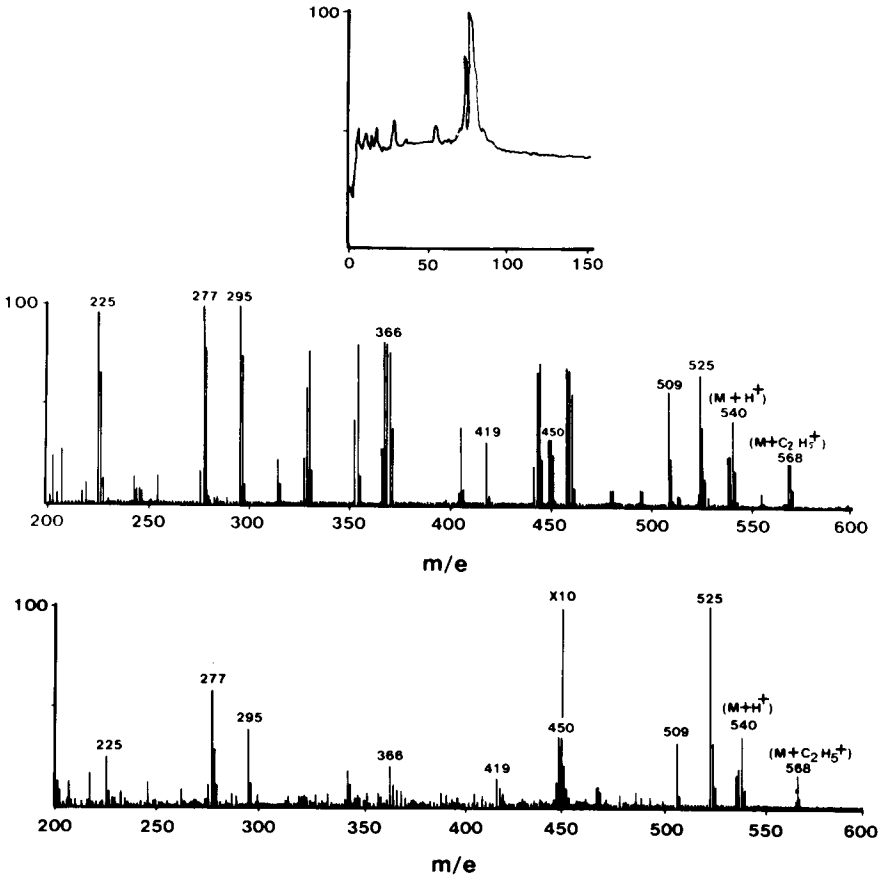

Figure 3. Chemical ionization (CH_4) - mass spectrum (middle panel) of the major product recovered from the medium after incubation of arachidonic acid (167 μM) with toadfish red blood cells corresponding to the red cell content of 10 ml of original blood volume. The mass spectrum of authentic prostaglandin E_2 (lower panel) is shown for comparison. The toadfish red cell metabolite eluted from the 3% OV-1 cloumn in 77 seconds (upper panel). Retention time of prostaglandin E_2 standard was 77 seconds.

To confirm that the toadfish erythrocytes convert arachidonic acid to prostanoid products, red cell suspensions were incubated with unlabelled arachidonic acid at a higher concentration (50 μg/ml) and the products analyzed by gas chromatography-chemical ionization mass spectrometry. Only one major product was detected, whose elution time from the OV-1 inlet column and chemical ionization mass spectrum, were characteristic of prostaglandin E_2 (Fig 3). No prostaglandin $F_{2\alpha}$ was detected in these experiments; it is possible that the capacity of the red cell to generate this product is limited, and saturated at the high substrate concentration employed.

The significance of this metabolic capacity of circulating toadfish erythrocytes, either for the red cell itself or for cardiovascular functions in the fish, remain to be determined. It is of interest that human erythrocytes undergo a measurable change in osmotic fragility after exposure to very low concentrations of prostaglandins (9). Prostaglandin E_2 is known to modulate the activity of adenylate cyclase in a variety of tissues, including the rat erythrocyte (10,11). In any case, the generalization that red blood cells lack the capacity to generate prostaglandins cannot be applied to all species.

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