PROSTAGLANDIN PRODUCTION BY THE KIDNEY OF THE GOLDFISH (CARASSIUS AURATUS)

J. Lowenstein and J. Zadunaisky (with the technical assistance of Ben Lowenstein). Departments of Medicine and Physiology, NYU Medical Center, New York, N.Y.

The kidney is one of the most active organs in the production of prostaglandins. The wide range of prostaglandins, leukotrienes, and epoxygenase products synthesized by the kidney (Schlondorff and Ardaillou, Kidney Int. 29:108-119, 1986) and the known vasoactive properties of many of these eicosanoids suggest a possible role for renal prostaglandins in the control of the circulation of the kidney (Dworkin, Ichikawa and Brenner, Amer J. Physiol. 244:F95-F104, 1983). It has been observed that sea water adaptation in marine teleosts is associated with a marked reduction in glomerular filtration rate (Brown et al, Pflugers Archiv 377:101-108, 1978). We (Bull MDIBL 25:66-69, 1985) and others (Elger and Hentschel, Cell Tissue Res. 220:73-85, 1981) have reported that adaptation of the goldfish (Carassius auratus) to dilute sea water is associated with a marked reduction in the number of identifiable glomeruli in renal tissue. We sought to examine the possibility that the renal hemodynamic responses to sea water adaptation in Carassius auratus might be mediated by an alteration in renal prostaglandin synthesis.

Methods: Goldfish, weighing 127 to 186 grams, were maintained in fresh water ([Na+]=1.0 mEq/l, [K+]=0.8 mEq/l) in a large aerated tank. Sea water adaptation was accomplished by transfer directly into 1/3 sea water ([Na+]=143 mEq/l, [K+]=3.0 mEq/l). All fish tolerated this transfer and were studied 24-28 days after sea water adaptation. After pithing, the kidneys were removed, dissected free of adherent loose connective tissue, and homogenized in 2-3 ml of iced Ringer's solution in a glass tube with a teflon plunger (5-8 strokes). Aliquots of the crude homogenate (average protein concentration, 7mg/250 ul) were incubated in a final volume of 500 ul with either $^{3}H$- arachidonic acid (83.8 Ci/mmole, New England Nuclear) or $^{14}C$-eicosapentanoic acid (463.4 Ci/mmole, New England Nuclear), in the presence of reduced glutathione (1.25 mM). Parallel incubations were carried out with homogenate which had been heated to 100°C for 10 minutes and homogenates to which were added either indomethacin (0.4-1.0 mM in ethanol) or nordihydroguaiaretic acid, NDGA, (0.05 mM in ethanol). Incubations were carried out at room temperature with gentle mixing for 30 minutes and were terminated by the addition of 25 ul 1N HCl. The incubation mixture was extracted with 1.5 ml ethyl ether and centrifuged at 7000 rpm in a refrigerated centrifuge. An aliquot of the supernatant layer was taken for scintillation counting (Optifluor, Packard Instrument Co.); the remainder was blown to dryness in a stream of nitrogen, taken up in ethyl ether and spotted on silica gel plates (Eastman Kodak, Rochester, NY) for chromatographic separation. A mixture of authentic prostaglandins consisting of 6 keto PGF1 , PGF2 , PGE2 and thromboxane B2 was spotted on each chromatographic plate as a reference. In some instances radiolabelled

hydroxyeicosatetraenoic acids (HETE) and leukotriene $B_4$ (LTB4) were cochromatographed as references.

Ascending thin layer chromatography was carried out using the organic phase of a solvent system consisting of ethyl acetate, isooctane, acetic acid and water (11:5:2:10 System B) or a solvent system consisting of benzene, diethyl ether, ethanol, and acetic acid (50:49:2:0.2 System G) as described by van Praag and Farber (Prostaglandins, Leukotrienes and Medicine 12:29-47, 1983). After drying, the reference lane of the chromatogram was developed by brief exposure to iodine vapor and the remainder of the chromatogram was sprayed with Enhance$^R$ (New England Nuclear) and exposed to Kodak XAR-2 film for 7-14 days. After identification of areas corresponding to the authentic prostaglandin standards or to radioactive leukotriene or HETE standards, the chromatogram was cut into segments approximately 1 sq cm, placed into scintillation vials and shaken briefly with 7 ml of Optifluor. Scintillation counting was performed and the quantity of each metabolite formed was expressed as a percent of the total radioactivity.

Results: Solvent system B yielded a clear separation of the prostaglandin standards, LTB4, and 5-HETE; the migrations 12- and 15-HETE were almost identical (Fig 1). Solvent G did not separate the prostaglandins which remained at the origin, but allowed identification of the lipoxygenase products and a clear separation of unreacted substrate (arachidonic acid or eicosapentanoic acid) and labelled fatty acids which migrated more rapidly (Fig 2).

Kidney homogenates derived from goldfish in fresh water produced a variety of radioactive metabolites. The overall pattern of incorporation of substrate into radioactive metabolites appeared to be similar with $^3$H-arachidonic acid and $^{14}$C- eicosapentanoic acid. This finding confirms the report of Herman, Zimmerman, and Doolittle (Gen. and Comp. Endo. 54:478-485, 1984) that goldfish heart homogenate utilized both arachidonic acid and eicosapentanoic acid as substrates for prostaglandin synthesis. Comparison of the migration with authentic prostaglandin standards and radioactive leukotriene standards suggests that the predominant eicosanoids were products of the lipoxygenase pathway. This was confirmed by the observation that while as much as 1.0 mM indomethacin, an inhibitor of cyclooxygenase, had little effect on the production of radioactive metabolites, NDGA, an inhibitor of lipoxygenase, reduced the incorporation of radioactive substrate into metabolites which comigrated with LTB4, 5-HETE, 12-HETE, and 15-HETE and increased the proportion of unreacted substrate.

The preliminary findings reported here do not permit us to conclude whether sea water adaptation is attended by a shift in the pattern of renal prostaglandin or leukotriene synthesis in the goldfish. The overall incorporation of radioactive substrate into prostaglandins and eicosanoids appears to be greater in sea water adapted fish as evidenced by a smaller proportion of the


Fig. 1 Silica gel chromatogram (Solvent B)
The markers at the left indicate the positions, in ascending order, of authentic 6 keto $PGF_1\alpha$, $PGF_2\alpha$, thromboxane $B_2$, and $PGE_2$. The first lane on the left (unmarked) represents renal homogenate incubated with $^{14}$-C eicosapentanoic acid (EPA) without addition of inhibitors. "+INDO" and "+NDGA" represent incubations carried out in the presence of 0.4 mM indomethacin and 0.05 mM NDGA respectively. "100°C" represents incubation following heat inactivation of homogenate enzymes. $^{14}$C-15 HETE $^{14}$C-5 HETE, $^{3}$H-12 HETE and $^{3}$H $LTB_4$ references were a gift from Dr. S. Shak.

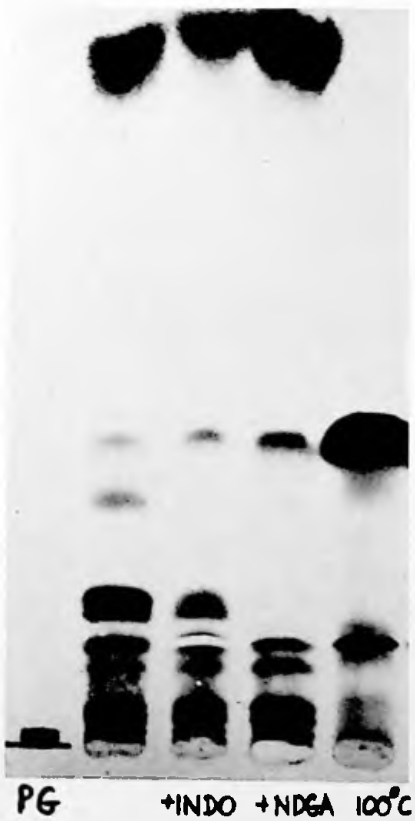


Fig. 2 Silica gel chromatogram (Solvent G)
The marker at the left indicates the position of the 4 reference prostaglandins (6 keto $PGF_1\alpha$, $PGF_2\alpha$, thromboxane $B_2$, and $PGE_2$) which fail to migrate in this solvent system. The large radioactive spots present at the top of the control, +INDO, and +NDGA lanes, and absent in the supernatant following heat inactivation probably represent labelled fatty acids.

radioactive label remaining as unreacted substrate (Table 1, Solvent B) and a smaller fraction of the radioactive label appearing as free fatty acid (Table 1, Solvent G).

TABLE I

DISTRIBUTION OF RADIOACTIVE METABOLITES

SOLVENT SYSTEM B

| | 6KETO $PGF_1\alpha$ | $PGF_2\alpha$ | TBX | $PGE_2$ | $LTB_4$ | 5 HETE | 12 HETE 15 HETE | UNREACTED SUBSTRATE |
|---|---|---|---|---|---|---|---|---|
| FRESH WATER | | | | | | | | |
| (n=5) | 2.14±1.51 | 2.04±0.86 | 2.06±0.89 | 4.16±1.83 | 5.48±1.61 | 5.90±1.20 | 25.2±15.8 | 37.2±17.3 |
| +INDO | 2.94±1.89 | 2.02±1.13 | 2.32±0.83 | 4.54±2.07 | 5.38±1.61 | 6.06±1.69 | 21.5±12.6 | 35.8±17.7 |
| +NDGA | 0.78±0.33 | 0.46±0.30 | 0.64±0.34 | 1.06±0.69 | 2.46±1.52 | 2.74±1.18 | 7.8±5.8 | 76.1±6.90 |
| SEA WATER | | | | | | | | |
| (n=2) | 4.9 - | 3.1 - | 3.7 - | 3.0 - | 8.3 - | 6.7 - | 14.2 - | 22.1 - |
| +INDO | 4.3 - | 2.6 - | 3.1 - | 2.7 - | 9.2 - | 6.6 - | 18.8 - | 26.7 - |
| +NDGA | 2.1 - | 1.4 - | 1.6 - | 1.9 - | 6.0 - | 5.1 - | 12.5 - | 53.0 - |

SOLVENT SYSTEM G

| | PG | 5 HETE 12 HETE $LTB_4$ | 15 HETE | UNREACTED SUBSTRATE | FATTY ACIDS |
|---|---|---|---|---|---|
| FRESH WATER | | | | | |
| (n=5) | 23.1±10.8 | 10.3±3.1 | 27.2±11.2 | 5.8±2.1 | 29.6±2.8 |
| +INDO | 24.8±11.7 | 10.4±3.6 | 28.8±13.0 | 6.0±1.2 | 24.6±3.0 |
| +NDGA | 17.3±11.6 | 8.7±4.2 | 14.4±12.5 | 21.7±20.6 | 65.9±14.9 |
| SEA WATER | | | | | |
| (n=5) | 22.9±4.5 | 17.1±8.6 | 13.3±10.7 | 6.1±2.8 | 20.7±5.8 |
| +INDO | 23.6±4.0 | 19.2±8.3 | 11.8±8.8 | 5.9±1.5 | 21.3±15.7 |
| +NDGA | 12.7±5.0 | 9.7±7.3 | 3.9±4.7 | 11.0±8.2 | 49.4±15.7 |

The values shown are mean± SD. PG= prostaglandins, TBX = thromboxane, LTB4 = Leukotriene B4, HETE = hydroxyeicosatetraenoic acid. Indomethacin (+INDO) was added to a final concentration of 0.4-1.0 mM. Nordihydroguaiaretic acid (+NDGA) was added to a final concentration of 0.05 mM.