Metabolism of 3,4-Benzpyrene in Rainbow Trout (*Salmo Gairdneri*)

by M. G. PEDERSEN and W. K. HERSHBERGER
College of Fisheries
and
M. R. JUCHAU
Department of Pharmacology
School of Medicine
University of Washington
Seattle, Wash. 98195

Aryl hydrocarbons are common environmental contaminants originating from many types of industrial processes. Limited knowledge is available on the metabolic disposition of such xenobiotics encountered by fish in the aquatic habitat. Only during the last decade has evidence for the biotransformation of any foreign compounds by fish been made available (1,2,3,4). Many of these studies have utilized the rainbow trout (Salmo gairdneri) as a test organism because of its wide range and economic importance. Benzpyrene hydroxylase, an enzyme system also referred to as aryl hydrocarbon hydroxylase (AHH), is a mixed function oxidase system felt to be an important factor in mechanisms of chemical carcinogenesis. It has been studied extensively in mammalian systems, but has not been investigated in fish. This presentation represents a preliminary investigation of the biotransformation of 3,4-benzpyrene in various tissues of the rainbow trout.

Experimental

Anadromous rainbow trout (steelhead trout), originating from the hatchery of the Washington State Department of Game at Aberdeen, Washington, were maintained in freshwater troughs at temperatures ranging from 10° to 15°C. Fish were fed trout pellets prepared at the University of Washington (5). Fish ranged in length from 15 to 20 cm total length and from 2 to 3 years old.

Fish were killed by cervical dislocation and the livers and other tissues were immediately removed and placed on ice. Blood was taken with a syringe from the heart. Tissues from several fish were pooled and homogenized in a teflon-glass homogenizer with cold 1.15%

Bulletin of Environmental Contamination & Toxicology,
Vol. 12, No. 4 © 1974 by Springer-Verlag New York Inc.

KCl as follows: blood - 1:3 (v/v); posterior kidney - 1:4 (w/v); heart - 1:9 (w/v); muscle - 1:3 (w/v); gill - 1:2 (w/v); and liver - 1:2 (w/v). In the case of blood, whole homogenates were employed in the enzyme assays. Other tissue homogenates were centrifuged at 10,000 x g for 20 minutes in an I.E.C. refrigerated centrifuge. The resulting 10,000 x g supernatant fractions were utilized in the incubation systems, except for hepatic 10,000 x g supernatant fractions which were further diluted 1:29 with 1.15% KCl. In some cases the diluted liver supernatant fractions were stored for 2-3 weeks at -85°C. This resulted in approximately a 10% loss in specific activity. Some of the hepatic 10,000 x g supernatant fractions were further centrifuged at 104,000 x g for one hour in a Spinco Model L ultracentrifuge to obtain microsomal and soluble fractions.

Various fish enzyme preparations were incubated in duplicate with 3,4-benzpyrene in a Dubnoff metabolic shaking incubator, usually at 28.5°C for 10 to 30 minutes. Unless otherwise stated, reaction mixtures contained 1.0 ml enzyme, 0.025 mg 3,4-benzpyrene dissolved in 0.05 ml acetone, 0.7 mg NADPH, and 0.1 M potassium phosphate buffer (pH 7.5) in a total volume of 4.0 ml. Optimum incubation conditions and cofactor requirements for fish hepatic aryl hydrocarbon hydroxylase activity were determined and utilized in these experiments.

In experiments designed to examine inducibility of the fish hepatic AHH system, three groups of 10 fish were treated as follows: group one was injected intraperitoneally with 10 mg of 3-methylcholanthrene in 0.2 ml corn oil 48 hours prior to sacrifice; group two was injected with corn oil only; and group three was untreated.

The assay method used for determination of benzpyrene hydroxylase activity was that described previously (6). Protein concentrations were determined by the methods of Lowry, et al. (7)

Results

Incubation of 3,4-benzpyrene with homogenates of various trout tissues showed that the liver, posterior kidney, and heart were capable of converting the substrate to its 8-hydroxy derivative (Table I). Activity

TABLE I

Tissue Distribution of Benzpyrene Hydroxylase Activity[1]

Tissue preparation	umoles 8-hydroxybenzpyrene formed/gm protein/minute
Blood	Not detectable
Gill	Not detectable
Heart	0.006
Posterior kidney	0.026
Liver	2.404
Muscle	Not detectable

[1]Mixtures were incubated with shaking for 30 minutes at 28.5 C under an atmosphere of 100% oxygen.

was not detectable in any of the other tissues examined. Homogenates or homogenate subfractions which had been pre-exposed to high temperatures (100°C for 5 minutes) were completely inactive.

Rates of hydroxylation of 3,4-benzpyrene effected by preparations of trout liver 10,000 x g supernatant fraction were found to proceed more than 26 times more rapidly than those observed in analogous preparations from male Sprague Dawley rat livers (6), when assayed under their respective optimal conditions. Fish hepatic microsomal fractions exhibited slightly higher specific activities than corresponding 10,000 x g supernatant fractions, while the activity of the fish hepatic 104,000 x g supernatant fractions showed less than 1/40 of the activity observed in 10,000 x g supernatant fractions.

The maximal rate of hepatic AHH in the 10,000 x g supernatant fraction was found to be 4.92 umoles 8-hydroxybenzpyrene formed/gm protein/minute, and was linear for 10 minutes. Reaction velocities decreased gradually to 2.40 umoles product formed/gm protein/minute after 30 minutes of incubation.

The effect of pH on hepatic AHH is shown in figure 1. The optimum pH was found to be approximately 7.5. Trout hepatic AHH activity was tested at several incubation temperatures ranging from 5°C to 35°C. A temperature of 28.5°C yielded the highest activity (figure 2).

NADPH was found to be a necessary cofactor of the hepatic reaction, the activity being 10 times that of reactions in which NADH was substituted for NADPH. Additions of 0.24 M glucose-6-phosphate did not consistently enhance the rate of the reaction. Additions of 0.13 M $MgSO_4$ and/or 0.25 M nicotinamide slightly inhibited the reaction. The system was saturated with NADPH at concentrations above 10^{-4} M final concentration. Saturating concentrations of substrate were those above 10^{-5} M. Acetone did not significantly inhibit the reaction at concentrations below 9×10^{-5} M.

Incubations carried out under 100% nitrogen or 100% carbon monoxide exhibited approximately one third of the hepatic AHH activity observed in systems in which 100% oxygen was utilized. The possibility can not be ruled out that sufficient oxygen may have been dissolved in the reaction flasks to partially account for the activity observed in incubations utilizing nitrogen or carbon monoxide.

The results of the induction experiments indicated that the hepatic benzpyrene hydroxylase activity of trout pretreated with 3-methylcholanthrene was approximately four times that of the activity of both the untreated controls and the trout treated with corn oil. As compared to the controls, no statistically significant increase in the quantity of protein per gram of liver was observed in 3-methylcholanthrene pretreated fish ($P > 0.5$).

Discussion

In view of the fact that other investigators (1,4, 8) have found that mammalian hepatic systems appear to be several times more effective than trout hepatic systems in the biotransformation of certain drugs, it is remarkable that the trout liver 10,000 x g supernatant fraction exhibited approximately 26 times the AHH activity of male rat liver 10,000 x g supernatant.

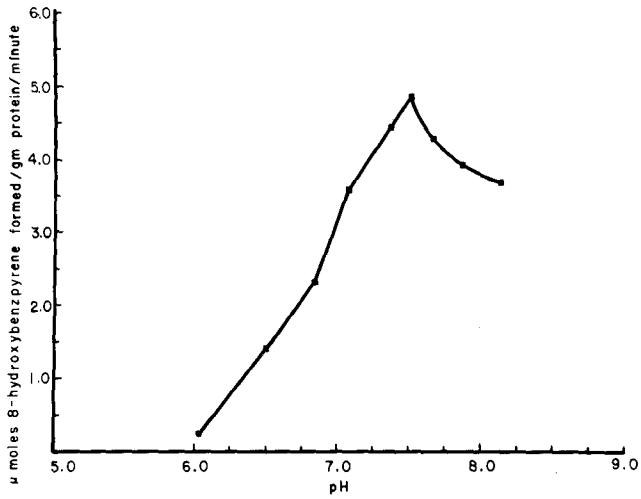

Figure 1. Relationship of pH to benzpyrene hydroxylase activity in trout hepatic 10,000 x g supernatant fractions. Incubation flasks contained 0.7 mg NADPH, 0.01 mg 3,4-benzpyrene dissolved in 0.02 ml acetone, 1.0 ml of homogenate subfraction, and 0.1 M potassium phosphate buffer in a total volume of 4.0 ml. Incubations were carried at 28.5°C for 10 minutes under 100% oxygen.

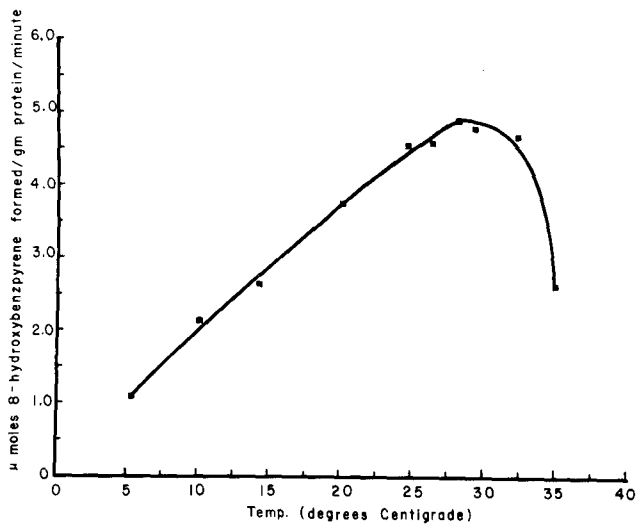

Figure 2. Relationship of temperature to benzpyrene hydroxylase activity in trout hepatic 10,000 x g supernatant fractions. Incubation conditions were the same as described in figure 1. In all cases the pH was 7.5.

In addition, these same investigators observed little evidence for induction of other trout hepatic enzyme systems. However, in the rainbow trout, the hepatic AHH system (which has not heretofore been studied in fish) the evidence strongly suggests that the enzyme system was markedly induced by a single dose of 3-methylcholanthrene.

Whether this information can be sucessfully utilized as a determinant in the economy of this species of fish remains to be investigated. In view of the importance of the AHH system as a determinant of toxicity, it would appear that extensive additional investigations of this enzyme system in fish would be warranted.

Finally, the results also re-emphasize the need for attention in optimization of reaction conditions in studies of this nature. Many of these parameters were found to be considerably different from those observed in mammalian systems.

Acknowledgements

This research was supported in part by N.I.C.H.D. grant HD-04839. The authors are indebted to Mr. Jim Mighell of N.M.F.S., Seattle, Washington for assistance in obtaining fish used in these studies, and to Mr. Ken Symms for review of the manuscript.

References

1. BUHLER, D.R. and RASMUSSON, M.E., Comp. Biochem. Physiol 25, 223 (1968)

2. ADAMSON, R.H., Fed. Proc. 26,4,1047 (1967)

3. LUDKE, J.L., GIBSON, J.R., and LUSK, C.I., Toxicol. Appl. Pharmacol. 21,89 (1972)

4. DEWAIDE, J.H. and HENDERSON, P.T., Comp. Biochem. Physiol. 32,489 (1969)

5. RAMOS, H.A., M.S. Thesis, Univ. of Wash. 56 p. (1970)

6. JUCHAU, M.R., PEDERSEN, M.G. and SYMMS, K.G., Biochem. Pharmacol. 21,2269 (1972)

7. LOWRY, O.M., ROSENBROUGH, N.J., FARR, A.L., and RANDALL, R.J., J. Biol. Chem. 193,265 (1951)

8. ADAMSON, R.H., DIXON, R.L., FRANCIS, F.L., and RALL, D.P., Proc. Natl. Acad. Sci. U.S. 54,1386 (1965)