COMPARISON OF BENZO[a]PYRENE HYDROXYLASE (ARYL HYDROCARBON HYDROXYLASE, AHH) ACTIVITIES IN HEPATIC MICROSOMES FROM UNTREATED AND 1,2,3,4-DIBENZANTHRACENE (DBA)-INDUCED MALE LITTLE SKATES (*Raja erinacea*)

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The discharge of pesticides, urban and industrial wastes, and other foreign organic chemicals into our waterways poses a potential threat to estuarine and marine ecosystems as well as to humans who use these species as direct or indirect food sources. The metabolism and disposition of chemicals such as benzo[a]pyrene, a carcinogen, by marine animals are of some importance since residues of this polycyclic aromatic hydrocarbon are found in certain marine shellfish (Dunn and Stich, Bull. Environ. Cont. Toxicol., 15:398, 1976).

In this report several enzymological properties of liver AHH activity are compared in microsomes prepared from untreated and DBA-pretreated skates. The effect of α-naphthoflavone (ANF) addition (*in vitro*) on hepatic microsomal AHH activities in untreated and induced skates is also described.

Male little skates (700-1300 g) were used throughout. Induced skates were administered DBA (10 mg/kg) in corn oil (12.5 mg/ml) by I.P. injection on days 1, 2, and 3 and were sacrificed on day 10. The skates were retained in a large floating dock moored in Frenchman Bay during this period. Microsomes were prepared from liver homogenates and AHH activities were determined as previously described (Pohl, Bend, Devereux, and Fouts, Bull. MDIBL, 13:94, 1973). One fluorescence unit (FU) equals the fluorescence intensity of 3 µg/ml quinine sulfate · $2H_2O$ in 0.1 N sulfuric acid (excitation λ 425 nm, emission λ 555 nm).

AHH activities of hepatic microsomes from DBA-pretreated skates were about 35-fold higher than those in microsomes from untreated skates (8.08 ± 4.32 FU/min/mg microsomal protein vs. 0.23 ± 0.06 FU/min/mg protein, mean SD, n = 4). This induction data is similar to that previously reported (Pohl, Fouts, and Bend, Bull. MDIBL, 15: in press).

TABLE 1

SOME PROPERTIES OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN HEPATIC MICROSOMES FROM CONTROL AND DBA-PRETREATED LITTLE SKATES (Raja erinacea)

PARAMETER		CONTROL SKATES	DBA-PRETREATED SKATES[1]
Temperature-Activity Relationship	at 20°	Linear for at least 30 min[2]	Linear for at least 20 min
	at 25°	Linear for at least 20 min	Linear for at least 15 min
	at 30°	Linear for at least 10 min	Linear for at least 10 min
Microsomal Protein Concentration-Activity Relationship (at 25°)		Linear to at least 1.6 mg/ml incubation mixture[3]	Linear to 1.2 mg/ml incubation mixture
pH-Activity Relationship (at 25°)		Broad Optimum pH 7.4-8.0	Broad Optimum pH 7.4-8.0
Benzo[a]pyrene Concentration-Activity Relationship (at 25°)		Enzyme-Saturating Concentration, 0.06 mM Apparent K_m 0.005, 0.006 mM[4] Apparent V_{max} 0.20, 0.21[5]	Enzyme-Saturating Concentration, 0.06 mM Apparent K_m 0.008, 0.010 mM[4] Apparent V_{max} 4.2, 6.9[5]

[1] Skates were treated (I.P.) with 1,2,3,4-dibenzanthracene (10 mg/kg) on days 1,2, and 3 and sacrificed on day 10.

[2] Data from hepatic microsomes of a single male skate. All experiments were repeated at least once and similar results were obtained.

[3] Final incubation mixture volume was 2.5 ml.

[4] Experiments performed with microsomes from 2 different control and induced skates.

[5] FU/min/mg microsomal protein.

Table 1 summarizes some of the characteristics of hepatic microsomal AHH activity in both control and DBA-induced skates. The linearity of product formation was studied at various temperatures (20, 25, and 30°) since it is well known that *in vitro* temperature optima for microsomal mixed-function oxidase activities in fish tend to be lower than those in mammals and are often substantially higher than environmental (water) temperature (Pohl, Bend, Guarino, and Fouts, Drug Metab. Disp. 2:545, 1974). The shorter periods of linearity observed with microsomes from the induced skates at 20° and 25° may be related to the much faster metabolism of benzo[a]pyrene (relative to control animals). Following these initial experiments, incubation conditions were 25°C for 15 min throughout.

Product formation was linear with increasing microsomal protein concentration to at least 4.0 mg protein per 2.5 ml incubation mixture in control animals (the highest concentration studied) and to about 3.0 mg protein per incubation mixture in induced animals. This slight apparent difference may again be related to more rapid substrate disappearance (per mg of microsomal protein) with the DBA-pretreated fish.

There was no obvious difference in the pH optima for AHH activity in control and DBA-induced hepatic microsomes. Both were quite broad over pH 7.4-8.0.

Enzyme-saturating concentrations of benzo[a]pyrene (BP) were the same in hepatic microsomes from control and polycyclic aromatic hydrocarbon-pretreated skates (0.06 mM). Kinetic constants were obtained from Lineweaver-Burk plots of AHH activities recorded in the presence of increasing BP concentrations (0.001-1.0 mM). These plots were linear. The apparent V_{max} was much higher (20- to 30-fold) in microsomes from DBA-pretreated skates than in those from control animals, whereas the apparent K_m values were similar in induced and control fish.

The effect of adding ANF (*in vitro*) on AHH activity of hepatic microsomes from control and induced skates was also studied (Table 2). An obvious difference between untreated and DBA-pretreated skates was

TABLE 2

IN VITRO EFFECT OF α-NAPHTHOFLAVONE ON BENZO[a]PYRENE HYDROXYLASE (AHH) ACTIVITY IN HEPATIC MICROSOMES PREPARED FROM UNTREATED (CONTROL) AND 1,2,3,4-DIBENZANTHRACENE-PRETREATED LITTLE SKATES (Raja erinacea)

α-NAPHTHOFLAVONE ADDED (M)	AHH ACTIVITY (FU/min/mg protein) CONTROL SKATE	DBA - SKATE[1]
0 (Control)	0.23[2]	5.23[2]
10^{-7}	0.23	5.08 (-3%)[3]
10^{-6}	0.24 (+4%)	3.88 (-26%)
10^{-5}	0.21 (-9%)	3.30 (-37%)
10^{-4}	0.73 (+217%)	1.16 (-78%)
10^{-3}	0.64 (+178%)	0.79 (-85%)

[1] Skate was treated (I.P.) with DBA (10 mg/kg) on days 1, 2, and 3 and sacrificed on day 10.

[2] Data from a single experiment. The experiment was repeated twice and similar results were obtained all three times.

[3] Values in parentheses give the amount of inhibition (-) or stimulation (+) of AHH activity as α-naphthoflavone was added to the incubation mixture.

observed. The higher concentrations of ANF tested (0.1-1.0 mM) caused significant stimulation of AHH activity in control animals (about 3-fold) but inhibited AHH activity in DBA-induced skates (as much as

85%). Similar results were reported earlier (Wiebel, Leutz, Diamond, and Gelboin, Arch. Biochem. Biophys. 144:78, 1971) for 3-methylcholanthrene (3-MC) pretreated and control rats. In rats the response is due to the differential effects of ANF on cytochrome P-450 (control) or cytochrome P-448 (3-MC treated) catalyzed AHH activities; cytochrome P-450-dependent AHH is stimulated by higher concentrations of ANF, whereas cytochrome P-448-dependent AHH is inhibited (Wiebel and Gelboin, Biochem. Pharmacol. 24:1511, 1975).

This is interesting in respect to AHH activity in the little skate since DBA and 3-MC treatment do not induce the apparent formation of cytochrome P-448 in hepatic microsomes (Pohl, Fouts, and Bend, Bull. MDIBL, 15: in press). However, it is possible that an altered form of cytochrome P-450 is synthesized in skate liver in response to type II inducers, as evidenced by the inhibition of AHH activity by ANF, but that this hemoprotein cannot be distinguished spectrally from the cytochrome P-450 present in hepatic microsomes of untreated fish. The hepatic microsomal mixed-function oxidase system from DBA-induced skates is currently being separated into cytochrome P-450, NADPH-cytochrome c reductase and lipid fractions, as described previously for untreated skates (Bend, Pohl, Arinc, and Philpot, Proc. Third Inter. Symp. on Microsomes and Drug Oxidatives, Pergamon Press, in press), in order to investigate this question in more detail.

This investigation has demonstrated that AHH activity in hepatic microsomes from control and DBA-induced little skates can be differentiated by carrying out the incubations in the presence of ANF (as well as by the much higher AHH activities in the induced skates). We are interested in the potential utilization of data such as this in monitoring for polycyclic aromatic hydrocarbon or TCDD pollution (potent type II inducers) in the marine environment since fish are able to accumulate these lipophilic compounds in their livers. Many pollutants of this type are toxic to humans.

RENAL EFFECTS OF ANGIOTENSIN II IN *Lophius americanus*

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The direct effects of angiotensin II on urine flow and sodium excretion rates in mammals are exceedingly variable. As a first general rule, diuresis and natriuresis result when the dose administered is relatively large (or when the dose is small, but endogenous levels are presumed to be high, e.g., during sodium depletion). Explanations for these effects fall into two main categories: hemodynamic and tubular. Hemodynamic. Angiotensin II is a potent vasoconstrictor. Changes in urine flow and sodium excretion are often directly related to changes in blood pressure, independently of changes in whole kidney glomerular filtration rate. Further, whole kidney glomerular filtration rate can be modified by angiotensin, the direction of change being determined by the site--afferent or efferent arteriolar--where angiotensin's vasoconstrictor effect predominates. Still further, the distribution of glomerular filtration between different populations of nephrons might be affected by angiotensin II. Tubular. Stop flow experiments in dogs and some micropuncture experiments in rats suggest that angiotensin directly inhibits the reabsorption of filtered sodium. As a second general rule, smaller doses of angiotensin cause antidiuresis and sodium retention. Because of the relatively short time-lag, aldosterone is thought not to be the mediator of these effects, although its secretion rate is stimulated by angiotensin II. The same sorts of explanations