Comparative Metabolism of Benzo(a)pyrene and Covalent Binding to Hepatic DNA in English Sole, Starry Flounder, and Rat

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ABSTRACT

Metabolism of benzo(a)pyrene (BaP) in vivo and in vitro was studied using two benthic fish species, English sole (Parophrys vetulus) and starry flounder (Platichthys stellatus), and Sprague-Dawley rats. At 24 h after administration of BaP (7.9 μmol/kg of body weight) to fish either p.o. (Experiment 1) or i.p. (Experiment 2), the specific activity of binding of BaP metabolites to hepatic DNA (pmol of BaP equivalent per mg of DNA) was higher in sole [21.0 ± 3.2 (SE) in Experiment 1; 28 ± 5 (SE) in Experiment 2] than in flounder (0.5 ± 0.1 in Experiment 1; 14 ± 4 in Experiment 2). Treatment of bile with β-glucuronidase and arylsulfatase released a significantly higher proportion of 7,8-dihydroxy-7,8-dihydro-BaP (BaP 7,8-diol) from sole bile than from flounder bile in both experiments. However, the rate of BaP metabolism and rate of formation of BaP 7,8-diol by hepatic microsomes were comparable for both fish species. Thus, the differences in both the level of DNA binding and the concentration of BaP 7,8-diol in bile of BaP-exposed sole and flounder were apparently due to differences in detoxification, rather than formation, of BaP 7,8-oxide and BaP 7,8-diol-9,10-epoxide.

The rate of formation of BaP 7,8-diol by rat liver microsomes (28 ± 1 pmol of BaP 7,8-diol formed per min per mg of protein) was comparable to that by hepatic microsomes from both fish species (50 ± 10 for sole and 33 ± 6 for flounder), although the rate of BaP metabolism (600 ± 200) was approximately 3 times greater than that by the fish species (190 ± 60 for sole and 180 ± 40 for flounder). Thus, greater proportion of BaP was converted to BaP 7,8-diol by liver microsomes of fish species than rat. These differences in BaP metabolism in vivo help explain, in part, the substantially lower binding (0.3 ± 0.1; Experiment 2) for hepatic DNA in BaP-exposed rat than that in either sole or flounder.

INTRODUCTION

Increasing evidence indicates that fish may serve as excellent models to study biochemical processes involved in chemical carcinogenesis (1). Considerable epizootiological evidence shows that bottom fish species exhibit a wide range of susceptibility to hepatocarcinogenesis in chemically polluted estuaries. For example, of the pleuronectid fish species studied by Malins and coworkers (2), English sole (Parophrys vetulus) and rock sole (Lepidopsetta bilineata) exhibit a high frequency of liver cancer when sampled from chemically polluted estuaries in Puget Sound, WA, whereas starry flounder (Platichthys stellatus) exhibits a low frequency of liver cancer (3). In other field studies, winter flounder (Pseudopleuronectes americanus) in Boston Harbor, MA (4), and brown bullhead (Ictalurus nebulosus) in Niagara River, NY (5), show a high incidence of liver neoplasia. Laboratory studies with salmonid fish (6, 7) have demonstrated that the Mount Shasta strain of rainbow trout (Salmo gairdneri) is susceptible to both BaP(2) and aflatoxin B1-induced carcinogenesis; however, coho salmon (Oncorhynchus kisutch) is relatively resistant to these carcinogens (7). As many xenobiotics exert their carcinogenic effects only after metabolic activation, detailed studies on activation and detoxication of carcinogens in various fish species may provide valuable insights into whether there is a biochemical basis for the differences among fish species in their susceptibility to chemical carcinogens.

Considerable debate continues, however, about the validity of using biochemical parameters, such as covalent binding of a carcinogen to DNA or metabolite profiles of a procarcinogen, to measure quantitatively the carcinogenic potency of a compound or susceptibility of a tissue or species. However, it is generally agreed that information from such studies, if used prudently, can be useful in our attempts to understand species-specific differences to chemically induced carcinogenesis (8). Accordingly, in this paper we have evaluated the ability of two closely related fish species, English sole and starry flounder (9), and Sprague-Dawley rat—a species shown to be generally resistant to BaP-induced hepatocarcinogenesis (8)—to metabolize BaP in vivo and in vitro. BaP is present in high concentrations in sediments where benthic fish, such as English sole (2) and brown bullhead (5), exhibit high frequency of liver lesions. Moreover, Krahn et al. (10) have demonstrated a positive correlation between levels of aromatic compounds fluorescing at BaP wavelengths in bile and the frequency of liver neoplasia in English sole sampled from contaminated estuaries. Furthermore, our laboratory studies (11) with English sole have demonstrated that PAHs, such as BaP, present in estuarine sediment are readily available for uptake by these fish.

MATERIALS AND METHODS

Chemicals

Soluene-350, Dimilume-30, and Insta-Gel were purchased from Packard Instrument Co., Downers Grove, IL. BaP, sodium p-aminosalicylate, sodium dodecyl sulfate, RNase A (R-4875), β-glucuronidase (G-8132), GSH (G-4251), and protease (P-5005) were purchased from Sigma Chemical Co., St. Louis, MO. To inactivate any contaminating DNases, the RNase was dissolved in water (10 mg/ml) and heated at 80-85°C for 15 min, whereas the protease was dissolved in water (10 mg/ml), self-digested for 1 h at 37°C, heated for 2 min at 80°C, and then quenched on ice (12). The RNase and protease solutions were stored at −20°C. Aluminum oxide (70–230 mesh ASTM) was obtained from Merck (Darmstadt, Federal Republic of Germany). Generally labeled [3H]BaP and [7,10-14C]BaP were purchased from Amersham, Arlington Heights, IL. The radiolabeled and unlabeled BaP was purified as described previously (13). [7,10-14C]BPDE and unlabeled BaP metabolite standards were obtained from Midwest Research Institute, Kansas City, MO. The m-cresol was distilled under reduced pressure. All other chemicals were of reagent or analytical grade and used without further purification.
BaP METABOLISM IN BENTHIC FISH AND RATS

Animals and Treatment

Experiment 1. English sole (16 ± 1 cm, 29 ± 2 g) were obtained from Useless Bay (47°59′ north, 122°30′ west), and starry flounder (23 ± 1 cm, 130 ± 20 g) were from the Nisqually River basin area (47°6′ north, 122°41′ west) of Puget Sound, WA. Based on sediment chemistry, these two areas are considered as reference (uncontaminated) sites. Moreover, bile of fish sampled from the reference areas shows undetectable levels of aromatic compounds fluorescing at BaP wavelengths (10, 14). These results, along with the fact that pleuronectid fish are considered territorial (3, 15), indicate that our test fish were not exposed to appreciable levels of chemical inducers prior to BaP exposure in the laboratory. The fish were force-fed gelatin capsules containing [3H]BaP in corn oil (7.9 μmol/kg of body weight; 2 ml of corn oil per kg of body weight).

Experiment 2. English sole (20 ± 3 cm, 80 ± 50 g) and starry flounder (19 ± 4 cm, 90 ± 60 g) were caught off the Nisqually River basin area. The fish were given injections of a single i.p. dose of [3H]BaP dissolved in acetone (7.9 μmol/kg of body weight; 1 ml of acetone per kg of body weight). Female Sprague-Dawley rats (180 ± 20 g) were purchased from Tdler Labs, Inc., Bellevue, WA. A diet of standard Purina chow and water was given ad libitum for 1 wk prior to exposure. Three rats were given injections i.p. with a single dose of [3H]BaP dissolved in acetone (7.9 μmol/kg of body weight; 1 ml of acetone per kg of body weight). Livers from untreated rats and both fish species were excised and stored at −80°C prior to preparation of microsomes.

Because water temperature exerts significant influence on uptake and metabolism of xenobiotics in poikilothermic organisms, both experiments were conducted in summer months when water temperature was 14 ± 1°C. Fish were held in flowing seawater (28%) for 1 wk and fed minced clams and krill during acclimatization (16). Analysis of the food shows no detectable levels of PAHs or polychlorinated biphenyls. The feeding was stopped 2 h prior to exposure. At 24 h postexposure, fish from both experiments were killed by a blow to the head, whereas rats were asphyxiated with CO2. Livers were removed from fish and rat, and gall bladders were removed from fish; all samples were stored at −80°C until analysis.

In Vivo Metabolism of BaP

BaP-derived radioactivity in liver was determined after solubilizing a portion of liver (approximately 0.05 g in 0.5 ml of Soluene-350 followed by addition of Dimilum-30, whereas Insta-Gel was added to 5-μl aliquots of fish bile; radioactivity was measured using a Packard Model 300C liquid scintillation spectrometer.

Enzymatic hydrolysis of bile was performed according to Varanasi and colleagues (17, 18) with some modifications. Three enzymatic hydrolyses of 5 μl of bile samples were done per fish. The first aliquot was treated with 4000 units of β-glucuronidase containing arylsulfatase activity in 0.2 m sodium acetate buffer (pH 4.5). The second aliquot was treated with 4000 units of β-glucuronidase in 1 ml of 0.1 m sodium phosphate buffer (pH 6), which was used to inhibit any arylsulfatase activity. The third aliquot was treated with 100 units of arylsulfatase in 1 ml of 0.2 m sodium acetate buffer containing 20 mM D-saccharic acid-1,4-lactone to inhibit any β-glucuronidase activity. A fourth aliquot was placed in 1 ml of either sodium acetate or sodium phosphate buffer. The samples were incubated at 37°C for 2 h and then extracted with methanol and chloroform, to which an antioxidant (0.5 mg of BHT per ml of chloroform) was added (18). The solvent was evaporated under nitrogen gas, and the residue was dissolved in methanol and analyzed by reverse-phase HPLC as described below.

Chromatography of Water-soluble Metabolites

[14C]BaP and [14C]BPDE were incubated with GSH in the presence of sole liver microsomes and cytisol to provide GSH conjugate standards as described earlier (19). The GSH conjugate standards were characterized by thin-layer chromatography, and their Rf values were comparable to those reported by Zaleski et al. (20). These GSH conjugate standards and several samples of water-soluble conjugates remaining after enzymatic treatment of bile were further analyzed via aluminum oxide column chromatography as described in an earlier report (19).

In Vitro Metabolism of BaP

Hepatic microsomes were prepared from livers of sole, flounder, and rat (21) and stored at −80°C until use. Each fish microsomal preparation represented 3–5 livers. BaP metabolism in vitro was performed using 80 nmoI of BaP per ml of reaction volume as previously described (21) except that the reaction was stopped by the addition of methanol. Assays with fish and rat liver microsomes were run for 15 min at optimum temperatures of 25°C for fish and 37°C for rat (13). After the reaction was stopped, BaP and metabolites were extracted from the incubation mixture with methanol and chloroform, to which BHT was added. BaP and the metabolites were separated by HPLC as described below.

HPLC of BaP Metabolites

Reverse-phase HPLC analyses were performed on a Varian 5020 instrument equipped with a Perkin-Elmer HCD ODS/Sil-X 5-μm column (0.26 × 25 cm). Metabolite standards, added to each sample, were detected using a VUV-10 variable UV detector (λ = 280 nm) and a Varian Fluorochrome fluorescence detector (excitation, 340–380 nm; emission, >423 nm) connected in tandem. Using a nonlinear gradient starting at 80% Solvent A (0.005% glacial acetic acid in water, v/v) and 20% Solvent B (methanol) that was held isocratic for 0.5 min, the gradient was changed as follows: 20–60% Solvent B in 12 min; 60–70% Solvent B in 12 min; 70–100% Solvent B in 10 min; and then held at 100% Solvent B for 8 min. The flow rate was 1.0 ml/min, and the HPLC column temperature was 35°C. Fractions were collected at appropriate intervals (see Figs. 1 and 2 for details), and radioactivity was determined by LSS.

The fractions eluting between 12 and 23 min from the reverse-phase HPLC were isolated, and the solvent was removed under nitrogen gas. The residue was dissolved in tetrahydrofuran-cyclohexane (1:3, v/v), and diol standards were added to the mixture prior to further analysis by normal-phase HPLC using a Partisil-5 silica 5-μm column (0.26 × 25 cm), with cyclohexane and ethanol as the mobile phase. Starting at 2% ethanol, the gradient was changed as follows: 2–4% ethanol in 25 min; held isocratic for 7 min; 4–10% ethanol in 5 min; and then held isocratic for 10 min. The flow rate was 1.0 ml/min, and column temperature was 40°C, and the absorbance was monitored at 280 nm.

The average recovery of BaP-derived radioactivity from the reverse-phase and normal-phase HPLC was greater than 90%.

Determination of Covalent Binding of BaP to Hepatic DNA and Protein

Crude DNA and protein were isolated from individual livers from Experiment 1 and from individual or 2-pooled livers from Experiment 2 as previously described (22), and the DNA was further purified by the method of Pruess-Schwartz et al. (23). For Experiment 1, the crude DNA samples from flounder or sole were pooled prior to further purification, whereas for Experiment 2, the DNA samples were purified individually. The DNA was dissolved in 2 ml of deionized, distilled water, and the purity of the DNA samples from both experiments was determined by taking ratios of A260/A280 and A260/A230, which had values of 1.8 ± 0.1 and 2.4 ± 0.1, respectively (12). The concentration of DNA was determined by its absorbance at 260 nm, and radioactivity in DNA was determined after acid hydrolysis of the DNA solution. Insta-Gel was added to each sample prior to analysis by LSS.

Individual hepatic protein samples of flounder and sole in Experiment 1 were purified as described previously (22). The purified protein (approximately 20 mg) was dissolved in 1 ml of 1 N NaOH with gentle heating at 40°C. Concentration of protein was determined by the method of Lowry et al. (24), and the radioactivity bound to protein was determined by LSS after addition of 10 ml of Insta-Gel and neutralized using 2.4 N HCl.

Statistical Analysis of Data

For in vivo experiments, concentration data were log transformed, whereas data presented as percentages were arcsin transformed prior to statistical analyses. Data within a treatment (i.e. or p.o.) were
analyzed by one-way ANOVA or Student's t test, whereas data between treatments were analyzed by two-way ANOVA. For the in vitro experiment, rate data were square root transformed, and proportions of metabolites were arcsin transformed. Differences were assessed by one-way ANOVA and the Student-Neuman-Keuls test (25). Differences were considered significant at \( P < 0.05 \).

RESULTS

BaP Metabolism in Vivo

Covalent Binding to Hepatic Macromolecules. From an earlier time course study (22) with English sole exposed to BaP p.o. and a recent study\(^5\) in which fish were exposed to BaP via i.p. injection, we determined that the maximum concentration of BaP-derived radioactivity in liver and the maximum value for covalent binding of BaP intermediates to hepatic DNA are measured between 8 and 24 h after BaP administration. At 8 h, however, variation among individual measurements is considerably higher than at 24 h; therefore, in this study all measurements were made at 24 h. In Experiment 1, when BaP was administered to fish p.o., less than 1% of the administered dose was present in the liver of either species at 24 h (Table 1). Accurate determination of covalent binding of BaP intermediates to DNA for individual fish was not feasible due to low level of radioactivity in the liver. Therefore, DNA was isolated from each fish liver and pooled to obtain one value of covalent binding for each species. Covalent binding of BaP intermediates to hepatic protein was measured for individual fish and the average value for sole liver \((1.8 \pm 0.3 \text{ pmol of BaP equivalent per mg of protein})\) was significantly higher than that for flounder liver \((0.6 \pm 0.4 \text{ pmol of BaP equivalent per mg of protein})\); in agreement with this finding, covalent binding of BaP intermediates to hepatic DNA was 4 times higher in sole than in flounder (Table 1).

In Experiment 2, when BaP was administered i.p., a substantially \((P < 0.05)\) larger percentage of the administered dose was present in livers of both English sole and starry flounder at 24 h than the corresponding values for each species in Experiment 1 (Table 1). Moreover, concentration of BaP-derived radioactivity in bile of both fish species was about 8 times higher than the respective concentrations in bile of fish in Experiment 1 (Table 2).

Assessment by one-way ANOVA revealed a significantly higher concentration of BaP-derived radioactivity in liver of sole than in flounder liver in Experiment 2; the covalent binding of BaP intermediates to hepatic DNA was also significantly higher in sole than in flounder (Table 1).

\(^5\) Unpublished data.
The results show that the binding of BaP metabolites for rat liver DNA was about 40 and 80 times lower than the values for starry flounder and English sole, respectively (Table 1); BaP-derived radioactivity in rat liver was 20–30 times lower than that in livers of both fish species. The hepatosomatic indices [(liver weight/body weight) x 100] for sole, flounder, and rat were 0.90 ± 0.08, 0.93 ± 0.04, and 5.3 ± 0.3, respectively.

Composition of BaP Metabolites in Bile. Concentrations of BaP-derived radioactivity in bile (>99% of radioactivity was present as metabolites) of fish were about 20–50 times greater than the concentrations in the corresponding livers (Tables 1 and 2), regardless of the route of BaP administration. About 2–3% of radioactivity in bile was present as unconjugated metabolites. Before performing enzymatic hydrolysis of all bile samples, a few samples, selected at random, were treated with either β-glucuronidase or arylsulfatase to determine the proportions of glucuronide and sulfate conjugates. In agreement with our earlier studies of naphthalene and BaP metabolism (17), we found that the proportion of glucuronide conjugates was 8–10 times higher than the proportion of sulfate conjugates in both experiments and with both fish species. Subsequently, individual bile samples were treated with both β-glucuronidase and arylsulfatase together, and the organic solvent-soluble metabolites released after enzymatic treatment were analyzed by both reverse- and normal-phase HPLC to identify and quantify individual Phase I metabolites. Water-soluble metabolites remaining after enzymatic hydrolysis of bile were tentatively characterized as GSH conjugates by both thin-layer and aluminum column chromatographies as described previously (19).

Metabolites Released after Enzymatic Hydrolysis of Bile. Both reverse- and normal-phase HPLC were used to separate and quantify metabolites released after enzymatic treatment of bile. Only those metabolites coeluting with standards and characterized in our earlier study by fluorescence spectroscopy and mass spectrometry (26) were quantified in this study. The results show that 3- and 1-hydroxy-BaP and BaP 7,8-diol were the major metabolites present as glucuronide and sulfate conjugates in bile of both English sole and starry flounder (Fig. 1;
Table 3). Smaller proportions of BaP 9,10-diol and 9-hydroxy-BaP were also detected in bile of both fish species. Data show that about 60% of the organic solvent-soluble metabolites coeluted with the standards (Table 3), and the remaining radioactivity was largely due to a number of polar metabolites eluting during the first 20 min (Fig. 1). Thus, accurate quantification of the BaP diols could not be made from the reverse-phase HPLC analyses alone. Therefore, the fractions eluting from 12–23 min (Fig. 1) in the reverse-phase HPLC were collected and rechromatographed by normal-phase HPLC. The results show that the percentage of BaP 7,8-diol calculated from normal-phase HPLC analyses was within 5% of the corresponding value calculated from the reverse-phase HPLC analyses. However, the proportion of BaP 9,10-diol calculated from normal-phase HPLC analysis was substantially (40–60%) lower than that calculated from reverse-phase HPLC analysis, showing that since this diol elutes earlier than the BaP 7,8-diol in the reverse-phase HPLC, other polar metabolites coeluted with it. Additionally, it can be seen from Fig. 1 that virtually no radioactivity coeluted with the standard BaP 4,5-diol in normal-phase HPLC, which indicated that the radioactivity coeluting with BaP 4,5-diol standard in the reverse-phase HPLC was due to some other polyhydroxy derivative(s) of BaP. These results emphasize the need to use more than one chromatographic technique for accurate quantification of BaP metabolites.

In addition to the diols and phenols, a significant proportion of quinones was detected in reverse-phase HPLC whether or not the antioxidant BHT was added to the extraction solvents. In all samples, a higher proportion of radioactivity eluted at the retention time of BaP 3,6-quinone than that for BaP 1,6-quinone; very little radioactivity was present in the fraction eluting with BaP 6,12-quinone. In addition, appreciable amounts of radioactivity eluted between BaP 7,8-diol and quinone standards in fraction y (Fig. 1). Our previous studies (26) suggest that this fraction may contain a metabolite characterized as 3,9-dihydroxy-BaP; however, none of the quinones or dihydroxy derivatives was further characterized in this study.

Data in Table 3 show that the proportion of BaP 7,8-diol released after enzymatic hydrolysis of bile was significantly greater in English sole than that in starry flounder, regardless of the mode of BaP exposure. Concentrations of BaP 7,8-diol conjugates (sulfates and glucuronides) in bile of English sole for Experiments 1 and 2 were 41 ± 8 and 350 ± 50 pmol of BaP 7,8-diol equivalent per mg of bile (dry weight); these values were significantly higher than the corresponding values, 20 ± 10 and 260 ± 50 pmol of BaP 7,8-diol equivalent per mg of bile (dry weight), for starry flounder. No consistent differences in proportions of other metabolites were observed for the two species.

**BaP Metabolism in Vitro**

The data in Table 4 show that the overall conversion of BaP by liver microsomes of untreated flounder and sole proceeded at the same rate. In addition, the profiles of metabolites were similar for both fish species; BaP 7,8-diol, BaP 9,10-diol, 3-hydroxy-BaP, and 1-hydroxy-BaP were the major metabolites formed, whereas 9-hydroxy-BaP and BaP quinones were formed in smaller amounts (Table 4). Moreover, the results show that the small amount of radioactivity coeluting with the BaP 4,5-diol standard in reverse-phase HPLC did not coelute with the standard in normal-phase HPLC.

Comparison of the rates of formation of individual metabolites revealed that each metabolite was formed at essentially the same rate by liver enzymes of both fish species. For example, BaP 7,8-diol was formed at a comparable rate in both flounder (33 ± 6 pmol/min/mg of protein) and sole (50 ± 10 pmol/min/mg of protein) (Table 4). Because very little 7-hydroxy-BaP was formed in vitro, it appears that the formation of the BaP 7,8-diol is rate limiting and that subsequent formation of BaP 7,8-diol is fast. Thus, the present results indicate that the rates of formation of BaP 7,8-diol by both sole and flounder liver microsomes were essentially the same.

The rate of overall metabolism of BaP by hepatic microsomes of untreated rat was significantly greater than that by either fish species. However, the rate of formation of BaP 7,8-diol by rat liver microsomes was comparable to that of either flounder or sole (Table 4). Therefore, only 6.7% of the metabolized BaP was present as BaP 7,8-diol in rat liver microsomes compared to a value of 22–26% for either fish species. Normal-phase HPLC of the diols formed with rat liver microsomes showed that a considerable percentage of radioactivity coeluted with the BaP 4,5-diol standard. In addition, both 1- and 3-hydroxy-BaP were formed at equal rates by both fish species and rat; however, 9-hydroxy-BaP was formed at a substantially higher rate with rat hepatic microsomes than with fish hepatic microsomes (Table 4).
DISCUSSION

English sole underwent a greater (2- to 4-fold) degree of chemical modification of hepatic DNA than did starry flounder when both fish species were exposed to the same dose of BaP, regardless of the mode of BaP exposure. Higher binding of BaP intermediates to hepatic DNA in sole was accompanied by higher concentrations of glucuronide and sulfate conjugates of BaP 7,8-diol in bile. Our previous results (27, 28) showing that sole liver microsomes metabolized BaP essentially to a single adduct, namely (+)-anti-BPDE-dGuo, suggest that the higher binding of BaP intermediates to hepatic DNA in sole in the present study may be mainly due to more BPDE available for binding to hepatic DNA in sole than in flounder. It appears from studies with mammals that the formation of BaP 7,8-oxide is the rate-limiting step (29), and the subsequent formation of BaP 7,8-oxide and BPDE occurs readily. Our results show that both the rate of BaP metabolism and the rate of formation of BaP 7,8-oxide by sole liver microsomes were essentially the same as the corresponding values for flounder, which indicates that the rates of formation of BaP 7,8-oxide were comparable for both species. Thus, the higher concentration of the conjugates of BaP 7,8-oxide in sole bile, accompanied by the higher binding of BaP to sole liver DNA, suggests that more efficient conjugation of BaP 7,8-oxide with GSH occurred in flounder liver than in sole liver. In support of this conjecture, our preliminary data show significantly higher (approximately 3-fold) activity of GST in flounder liver than in sole liver. The more effective conjugation of BaP 7,8-oxide with GSH in flounder liver could result in less BaP 7,8-oxide available for conjugation with glucuronic acid and subsequent release into bile, as well as less BPDE formed. In addition, BPDE could also be more effectively conjugated with GSH in flounder liver than in sole liver, thereby further reducing available BPDE for binding to hepatic DNA in flounder. It should be noted that both BaP 7,8-oxide and BPDE are shown to be substrates for mammalian GST isozymes, with BPDE being a better substrate than BaP 7,8-oxide (30). Studies with mammalian systems show that both cellular GSH level and GST activity are inversely related to DNA binding of BaP metabolites (30–33); however, there appears to be a better correlation between GST activity and DNA modification. Although the more effective detoxification of epoxides by GSH in flounder than sole can explain the present results, it should be emphasized that the efficiency of GSH conjugation apparently depends on substrate specificities of GST isozymes (33, 34) and, thus, cannot be predicted only from total GST activity. Moreover, no information is available at present on how exposure to contaminants affects the hepatic GST activity in juvenile English sole or starry flounder. Recent studies, however, with spawning English sole (21) show that, while hepatic AHH activity is induced within a day after exposure to chemicals extracted from contaminated sediments, GST activity is not induced for several days. A similar observation is also reported for rainbow trout (35). Hence, further studies are needed to: (a) characterize isozymes of hepatic GST in uninduced and induced fish; (b) identify the adducts of BaP intermediates with both GSH and hepatic DNA; and (c) measure differences in the rates of excision-repair of modified DNA in these two fish species. Such studies should provide a clearer understanding of the present results showing a higher level of chemical modification of hepatic DNA in BaP-exposed sole than in flounder.

To evaluate if route of exposure of BaP (i.p. versus p.o.) had a significant influence on BaP metabolism by the fish species, data from the two treatments were analyzed by two-way ANOVA. The results show that the level of chemical modification of hepatic DNA and the level of BaP metabolites in bile were higher in fish species exposed to BaP i.p. (Experiment 2) than the corresponding values when the same dose of BaP was administered p.o. (Experiment 1), whereas the types of conjugates and the profiles of Phase I metabolites released on hydrolysis of bile were similar in both experiments. In Experiment 1, considerable proportion of the dose may have been directly excreted from the digestive tract prior to absorption, and hence the amount reaching the liver should be considerably lower in Experiment 1 than in Experiment 2. Furthermore, our earlier study (22) showed that, when a low dose (0.1 mg of BaP per kg of body weight) of BaP was administered p.o., the level of binding of BaP intermediates to hepatic protein in juvenile English sole was about 20-fold lower than the binding value obtained for sole in Experiment 1 (2 mg of BaP per kg of body weight). Thus, these experiments indicate that the difference in the effective dose of BaP reaching the liver, rather than the route of entry of BaP, appears to determine the level of modification of hepatic macromolecules in these fish. Nevertheless, since these experiments involved only a single exposure to BaP, it remains to be seen how continuous exposure of these fish species to contaminants (e.g., long-term exposure to contaminated sediment to mimic environmental exposure) affects their ability to activate and detoxicate carcinogens.

The present results, showing that a greater proportion of the administered BaP was bound to hepatic DNA in both fish
species than in rat, can be explained, in part, by the fact that BaP 7,8-diol was the major diol formed in the liver of both fish species, whereas this diol is reported to be a minor metabolite formed in rat liver (36). The results of the in vivo metabolism are supported by our studies on BaP metabolism by hepatic microsomes of fish species and rat showing that BaP 7,8-diol constituted only 6.7% of total metabolites formed by rat, whereas this diol comprised as much as 22–26% of total metabolites formed by sole or flounder. Moreover, the finding that rat liver microsomes metabolized BaP 3–4 times faster than sole and flounder liver microsomes helps explain the results of Experiment 2 in which rat liver contained substantially lower levels of BaP-derived radioactivity at 24 h than did livers of both fish species. While comparing our results of in vivo and in vitro studies, it should be remembered that the microsomal studies were performed with untreated animals, whereas a certain degree of induction may have occurred in the test organisms in the in vivo experiments. However, it is reported that remarkably similar metabolite profiles are obtained with hepatic microsomes of both uninduced and PAH-induced southern flounder (37). Further, the proportion of BaP 7,8-diol formed by fish liver microsomes is consistently higher than that produced by hepatic microsomes from either uninduced or PAH-induced rats (37). Thus, the result that in rat liver relatively small proportions of BaP were converted into BaP 7,8-diol, the precursor of BPDE, helps explain the very low level of binding of BaP intermediates to rat liver DNA compared to sole or flounder liver DNA. In addition, differences in both the levels of hepatic GST activity and rates of excision-repair of BaP-modified DNA should also contribute significantly to the observed differences in binding levels between rat and fish species. Hepatic GST activity is reported to be considerably higher in rat (31) than that in either English sole or starry flounder. Furthermore, our earlier results (22), showing that high levels of modification of hepatic DNA in juvenile English sole persisted for about 2 wk after force-feeding of BaP, support the results (38, 39) shown that fish cells have a very low rate of excision-repair compared to rodent cells.

The present finding that the modification of hepatic DNA in both English sole and starry flounder was substantially higher than that for rat suggests that both fish species may be more susceptible to BaP-induced hepatocarcinogenesis than rat, if it is assumed that the level of DNA binding of carcinogenic PAHs is a good measure of their carcinogenic potential (40, 41). At present there are no laboratory studies on comparative tumorigenic susceptibility of these fish to hydrocarbon exposure. However, BaP is shown to be a hepatocarcinogen in the Mount Shasta strain of rainbow trout exposed to BaP p.o. or i.p. (6), and the covalent binding of BaP intermediates (42) to trout hepatic DNA is comparable to the values for sole and flounder when exposed to the same dose of BaP. Because numerous factors (e.g., route and mode of carcinogen exposure, developmental age at exposure, diet, immunocompetence, nature and persistence of carcinogen-DNA adducts) influence the eventual response of an organism to carcinogens, the present study serves as an initial attempt to understand the observed differential susceptibilities of these feral fish species to chemically contaminated environments.

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<th>Species</th>
<th>AHH activity (μmol/mg protein)</th>
<th>Mean ± SE (μmol/mg protein)</th>
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<tr>
<td>English sole (n = 4)</td>
<td>190 ± 60</td>
<td>38 ± 8 (∆7,8-Oh)</td>
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<tr>
<td>Starry flounder (n = 3)</td>
<td>180 ± 40</td>
<td>38 ± 5 (∆7,8-Oh)</td>
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<tr>
<td>Sprague-Dawley rat (n = 3)</td>
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<td>64 ± 8 (∆15,16-Oh)</td>
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</table>

- ∆4- and 14-ohydroxy metabolites were present in all species. 
- ∆9- and 9-oxo metabolites were present in all species.
- ∆11- and 11-oxo metabolites were present in all species.
- ∆13- and 13-oxo metabolites were present in all species.
National Marine Fisheries Service. We also thank Herbert Sanborn for help in obtaining the fish and Dr. Melvin Eklund for assistance with the experimental protocol with rats.

Note Added in Proof

Our recent study shows that the rates of BaP metabolism by hepatic microsomes from BaP-induced English sole and starry flounder were 555 ± 50 and 630 ± 180 pmol of BaP converted per mg protein per minute, respectively, and the rates of formation of BaP 7,8-diol were 180 ± 20 and 190 ± 40 pmol BaP 7,8-diol formed per mg protein per minute, respectively.

REFERENCES


Comparative Metabolism of Benzo(a)pyrene and Covalent Binding to Hepatic DNA in English Sole, Starry Flounder, and Rat

Usha Varanasi, Marc Nishimoto, William L. Reichert, et al.


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