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RESPONSE OF HEPATIC AND RENAL MICROSOMAL MIXED-FUNCTION OXIDASES IN THE LITTLE SKATE, *Raja erinacea*, TO PRETREATMENT WITH 3-METHYLCHOLANTHRENE OR TODD (2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN)

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Previous reports from our laboratory have described several properties of the hepatic microsomal mixed-function oxidase system of the little skate, a marine elasmobranch (Bend, Pohl, Fouts, Bull. MDIBL 12: 12, 1972; 13: 9, 1973). The little skate system differs from the analogous mammalian system since it is not induced by pretreatment of the skates with phenobarbital or Aroclor 1254 (Bull. MDIBL 13: 9, 1973). Experiments conducted last summer indicated that the skate liver system did respond to pretreatment with 3-methylcholanthrene. In the present investigation we determined the effects of 3-methylcholanthrene (3-MC) (given by different routes of administration and following different dosage schedules) and of TCDD, the most potent inducer of the mammalian hepatic microsomal mixed-function oxidase system yet found, on skate hepatic and renal microsomal xenobiotic-metabolizing enzymes.

Little skates (500 - 1,100 g) were caught locally and stored in live cars until pretreatment. Oral doses of chemical were given by stomach intubation. Control skates received an equivalent dosage of vehicle only (DMSO or corn oil) by the same route as treated animals. Microsomes were prepared from livers and kidneys as previously described (Bull. MDIBL 12: 12, 1972). Aniline hydroxylase, benzphetamine demethylase, benzpyrene

hydroxylase, and 7-ethoxycoumarin deethylase activities were also assayed as previously described (Bull. MDIBL 12: 12, 1972; 13: 94, 1973). Aliquots of fresh skate liver were frozen immediately after sacrifice. These liver sections were transported to NIEHS (frozen in dry ice) where cytochrome P-450 analyses were conducted on microsomes prepared from the freshly thawed livers. Cytochrome P-450 was quantitated by the dithionite difference spectral technic using an Aminco DW-2 double-beam spectrophotometer (Bend et al., J. Pharmacol. Exp. Ther. 183: 206, 1972).

As shown in Tables 1 and 2 little skates pretreated with either 3-MC or TCDD showed variable increases in hepatic microsomal benzpyrene hydroxylase activity. Benzpyrene hydroxylase (or aryl hydrocarbon hydroxylase) is the hepatic enzyme activity which shows the greatest increase following polycyclic hydrocarbon administration to mammals. In some skates a biologically significant increase of benzpyrene hydroxylase activity (up to fourteen-fold) was noted following 3-MC or TCDD administration; in others, there was no change from control rates. There was considerable variability of enzyme activities in control (nontreated) skates. This appeared to be due to individual differences and was not unexpected in a wild population. Similar results were also noted for hepatic mixed-function oxidase activities in humans (Davies, Thorgeirsson, Breckenridge, Orme, Drug Metab. Disp. 1: 411, 1973). The variation in drug-metabolizing activities found in control skate liver microsomes may also be related to prior induction of the skate system by organic pollutants present in the marine environment.

One of the most characteristic responses of rat and rabbit hepatic microsomal cytochrome P-450, following administration of polycyclic hydrocarbons, is a 2 nm shift in the wavelength of maximum absorption of the reduced CO-bound hemoprotein (from 450 to 448 nm). No such shift was

observed in skates after 3-MC or TCDD pretreatment. There was considerable variation in the microsomal cytochrome P-450 content of treated and control skates (Table 1 and 2). This is probably due to the procedures used (i.e., storage in the frozen state prior to assay of the cytochrome content). At periods of 21 days or longer between sacrifice and assay, there appeared to be considerable decreases in amounts of cytochrome P-450 and concomitant increases in cytochrome P-420. This emphasizes the importance of assaying skate microsomal P-450 (and possibly P-450 of other fish) in fresh liver if at all possible.

Renal microsomes from skates pretreated with 3-MC also showed variability of response (data not shown). In some treated skates there was no apparent increase in benzpyrene hydroxylase activity (vs controls), while in other treated skates there appeared to be an approximate doubling of activity (0.04 to 0.08 units/min/mg microsomal protein). In one experiment with skates given TCDD (1 µg/kg on day 1, sacrificed on day 10), almost tenfold induction of renal microsomal AHH was observed (0.04 to 0.35 units/min/mg microsomal protein).

There are several explanations for the occasional lack of benzpyrene hydroxylase induction in hepatic microsomes prepared from 3-MC-treated skates. The induction of aryl hydrocarbon hydroxylase is known to be under genetic control in mice, where some strains (C-57 Bl/6J) show increased benzypyrene hydroxylase activity but others (DBA) are unaffected (Nebert, Considine, Kon, Drug Metab. Disp. 1: 231, 1973). Consequently it would seem reasonable that the response or lack thereof in some 3-MC pretreated skates may be due to a heterogeneity of the genetic pool of these skates. Another possibility is that there were wide variations in absorption of 3-MC or TCDD by the skates following oral or IP pretreatment. This possibility can and will be tested

TABLE 1

EFFECT OF PRETREATMENT WITH 3-METHYLCHOLANTHRENE ON LITTLE SKATE HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE ACTIVITIES AND CYTOCHROME P-450 CONTENT

DOSE (mg/kg)	ROUTE	DAY	CYTOCHROME P-450 CONTENT[1]	7-ETHOXYCOUMARIN DEETHYLASE[2]	ANILINE HYDROXYLASE[2]	BENZPHETAMINE DEMETHYLASE[2]	BENZPYRENE HYDROXYLASE[3]
0 (CONTROL)	ORAL	6-10	0.22±.05 (4)[4]	0.50±.09 (4)	0.41±.09 (4)	0.95±.15 (4)	0.06±.01 (4)
50	ORAL	6	0.12±.03 (3)	0.29±.05 (3)	0.20±.06 (3)	0.65±.17 (3)	0.42±.21 (3) (.17,.24,.84)
50	ORAL	8	0.13 (.03-.23)[5]	0.25 (.18-.31)	0.32 (.21-.42)	0.69 (.52-.86)	0.20 (.19-.21)
50	ORAL	10	0.31 (.19-.41)	0.42 (.28-.56)	0.26 (.22-.29)	1.02 (.81-1.22)	0.09 (.08-.10)
0 (CONTROL)	I.P.	4-10	0.26±.03 (5)	0.37±.04 (5)	0.48±.06 (4)	1.08±.12 (5)	0.12±0.4 (5)
20	I.P.	4	0.34±.06 (3)	0.38±.06 (3)	0.60±.07 (3)	1.33±.11 (3)	0.37±.17 (3) (.05,.43,.62)
20	I.P.	7	0.34 (.32-.35)	0.36 (.35-.37)	0.49 (.44-.53)	0.90 (.86-.94)	0.04 (0-.08)
20	I.P.	10	0.23 (.22-.24)	0.37±.08 (4)	--[6]	0.98±.09 (4)	0.40±.14 (4) (.19,.19,.43,.78)

[1]nmole cytochrome P-450/mg microsomal protein
[2]nmole product formed/min/mg microsomal protein
[3]units fluorescence/min/mg microsomal protein
[4]mean ± S.E.M. (N)
[5]mean (range)
[6]not measured

TABLE 2

EFFECT OF PRETREATMENT WITH TCDD (1 µg/kg, I.P.) ON LITTLE SKATE HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE ACTIVITIES AND CYTOCHROME P-450 CONTENT

DAY OF SACRIFICE (TREATED ON DAY 1)	CYTOCHROME P-450 CONTENT[1]	7-ETHOXYCOUMARIN DEETHYLASE	ANILINE HYDROXYLASE[2]	BENZPHETAMINE DEMETHYLASE[2]	BENZPYRENE HYDROXYLASE[3]
CONTROLS--NO TCDD					
6-10	0.28±.05 (4)[4]	0.18±.03 (4)	0.28±.06 (4)	0.63±.18 (4)	0.11±.04 (4) (.05,.06,.08,.23)
6	0.22±.01 (3)	0.21±.04 (3)	0.28±.05 (3)	1.11±.28 (3)	0.08±.04 (3) (0,.10,.14)
10	0.37±.01 (3)	0.23±.04 (3)	0.44±.01 (3)	1.00±.27 (3)	0.32±.09 (3) (.20,.28,.49)

[1]nmole cytochrome P-450/mg microsomal protein
[2]nmoles product formed/min/mg microsomal protein
[3]units fluorescence/min/mg microsomal protein
[4]mean ± S.E.M. (N)

by the administration of radiolabeled 3-MC and TCDD. A third possibility is that some skates may already have been partially induced by pollutants in the water and partial induction may have blocked response to other inducers.

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PRELIMINARY STUDIES OF BILE SECRETION IN THE ISOLATED PERFUSED LIVER OF THE SMALL SKATE, *Raja erinacea*

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Previous studies in the free-swimming small skate indicate that this species may be a useful model for the study of bile secretion and the hepatic uptake and excretion of organic anions (Bulletin, MDIBL, 1973). In the present report we describe our initial attempt to study these processes under more controlled conditions in the isolated perfused skate liver.

The gills of anesthetized 1 Kg skates were perfused with cold sea water while the common duct and portal vein collaterals were ligated and the gall-bladder cannulated with PE 260 tubing. A portal vein cannula was inserted (PE 260) which led from a reservoir containing Elasmobranch Ringers, pH 7.6 (Forster and Goldstein, Comp. Biochem. Physiol. 424: 3-12, 1972), that was oxygenated with 100 percent O_2.

After cutting away hepatic and portal vein attachments the liver was rapidly removed and placed on a perforated petri dish. Perfusion pressure was regulated by adjusting the level of the perfusate reservoir and the hepatic effluent was returned to the reservoir by means of a peristaltic pump. Twenty studies were carried out at ambient temperature (22-25°C).