CYTOCHROME P-450 INDUCTION DURING SPERMATOGENESIS IN THE DOGFISH SHARK (S. ACANTHIAS)

David M. Barnes and David S. Miller
Laboratory of Cellular and Molecular Pharmacology, NIH-NIEHS
Research Triangle Park NC 27709

In nature, animals are exposed to multiple types of toxicants, such as, heavy metals, organochlorines, and polycyclic aromatic hydrocarbons (PCAH). Thus, it is important to know how and when pollutants might interact. We wish to understand the mechanisms by which environmental pollutants disrupt spermatogenesis. The structural organization of the dogfish shark, Squalus acanthias, testis allows easy examination of cells at three distinct stages of development characterized as Zone 1 (pre-meiotic), Zone 2 (meiotic), and Zone 3 (post-meiotic; Callard et al., J. Exp. Zool. Suppl. 2:23, 1989). Tissue from each zone can be isolated easily and then cultured for extended periods. Previous experiments have shown that mercurials have stage-specific effects on protein synthesis in dogfish spermatocysts (Barnes and Miller Bull. MDIBL 31:32, 1992). To understand the physiological consequences of these effects, we would ultimately want to know, for example, how the function of individual metabolically important proteins is affected. For initial experiments, we have chosen to study the microsomal cytochrome P-450 system, an important determinant of drug and pollutant metabolism in many tissues. One distinguishing characteristic of this enzyme system is the massive induction of activity that can follow exposure to PCAH in vivo and in vitro. Here we report on patterns of substrate induction of cytochrome P-450 activity in developmentally staged dogfish spermatocysts.

In initial experiments animals (N=5) were injected IP with either 3 ml corn oil containing 20% DMSO (vehicle) or vehicle with 30 mg β-napthaflavone (BNF), an inducer of cytochrome P-450 enzyme activity. After 5 d, microsomes from testis and liver were prepared and cytochrome P-450 enzyme activity measured as the O-deethlyation of 7-ethoxyresorufin (ERF; Burke and Mayer Drug. Metab. Disp. 2:583, 1974). In liver, ERF deethylase activity was 0.02-0.6 nmol mg^{-1} min^{-1} in control animals, compared with 0.08-4.0 nmol mg^{-1} min^{-1} in treated animals. In testes, basal activity in Zone 1 (0.01-0.08 nmol mg^{-1} min^{-1}) was about 2 times that in Zones 2 and 3. With BNF injection, cytochrome P-450 activity increased about 10-fold in each zone, however the response was so variable that we cannot say which stage of spermatogenesis was affected most. The variability in response could be a result of variable and uncontrolled pollutant exposure prior to capture or variable BNF uptake *in vivo* .

For In vitro dosing experiments, spermatocysts were isolated and then cultured in Leibovitz L-15 media (Dubois and Callard J. Exp. Zool. 258:359, 1991) containing BNF. In control cysts, microsomal cytochrome P-450 activity in Zone 1 > Zone 3 > Zone 2 ($P<0.01$, paired t-test; Table 1). In spermatocysts from Zone 1, in vitro BNF exposure first increased then decreased P-450 activity; in Zones 2 and 3 BNF generally reduced P-450 activity (Table 1).

Comparison of results from in vivo and in vitro experiments indicates a marked effect of cyst culture on cytochrome P-450 enzyme activity. First, in all zones, control activities in vitro were at least an order of magnitude lower than in vivo values. Second, in Zone 1, BNF induction in vitro was modest compared to in vivo. Third, in Zones 2 and 3 in vitro exposure to BNF did not increase P-450 activity, but in vivo exposure did. These differences could be due to production of compounds that modify P-450 activity during culture or to direct inhibition of the enzyme by BNF. To further investigate these possibilities we are attempting to correlate changes in enzyme activity with changes in enzyme synthesis using Western blot analysis. Initial results indicate that dogfish shark P-450 could not be detected by a cytochrome P-450 antibody (1A1) known to crossreact with many species. We are currently screening additional antibodies to see which if any react with shark microsomal proteins. (This research was supported by a fellowship from the Lucille P. Markey Charitable Trust.)

Table 1. Effects of BNF Exposure In Vitro on Spermatocyst Microsomal Cytochrome P-450 Activity

BNF Concn (μg/ml)	Zone 1	Zone 2	Zone 3
0 (Control)	1.56 ± 0.29 (12)	0.39 ± 0.08 (11)	0.79 ± 0.15 (12)
2.5	1.84 ± 0.28 (4)	0.15 ± 0.02 (3)*	ND
5	4.28 ± 0.79 (13)*	0.59 ± 0.17 (12)	0.43 ± 0.10 (11)
10	0.92 ± 0.09 (4)*	0.11 ± 0.02 (4)*	0.12 ± 0.01 (3)*

Spermatocysts were cultured 4 days at 18°C in Leibovitz L-15 medium with 0-10 μg/ml BNF. Data are presented as pmole ERF converted/mg microsomal protein/min. Data given as mean ± SE(n), where n is the number of replicates. *Significantly different from controls, $P < 0.05$. ND, not determined.