Ovaries from postnatal day (PND) 4 B6C3F1 mice were incubated ovotoxicity using a novel neonatal mouse ovarian culture system. The present study was designed to evaluate the role of ovarian mEH in DMBA-induced ovotoxicity using a novel neonatal mouse ovarian culture system. Ovaries from postnatal day (PND) 4 B6C3F1 mice were incubated with DMBA (12.5nM–1 μM) for various lengths of time. Following incubation, ovaries were histologically evaluated or assessed for mEH protein or mRNA. Following 15 days of incubation, DMBA reduced (p < 0.05) healthy follicles at concentrations ≥ 12.5nM. At 1 μM DMBA, follicle loss and increased mEH protein were measured (p < 0.05) by 6 h. mRNA encoding mEH markedly increased after 2 days of incubation, and this increase preceded accelerated follicle loss at 4 days. Furthermore, follicle loss induced by DMBA was prevented when cyclohexene oxide (2mM), an mEH inhibitor, was added to DMBA incubations. These studies suggest that the PND4 mouse ovary is capable of bioactivating DMBA to its ovotoxic metabolite, and that ovarian mEH enzyme activity is likely involved. Furthermore, these observations support the use of a novel ovarian culture system to study ovary-specific metabolism of xenobiotic chemicals.

Key Words: DMBA; mEH; in vitro ovarian culture; ovary.

The polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene (DMBA) is attributed to its bioactivation by CYP1B1 to a 3,4-epoxide which is then hydrolyzed to form a 3,4-diol by microsomal epoxide hydrolase (mEH). Further epoxidation by CYP1A1 or 1B1 forms the ultimate ovotoxicant, DMBA-3,4-diol-1,2-epoxide. Studies suggest that the mouse ovary expresses these enzymes, and thus, may be capable of bioactivating DMBA to its ovotoxic metabolite. The present study was designed to evaluate the role of ovarian mEH in DMBA-induced ovotoxicity using a novel neonatal mouse ovarian culture system. Ovaries from postnatal day (PND) 4 B6C3F1 mice were incubated with DMBA (12.5nM–1 μM) for various lengths of time. Following incubation, ovaries were histologically evaluated or assessed for mEH protein or mRNA. Following 15 days of incubation, DMBA reduced (p < 0.05) healthy follicles at concentrations ≥ 12.5nM. At 1 μM DMBA, follicle loss and increased mEH protein were measured (p < 0.05) by 6 h. mRNA encoding mEH markedly increased after 2 days of incubation, and this increase preceded accelerated follicle loss at 4 days. Furthermore, follicle loss induced by DMBA was prevented when cyclohexene oxide (2mM), an mEH inhibitor, was added to DMBA incubations. These studies suggest that the PND4 mouse ovary is capable of bioactivating DMBA to its ovotoxic form, and that ovarian mEH enzyme activity is likely involved. Furthermore, these observations support the use of a novel ovarian culture system to study ovary-specific metabolism of xenobiotic chemicals.

Key Words: DMBA; mEH; in vitro ovarian culture; ovary.
DMBA destroyed follicles only in the treated ovary (Shirimizu and Mattison, 1985). Studies have shown that DMBA can be metabolized by ovarian and placental microsomes (Bengtsson et al., 1983; Miyata et al., 2002). Furthermore, studies by Becedas et al. (1993) suggest that rat granulosa cells in culture can metabolize DMBA to the carcinogenic, 3,4-diol-1,2-epoxide. Collectively, these findings suggest that the ovary is capable of bioactivating DMBA to the 3,4-diol-1,2-epoxide.

Even though studies indicate that the ovary is capable of bioactivating DMBA to the ovotoxicant, the role of ovarian enzymes, such as mEH, in the resulting toxicity of DMBA has not been assessed. Therefore, the present study was designed to evaluate the role of ovarian mEH in DMBA-induced ovotoxicity utilizing a novel in vitro ovarian culture system. Using this system, hepatic contribution to bioactivation of DMBA is removed, and ovary-specific capabilities can be assessed. The hypothesis is that ovarian mEH is involved in bioactivation of DMBA to the 3,4-diol-1,2-epoxide as evidenced by ovarian toxicity.

MATERIALS AND METHODS

Reagents. DMBA, cyclohexene oxide (CHO), bovine serum albumin (BSA), ascorbic acid (vitamin C), and transferrin were purchased from Sigma-Aldrich Inc (St Louis, MO). Dulbecco’s Modified Eagle Medium: nutrient mixture F12 (Ham) 1× (DMEM/Ham’s F12), Albumax, penicillin/streptomycin (5000 U/ml, 5000 µg/ml, respectively), Hanks’ Balanced Salt Solution (without CaCl₂, MgCl₂, or MgSO₄), mEH and β-actin custom-designed primers, and Superscript III One-Step RT-PCR System were obtained from Invitrogen Co. (Carlsbad, CA). Millicell-CM filter inserts were purchased from Millipore (Bedford, MA), and 48-well cell-culture plates were obtained from Corning Inc (Corning, NY). The mEH antibody (goat anti-rabbit) was purchased from Dako (Carpinteria, CA). Secondary antibody (horse anti-goat) and Cy-5-streptavidin were obtained from Vector (Burlingame, CA). YOYO-1 was purchased from Molecular Probes (Eugene, OR). RNeasy Mini kit, QIAshredder kit, RNeasy MinElute kit, and Quantitect SYBR Green PCR kit were purchased from Qiagen Inc (Valencia, CA). RNealater was obtained from Ambion Inc (Austin, TX).

Animals. Late gestation day pregnant mice (carrying B6C3F₁ litters) were purchased from Harlan Laboratories (Indianapolis, IN). All animals were housed one per cage in plastic cages, and maintained in a controlled environment (22 ± 2°C; 12-h light/12-h dark cycles). The animals were provided with a standard diet with ad libitum access to food and water and allowed to give birth. All animal experiments were approved by the University of Arizona’s Institutional Animal Care and Use Committee.

In vitro ovarian cultures. Postnatal day (PND) 4 female B6C3F₁ mice were killed by CO₂ inhalation followed by decapitation. Each ovary was removed, oviduct and excess tissue trimmed, and placed on a piece of Millicell-CM membrane floating on 250 µl of DMEM/Ham’s F12 medium containing 1 mg/ml BSA, 1 mg/ml Albumax, 50 µg/ml ascorbic acid, 5 U/ml penicillin/5 µg/ml streptomycin, and 27.5 µg/ml transferrin in a well in a 48-well plate previously equilibrated to 37°C. Using fine forceps, a drop of medium was placed to cover the top of the ovary to prevent drying. Ovaries were incubated with 1% DMSO (vehicle control), DMBA, and/or CHO at concentrations indicated in figure legends. One micromolar DMBA concentration was adapted from Matikainen et al. (2001). Plates containing ovaries were cultured at 37°C and 5% CO₂ in air. For those cultures lasting more than 2 days, media were removed and fresh media and treatment were replaced every 2 days. Four culture studies (three to five ovaries per treatment per culture) were conducted per experiment.

Histological evaluation of follicle numbers. Following incubation, ovaries were placed in Bouin’s fixative for 1.5 h, transferred to 70% ethanol, embedded in paraffin, serially sectioned (5 µm thick), and every sixth section was mounted. All ovarian sections were stained with hematoxylin and eosin. Healthy follicle populations containing oocytes were classified and counted in every 12th section. Unhealthy follicles were distinguished from healthy follicles by pyknosis of granulosa cells and intense eosinophilic staining of oocytes (Devine et al., 2002a). Follicle population classification was according to the procedure of Flaws et al. (1994) which was adapted from that described by Pedersen and Peters (1968). Briefly, primordial follicles contained the oocyte surrounded by a single layer of squamous-shaped granulosa cells, primary follicles contained the oocyte surrounded by a single layer of cuboidal-shaped granulosa cells, and secondary follicles contained the oocyte surrounded by multiple layers of granulosa cells. Total follicle loss for Figure 5 was calculated by subtracting the total number of follicles (primordial + primary + secondary) remaining following DMBA treatment at each time point from total number of follicles in vehicle control–treated group at that time point. These values were divided by the follicle numbers present in control ovaries, and the resulting value was multiplied by 100 to obtain a percentage, (Control – DMBA)/Control × 100.

Toluidine blue staining. This histological method was adapted from Tome et al. (2001). Briefly, following incubation, ovaries were fixed in 3% glutaraldehyde in 0.1M cacodylate (pH = 7.2) for 1.5 h, transferred to 1mM cacodylate, embedded in epoxy resin, serially sectioned (1 µm thick), and every 10th section was mounted. All ovarian sections were stained with toluidine blue for observation of pyknotic nuclei as a marker for apoptosis.

RNA isolation. Following 3 h, 6 h, 24 h, and 2 days of in vitro culture, ovaries (12 per pool) treated with vehicle control (1% DMSO) or DMBA (1µM) were stored in RNealater at ~ 80°C. Total RNA was isolated using RNeasy Mini kit. Briefly, ovaries were lysed and homogenized using a motor pestle followed by applying the mixture onto a QiAshredder column. The QiAshredder column containing ovarian tissue sample was then centrifuged at 14,000 rpm for 2 min. The resulting supernatant was applied to an RNeasy mini column, allowing the RNA to bind to the filter cartridge. Following washing, the RNA was eluted from the filter and concentrated using RNeasy MiniElute kit. Briefly, isolated RNA was applied to an RNeasy MiniElute spin column, and after washing, RNA was eluted using 14 µl of RNase-free water. RNA concentration was determined using a NanoDrop (λ = 260/280 nm; ND 1000).

First strand cDNA synthesis and real-time PCR. Total RNA (1 µg) was reverse transcribed into cDNA utilizing the Superscript III One-Step RT-PCR
System. cDNA was diluted (1:10) in RNase-free water. Two microliters of diluted cDNA were amplified on a Rotor-Gene 3000 using Quantitect SYBR Green PCR kit and custom-designed primers for mEH (forward primer: 5'-GGG TCA AAG CCA TCA GGC A 3'; reverse primer: 5'-CCT CCA GAA GGA CAC CAC TTT; Cannady et al., 2002) and β-actin (forward primer: 5'-ACG CAG CTC AGT AAC AGT CC 3'; reverse primer: 5'-TCC ATC CTG GCC TCA CTG TC 3'; NCBI GenBank accession number AK 151010). The regular cycling program consists of a 15-min hold at 95°C and 45 cycles of denaturing at 95°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 20 s at which point data were acquired. Product melt conditions were determined using a temperature gradient from 72 to 99°C with a 1°C increase at each step. There was no difference in β-actin mRNA between vehicle control and DMBA-treated ovaries. Therefore, each sample was normalized to β-actin before quantification.

Confocal microscopy. Following 6 h of in vitro culture, ovaries (three per group) treated with vehicle or DMBA (1 µM) were fixed in 4% buffered formalin for 2 h, transferred to 70% ethanol, embedded in paraffin, serially sectioned, and every 10th section was mounted. Sections were deparaffinized (approximately 10 sections per ovary) and incubated with primary antibody directed against mEH (goat anti-rabbit; 1:50 dilution) and Helium-neon laser projected through the tissue into a photomultiplier at 488 nm for YOYO-1 (green) and CY-5 (red), respectively. All images were captured with a 40× objective lens. Multiple readings were taken throughout the sections. Analysis was performed at control settings on the confocal microscope, in which 110 follicles per ovary were evaluated.

Statistical analysis. Comparisons were made using one-way ANOVA. When significant differences were detected, individual groups were compared with the Fisher’s protected least significant difference multiple range test. The assigned level of significance for all tests was p < 0.05.

RESULTS

Effect of Concentration on DMBA-Induced Follicle Loss

Follicle loss was evaluated in PND4 mouse ovaries following 15 days of incubation with various concentrations (12.5nM–1 µM) of DMBA (Fig. 2). Compared to vehicle control, DMBA reduced (p < 0.05) healthy primordial follicles at concentrations ≥ 12.5 nM (Fig. 2A). Healthy primary follicles were reduced (p < 0.05) at concentrations ≥ 25 nM DMBA (Fig. 2B). DMBA markedly reduced (p < 0.05) healthy secondary follicles at all concentrations (Fig. 2C). All healthy follicle populations were depleted by DMBA at concentrations ≥ 250 nM.

Time Course of DMBA-Induced Follicle Loss

Follicle loss was evaluated in PND4 mouse ovaries incubated with 1 µM DMBA for various time points in culture (Fig. 3). Relative to vehicle control, ovaries incubated with DMBA for 6 h, showed healthy primordial and primary follicle loss (p < 0.05). All healthy primordial and primary follicles were depleted in these ovaries by 8 days of culture (Figs. 3A and 3B). Secondary follicles in PND4 ovaries do not develop until 4 days. No healthy secondary follicles were observed in DMBA-treated ovaries at any time point (Fig. 3C). However, some unhealthy secondary follicles in DMBA-treated ovaries were observed between 4 and 15 days of culture (data not shown).

DMBA-Induced Cell Death

The type of cell death occurring in cultured ovaries following incubation with DMBA (4 days) was evaluated using toluidine blue staining (Fig. 4). Ovaries treated with DMBA contained...
pyknotic bodies in both the oocyte and granulosa cell nuclei. Some follicles in these ovaries also contained vacuoles along with pyknotic bodies (Figs. 4C and 4D). These morphological changes are characteristic of apoptosis (Kerr et al., 1972; Tome et al., 2001). Follicles containing pyknotic bodies that were rarely observed in control-treated ovaries were assumed to be undergoing the normal atretic process (cell death by apoptosis).

**Effect of DMBA on Ovarian Expression of mEH mRNA**

To investigate the effect of DMBA on ovarian mEH enzyme expression, the level of mEH mRNA in ovaries collected from PND4 mice following DMBA treatment was quantified using real-time PCR (Fig. 5A). mEH mRNA was detected in RNA isolated from ovaries in all treatment groups at all time points. Following incubation with 1 μM DMBA for 3, 6, or 24 h, the level of mEH mRNA did not change compared to that of the vehicle control–treated group. At 2 days, there was a 5.2-fold increase \( (p < 0.05) \) in mEH mRNA compared to vehicle control (Fig. 5A). Following 2 days of DMBA treatment, very few healthy follicles were observed; thus, not enough RNA could be isolated from ovaries to conduct real-time PCR reactions following this time point.

Figure 5B shows the total follicle loss expressed as a percentage throughout the 15-day DMBA incubation period. The rate of DMBA-induced follicle loss from 6 h to 2 days did not differ significantly. At 4 days, the rate of DMBA-induced follicle loss increased \( (p < 0.05) \), at which point it plateaued until 15 days of culture (Fig. 5B).

**Effect of DMBA on Ovarian Expression of mEH Protein**

To further evaluate the ovarian distribution of mEH, protein expression was visualized using confocal microscopy in ovaries collected from PND4 mice (Fig. 6). mEH protein was detected in all follicle populations present in PND4 ovaries (primordial and primary). Staining intensity for mEH in these follicles was highly localized to the oocyte cytoplasm. mEH protein was not detected in granulosa cells of either primordial or primary follicles (Figs. 6A and 6B). Following incubation with 1 μM DMBA for 6 h, mEH staining in primary oocytes increased \( (p < 0.05) \) compared to that of vehicle control ovary (Figs. 6C,
Interestingly, some diffuse mEH staining was detected in the primary oocyte nucleus in these DMBA-treated ovaries. There was no difference in staining intensity for mEH expression in primordial follicles between DMBA-treated and vehicle control ovaries (Fig. 6F). No Cy-5 staining was seen in immunonegative sections at \( \lambda = 647 \) nm (Fig. 6E).

**Effect of mEH Inhibitor on DMBA-Induced Follicle Loss**

Follicle loss was evaluated in PND4 mouse ovaries following 6 h of incubation with an mEH enzyme inhibitor, CHO (Fig. 7). CHO acts as an alternative substrate for mEH, and competitively inhibits mEH activity. Thus, metabolism of DMBA to the 3,4-diol-1,2-epoxide (active metabolite) is decreased (Oesch, 1973). CHO (2mM) did not affect primordial and primary follicle populations following 6 h of incubation. DMBA significantly \((p < 0.05)\) decreased primordial and primary follicles following 6 h in culture. Loss of primordial and primary follicles was prevented by the addition of 2mM CHO to DMBA incubations (Figs. 7A and 7B).

**DISCUSSION**

In addition to having carcinogenic properties, DMBA has been shown to be an ovarian toxicant. DMBA targets all follicle populations in the ovary following in vivo dosing leading to ovarian failure (Mattison, 1980; Weitzman et al., 1992).

Previous studies indicate that DMBA metabolism to the 3,4-diol-1,2-epoxide is required for carcinogenic and ovarian toxic effects of this compound (Sawicki et al., 1983; Shiromizu and Mattison, 1985; Vigny et al., 1985). Metabolic activation of DMBA to 3,4-diol-1,2-epoxide requires three sequential bio-activation steps mediated by CYP1B1, mEH, and CYP1A1/1B1 (Savas et al., 1997; Shimada et al., 2001). Although the liver is the primary organ participating in bioactivation of xenobiotics, extrahepatic organs such as the ovary have also been shown to be capable of bioactivation of xenobiotic chemicals. Both CYP1A1 and CYP1B1 are expressed in the ovary and mRNA for these CYP450s was induced in the ovary following a single dose of DMBA (Shimada et al., 2003). mEH enzyme is expressed in the ovary, and following dosing with the ovarian toxicant 4-vinylcyclohexene diepoxide, VCD, mEH activity, was induced in VCD-targeted follicle populations (Cannady et al., 2002). The study reported here provides...
additional evidence for the role of mEH in chemical-induced ovarian toxicity. Even though studies suggest that the ovary can metabolize DMBA, the role of ovarian metabolism in resulting ovotoxicity has not been evaluated in the absence of hepatic contributions.

Previous studies have shown that rat PND4 ovaries can be cultured up to 15 days utilizing an in vitro culture method (Devine et al., 2002a,b). Rat PND4 ovaries in culture remain healthy and retain their dynamic characteristics. For example, PND4 ovary does not contain secondary follicles; however, following 15 days of in vitro culture, follicles develop to the secondary stage. Furthermore, this culture system has been utilized to study the mechanism of ovotoxicity induced by the ovarian toxicant VCD. As with in vivo dosing, following 15 days of exposure, VCD selectively destroyed primordial and primary follicles via apoptosis in cultures of PND4 ovaries (Devine et al., 2002b, 2004). Thus, the in vitro ovarian cultures mimic VCD-induced follicle loss seen with in vivo dosing studies. Therefore, the current study was designed to evaluate a possible role of ovarian mEH in metabolism of DMBA using this novel ovarian culture system. By using this approach, the role of ovarian metabolism of DMBA and DMBA-induced ovotoxicity can be evaluated, independent of hepatic contributions.

In PND4 rat ovarian cultures, following 15 days of VCD exposure, unhealthy degenerating follicles as well as healthy follicles were observed. Unhealthy follicles were characterized by pyknosis of granulosa cells and intense eosinophilic staining of the oocytes (Devine et al., 2002a). In the present study, DMBA reduced healthy primordial and secondary follicles at concentrations ≥ 12.5nM following 15 days of incubation. Unlike primordial and secondary follicles, primary follicle loss was observed at concentrations ≥ 25nM. This observation is interesting because VCD also had less of an effect on primary follicles compared to that of primordial follicles in PND4 rat ovarian cultures (Devine et al., 2004). DMBA ≥ 250nM depleted all healthy ovarian follicles in culture following 15 days of incubation. At that time, follicles in ovaries incubated with DMBA had lost their shape, burst, and become part of the interstitial space (data not shown). A similar degree of ovotoxicity was seen in PND4 B6C3F1 ovaries incubated with 30µM VCD (15 days; Rajapaksa, Cannady, Sipes and Hoyer, unpublished data). Therefore, DMBA (250nM) induces ovotoxicity in the culture system at a much lower concentration compared to VCD (30µM). This greater potency of DMBA compared with VCD was also observed in an in vivo 15-day dosing study, where an equivalent degree of follicle loss (ED50) in mice was seen with 0.02 mg/kg DMBA compared with 80 mg/kg for VCD (Borman et al., 2000).

The highest concentration of DMBA (1µM) was then utilized to determine the shortest time point for follicle loss, to be used in studies for evaluating the role of mEH. Ovaries were incubated with 1µM DMBA or vehicle. Following 6 h in culture, DMBA decreased healthy primary and secondary follicles. Secondary follicles in PND4 ovaries begin to form after 4 days in culture. No healthy secondary follicles were observed in ovaries cultured with DMBA, at any time point. However, unhealthy secondary follicles were observed in DMBA-treated ovaries cultured ≥ 4 days. Therefore, DMBA targeted secondary follicles directly, rather than preventing recruitment from the primary to secondary stage. By 8 days, all healthy ovarian follicle populations were depleted in ovaries incubated with DMBA.

Ovarian toxicants such as VCD have been shown to induce follicle loss in rats via acceleration of the natural process of atresia (apoptosis; Springer et al., 1996a,b; Hu et al., 2001a,b). Therefore, to further examine the type of cell death caused by DMBA, ovaries were stained with toluidine blue following 4 days in culture. In ovaries treated with DMBA (1µM), pyknotic bodies were detected in granulosa cells and oocytes of ovarian follicles, while other follicles contained pyknotic bodies along with vacuoles. These morphological changes are characteristic of apoptosis (Kerr et al., 1971; Tome et al., 2001). Thus, it can be hypothesized that DMBA induces ovarian follicle death via apoptosis. Previous studies by Matikainen et al. (2001) have shown an increased expression of Bax (proapoptotic member of the Bcl-2 family of proto-oncogenes) protein in primordial and primary follicles in ovaries incubated with DMBA in culture. Furthermore, follicle loss was not observed in Bax null ovaries cultured with DMBA.
compared to wild-type ovaries. Thus, collectively the results of
these studies suggest that, as with other ovotoxicants, DMBA-
induced follicle loss is via an apoptotic-dependent mechanism.
Toluidine blue staining only affords a morphological assess-
ment. Studies are currently underway to mechanistically eval-
uate DMBA-induced apoptosis using molecular approaches.

An evaluation was made as to whether follicle loss induced
by DMBA involves ovarian mEH. Previous studies have shown
that the adult B6C3F1 mouse ovary expresses catalytically
active mEH, and this activity can be induced in the ovary
following administration of VCD (Cannady et al., 2002). There-
fore, expression of mEH protein in the PND4 ovary
was evaluated by confocal microscopy. mEH was seen to be
expressed in all follicle populations of the PND4 ovary.
Expression was highly concentrated in oocyte cytoplasm.
Unlike the adult B6C3F1 mouse ovary, mEH expression
was not observed in PND4 ovarian granulosa cells (Cannady et al.,
2002). Following DMBA treatment for 6 h, mEH expression
increased in oocytes of primary but not primordial follicles.
This is inconsistent with the observation that primordial
follicles were more sensitive to DMBA-induced toxicity than
primary follicles. Thus, it must be assumed that even though
DMBA is more highly bioactivated in primary follicles, DMBA
can diffuse into other ovarian compartments such as the
primordial follicle pool. Conversely, the basal level of mEH
expressed in primordial follicles may be sufficient to effect-
ively bioactivate DMBA, to cause localized ovotoxicity.

Even though ovotoxicity and increased mEH protein ex-
pression were observed by 6 h following DMBA treatment,
mRNA encoding mEH was not markedly increased until 2 days
in culture. Previous studies have shown an increase in mEH
protein level that could not be explained by an increase in gene
transcription, and thus, mEH is thought to be also regulated
post-transcriptionally (Kim and Kim, 1992; Simmons et al.,
1987). It is also possible that this discrepancy is due to a
decrease in protein degradation between the 6-h and 2-day
time points. Interestingly, the rate of follicle loss markedly
increased between 2 and 4 days. Therefore, the induction of
expression of mEH mRNA directly precedes a significant
increase in the rate of follicle loss at 4 days. The lag in time
between the marked increases in mRNA (2 days) for mEH and
follicle loss (4 days) likely reflects the time between mRNA
translation, mEH-stimulated bioactivation of DMBA, and onset
of follicle loss.

CHO, an mEH inhibitor, was used to block DMBA
bioactivation mediated by mEH. Incubation of ovaries with
CHO alone for 6 h did not affect follicle populations. However,
at that time DMBA induced follicle loss (primordial and
primary, $p < 0.05$). Loss of follicles induced by DMBA was
inhibited by coin cubation of ovaries with CHO. This observa-
tion provides functional support that ovarian mEH plays a role
in bioactivation of DMBA.

In summary, data presented here suggest that DMBA is
a highly potent ovarian toxicant. In these ovarian cultures, and
most likely in vivo, ovarian mEH plays a key role in the
bioactivation of DMBA. Ovarian mEH can be induced by
DMBA at the transcriptional and translational levels. Thus,
DMBA-induced expression of mEH appears to contribute to
the high level of potency of DMBA. These findings support an
extrahepatic role for target organ metabolism of xenobiotic
agents that could amplify potential hepatic effects. Addition-
ally, this study demonstrates that the in vitro PND4 whole ovary
culture system will be useful for investigating ovarian capa-
bilities for metabolism of xenobiotic chemicals.

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