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ETHYLENE GLYCOL MONOMETHYL ETHER (EGME) INHIBITS RAT EMBRYO ORNITHINE DECARBOXYLASE (ODC) ACTIVITY

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ABSTRACT

The effects of ethylene glycol monomethyl ether (EGME) on reproductive outcome in the rat, and on ornithine decarboxylase (ODC) activity in the rat embryo were evaluated. Dams (n=8) were treated by gavage on gestation days 6-12 (sperm = day 0) with 0, 25, 50 or 75 mg/kg EGME in 10 ml/kg distilled water. EGME had a dose-dependent effect on reproductive outcome. Gestation length was prolonged, and the number of litters delivered and neonatal body weight were reduced. Whole embryo ODC was measured on gestation days 9, 11, 13 and 15. ODC attained maximum activity in controls on day 11, increasing by more than an order of magnitude above the activity found on day 9. On day 11, a statistically significant dose-dependent inhibition of ODC activity was observed with the maximum dose of EGME inhibiting ODC activity 60 percent. On days 13 and 15, ODC activity declined markedly from peak values, and the dose-dependent inhibition was no longer evident. The study demonstrates a correlation between the inhibition of embryonic ODC activity by EGME and the effect of EGME on reproductive outcome.

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INTRODUCTION

Ornithine decarboxylase (ODC) has several characteristics that make it potentially useful as a biochemical marker in developmental toxicology. ODC is essential in mammalian cells for the conversion of ornithine to putrescine[1]. This is the rate limiting step in the metabolism of the polyamines, spermadine and spermine, which are involved in the synthesis of proteins and nucleic acids[2,3]. ODC activity is highest in rapidly growing tissues such as regenerating liver, tumors and embryos[4,5,6], and can be induced by various drugs, hormones, cyclic nucleotides and calcium[1,7,8]. The importance of ODC to development has been made evident by the action of DL-a-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC activity. Prenatal administration of DFMO arrests embryonic development in mice, rats and rabbits[9,10], while postnatal treatment with DFMO causes deficiencies in heart, brain and kidney growth in neonates[11]. In addition, a number of studies have demonstrated that the characteristic pattern of ODC activity in brain and heart during fetal and neonatal development is sensitive to prenatal exposure to drugs and alcohol[12,13,14,15]. Therefore, the importance of ODC activity to the developmental process, its sensitivity to prenatal exposures, and its responsiveness to physiological stimulation make ODC a potentially useful marker for evaluating the effects of reproductive toxins on cellular function during development.

Ethylene glycol monomethyl ether (EGME), also known as 2-methoxyethanol, is used as an anti-icing additive in various fluids and fuels, and as a solvent in many lacquers, enamels, varnishes, inks and dyes[16]. EGME is a teratogen in rats and mice and the developing cardiovascular system is particularly

sensitive[17,18,19,20]. The sensitivity of the cardiovascular system led to work examining the effect of prenatal EGME exposure on heart ODC activity in the neonatal rat[21]. ODC was found to be inhibited by EGME, but the inhibition was not associated with overt toxicity, and the use of a single EGME dose precluded the observation of a dose response.

The purpose of the present study was to further evaluate the ability of EGME to inhibit ODC activity during development. The hypothesis tested was that treatment of pregnant dams with EGME has a dose-dependent inhibitory effect on embryo ODC activity during organogenesis, and the inhibition of ODC activity is associated with the developmental toxicity of EGME.

MATERIALS AND METHODS

Experimental Animals

Female Sprague Dawley rats, time-mated by Charles River Breeding Labs (Wilmington, MA) were housed individually in wire mesh cages and provided rat chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum. Dams were maintained on a 12 hr light--12 hr dark photoperiod at 23 $\pm$ 2°C and 55 $\pm$ 15 % relative humidity.

Treatment

On gestation day 5 (sperm = day 0), 160 dams were randomized by weight and assigned to 5 groups. Four of these groups were to be sacrificed on gestation days 9, 11, 13 or 15 for ODC analysis of embryos. The fifth group was allowed to deliver or killed on gestation day 25 if they did not deliver. Each sacrifice group was subdivided into 4 EGME treatment groups of 8 dams each. On gestation days 6-12, dams were treated by gavage with 0, 25, 50 or 75 mg EGME/kg body weight in a constant dose volume of distilled water. Dams sacrificed on days 9 and 11 were not treated with EGME on day they were

killed. EGME (CAS 109-86-4) was purchased from (Cat. No. E182), and certified by (98 % pure), Fisher Scientific Company, Fair Lawn, New Jersey.

Reproductive Assessment

On gestation day 19, dams were transferred to box cages containing nonpyrogenic sawdust bedding. Boxes were examined each day at 5 P.M. for the presence of litters. The day litters were found was recorded as delivery day, and pups were counted and weighed. On gestation day 25, all dams that had not delivered were killed with 95 % CO_2 and uteri were examined for resorptions. Uteri were not stained as EGME normally causes late resorptions that are readily identified.

Embryo ODC Activity

Embryos were obtained from dams on gestation days 9, 11, 13 and 15 for determination of ODC activity following EGME treatment. Dams were killed (CO_2), weighed and the uterus excluding ovaries was removed. Implants were excised one at time. On days 9 and 11, embryos within embryonic sacs were harvested. On days 13 and 15, the embryos were large enough to disect free of all membranes. Each litter was weighed and homogenized in 10 volumes of 50 mM sodium phosphate buffer, pH 7.2. Three 1.5 ml aliquots of the homogenate were centrifuged at 13,000 xg for 10 minutes. The supernatant was frozen at -70 ^{o}C for not more than one week.

ODC Assay

The supernatant was thawed at room temperature and assayed for ODC activity by generating $^{14}CO_2$ from DL(1-^{14}C)-ornithine by a method previously described[21]. One-half ml of freshly prepared incubation medium was added to each 0.5 ml of supernatant. The incubation medium consisted of 50 mM sodium phosphate buffer, 0.1 mM EDTA, 5 mM dithiothreitol, 0.1 mM pyridoxal 5'-phosphate, and 0.5 uCi/ml DL(1-^{14}C)ornithine (specific activity, 55.7 mCi/mmole; radiochemical purity, 97 %,

Amersham, Arlington Heights, IL). $^{14}CO_2$ was captured in center wells (Kontes, Vineland, NJ) containing 0.1 ml hyamine hydroxide (Sigma, St. Louis, MO). Tubes were incubated for 60 min at 37°C, and the reaction was stopped with 2 M citric acid. $^{14}CO_2$ trapped in hyamine hydroxide was counted in 10 ml of Permafluor (Packard, Downers Grove, IL). ODC activity is presented as pmoles CO_2/30 min per mg cytosolic protein and per g embryonic tissue. Protein content of the supernatant was determined by the method of Bradford[22].

Statistical Analysis

Logistic regression was used to analyze the proportion of pregnant dams that delivered[23]. Embryo ODC activity, delivery day and number of live pups were analyzed by performing one-tailed nonparametric tests for trend with increasing level of the EGME treatment[24, 25]. Contrasts for detecting trends as well as comparisons of each EGME treatment to control were performed. All p-values are based on normal approximations to the test statistics. Body weights were analyzed by one-way ANOVA[26].

RESULTS

Maternal Toxicity

The EGME treatment levels did not appear to be maternally toxic as indicated by the absence of differences in maternal weight gain during and following treatment with EGME (Figure 1).

Reproductive Outcome

EGME had a dose-dependent effect on reproductive outcome. None of the pregnant dams treated with 75 mg/kg EGME, and only 50 % of pregnant dams treated with 50 mg/kg EGME delivered pups (Figure 2). The logistic regression indicated that there was a significant downward trend with increasing exposure in the proportion of pregnant dams delivering. A goodness-of-fit test

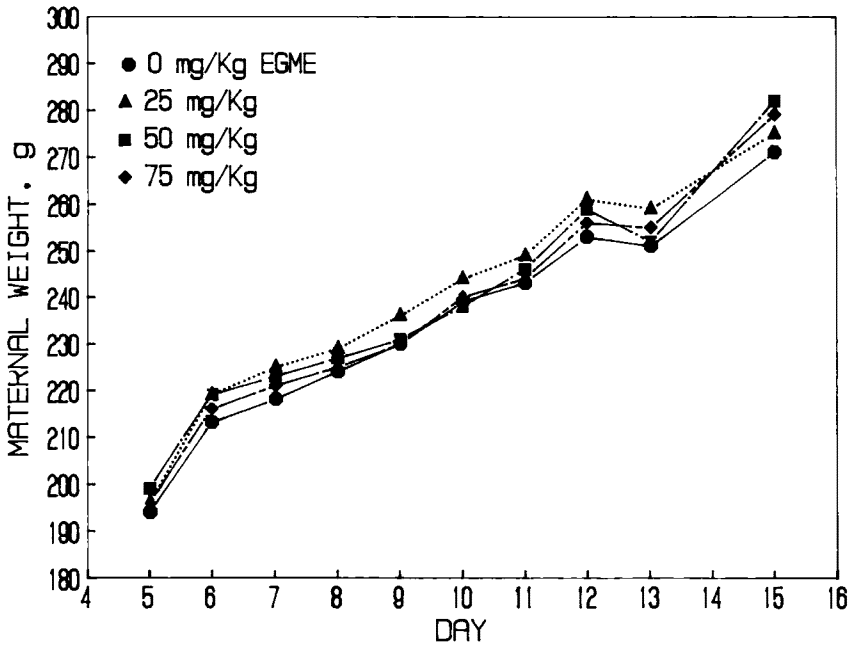

Figure 1. Maternal weight of dams treated on gestation days 6-12 with 0, 25, 50, and 75 mg/kg EGME. Values are means. SE of the means are obscured by the graph symbols.

indicated that the logit-linear model provided adequate fit to these data. Pup weight was significantly reduced, and although litter size was reduced in the 50 mg/kg EGME group, the test for downward trend was not significant (Table 1). Figure 2 illustrates that in addition to reducing the percentage of pregnant dams that delivered, EGME treatment also significantly prolonged gestation.

Embryo ODC Activity

ODC activity per g of embryonic tissue and per mg of cytosolic protein is shown in Figure 3. ODC activity peaked on day 11, increasing by an order of magnitude above the values

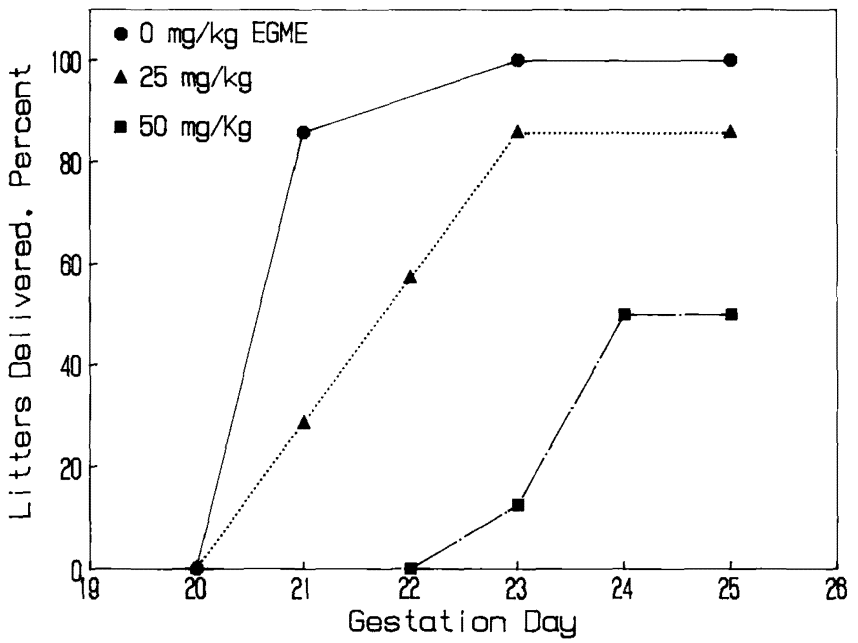

Figure 2. Cummulative percent of litters delivered by pregnant dams treated on gestation days 6-12 with 0, 25, 50 and 75 mg/kg EGME. No litters were delivered in the 75 mg/kg group. Trend analysis and logistic regression indicated that with increasing doses of EGME there was a significant ($P<0.001$) increase in gestation duration and a significant decrease in the proportion of pregnant dams that delivered.

TABLE 1. Reproductive Outcome in Dams Treated with EGME on Gestation Days 6-12 and Sacrificed on Day 25.

	Dose EGME (mg/kg)			
	0	25	50	75
Dams Treated	8	8	8	8
Dams Pregnant	7	7	8	6
Litter Size	10 ± 2	12 ± 1	7 ± 4	
Pup Wt (g)	9 ± 1	9 ± 1	*7 ± 1	

Dams are Totals. Litter Size and Pup wt are Mean ± SD and were recorded on gestation day 25.
* Significantly different than control (P 0.05).

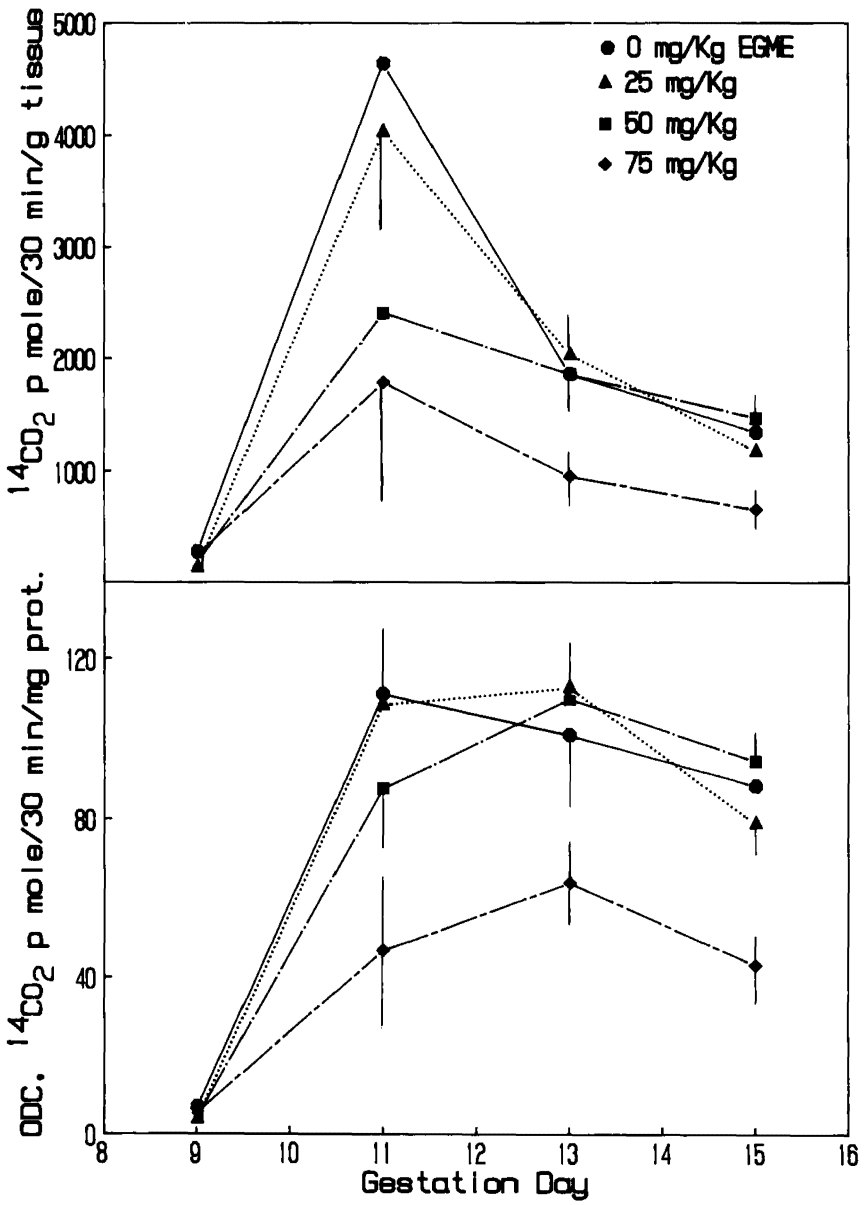

Figure 3. ODC activity per mg protien and per g tisuue in embryos from dams treated on gestation days 6-12 with 0, 25, 50, and 75 mg/kg EGME. Dams were not treated with EGME on days they were sacrificed. Values shown are the mean $\pm$ SE. Trend analysis indicated that there was a significant ($P<0.05$) dose-dependent decrease in ODC activity expressed per mg protein or per g tissue only on gestation day 11.

obtained on day 9 of gestation. When expressed per g of tissue, ODC activity on day 13 dropped to 20 % of that on day 11 due to the marked increase in tissue mass during this period (data not shown). When expressed per mg of cytosolic protein, the drop from peak activity on day 11 was more gradual.

A statistically significant downward trend in ODC activity with increasing EGME exposure was found only on day 11. On day 11, exposure to 75 mg/kg EGME decreased ODC activity per g of tissue by 60 %. On gestation days 13 and 15, exposure to 75 mg/kg EGME inhibited ODC activity by 48 and 50 percent respectively, but the dose dependent effect was no longer evident.

DISCUSSION

The EGME treatment used in the present study caused severe developmental toxicity in the absence of maternal toxicity. The high dose of EGME caused all litters to be completely resorbed. The intermediate dose reduced the number of litters and pup body weight, and prolonged gestation. These observations are consistent with previous developmental toxicity studies with EGME[17,18,19,20]. The present study also demonstrates an inhibition of ODC activity on gestation day 11, when activity is maximal, that is closely associated with the developmental toxicity of EGME. There is no evidence of a cause and effect relationship, but the dose-dependent inhibition of ODC activity corresponds directly with the dose-dependent effects of EGME on reproductive outcome. This finding gives support to the use of ODC activity as an early biochemical marker of developmental toxicity. It is important to note that the ODC activity is not a more sensitive marker of EGME treatment than offspring viability or body weight. The low dose of 25 mg/kg EGME was essentially a no-effect level for all the variables examined.

An important observation that is not evident from the data is that the majority of mothers in the high EGME dose group carried their litters to term. The sacrifice on gestation day 25 revealed many late resorptions and dead fetuses that were only in the early stages of resorption. Unfortunately, all dead pups, and early and late resorptions were recorded simply as resorbed litters and a precise break down is not available. Nonetheless, this observation indicates that the inhibition of ODC activity on day eleven does not necessarily signify embryo death, but rather an effect that prevents birth of viable offspring. This suggests that a significant portion of the fetal mortality may stem from an effect of EGME that interferes with parturition. Hardin[18] has suggested that the prolonged gestation following exposure to the glycol ethers could be due to impaired uterine smooth muscle contractility. How such an effect would be mediated by an EGME treatment on gestation days 6-12 is not apparent, but does warrent further investigation.

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