Effects of ethanol on the decidual cell reaction in rats

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Summary. The effects of ethanol on uterine sensitivity to induction of decidualization and deciduoma growth were determined. Rats were ovariectomized, given an oestrogen–progesterone regimen to optimize induction and growth of deciduoma and randomly assigned to one of three ethanol treatment groups: (i) days 1–4 (pre-induction/period of sensitivity), (ii) days 5–9 (post-induction/period of growth), (iii) days 1–9 (periods of sensitivity and growth); or to a control group not treated with ethanol (pair-fed to treated groups). Ethanol (0, 1, 2, or 4 g kg⁻¹) diluted in water was administered by stomach tube on the days prescribed. Decidualization was induced in one uterine horn by intraluminal injection of sodium phosphate buffer. Uterine sensitivity and decidual growth were assessed as cornu weight. Blood alcohol concentrations were measured by gas chromatography. Alcohol treatment reduced uterine sensitivity, but increased deciduoma growth. Blood alcohol concentrations rose to 133 mg% at 30 min, remained high for 90 min and declined to 82 mg% at 120 min. Thus, blood alcohol concentrations sufficient to induce mild intoxication in humans suppressed uterine sensitivity to decidualization and enhanced deciduoma growth in rats. As all ovarian steroid hormone support was exogenous, the effects of ethanol on deciduoma induction and growth were not due to alterations in the hypothalamic–pituitary–ovarian axis.

Keywords: decidualization; alcohol; implantation; rat

Introduction

Alcohol is associated with deleterious effects during all stages of pregnancy (Abel, 1990). In rats exposure to alcohol during pregnancy adversely affects fecundity (Tze & Lee, 1975; Testar et al., 1986) and gestational outcome (Anders & Persaud, 1980; Fernandez et al., 1983). Alcohol treatment impairs post-implantation embryo development (Sandor et al., 1980; Pennington et al., 1984) and increases the incidence of early resorption (Anders & Persaud, 1980). Such effects may result, in part, from alcohol-induced impairment of decidualization. This paper reports the results of experiments on the effects of alcohol on the decidual cell reaction in ovariectomized rats receiving an exogenous ovarian steroid hormone regimen optimal for supporting uterine sensitivity to deciduogenic stimulation and deciduomatal growth.

Materials and Methods

Animals

Nulliparous rats (Sprague–Dawley, Harlan), 200–250 g body weight, were maintained in individual cages in a controlled environment: room temperature (20–25°C), humidity (40–60%) and photoperiod (14 h light:10 h dark, lights on at 02:00 h). All alcohol-treated rats were provided with tap water and laboratory rat chow (R. Purina) ad libitum. Control rats were pair-fed to the high-dose, alcohol-treated group (i.e. daily food was limited to an amount

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equivalent to that consumed by the group treated with 4 g ethanol kg⁻¹. To compensate for calories received as alcohol by treated rats, controls received a daily dose of sesame oil (approximately 1 ml) in water via a stomach tube. Each dose (typically 3 ml) was isocaloric to 4 ml alcohol kg⁻¹ (assuming 7 calories g⁻¹ ethanol). Body weight and food consumption were measured daily.

**Ovariectomy and ovarian steroid treatment**

Animals were anaesthetized with ether and ovariectomized between 10:00 and 12:00 h. On the third day after ovariectomy, hormone treatment was initiated by subcutaneous injection of 500 µg oestrone in maize oil for two consecutive days (Yochim & DeFeo, 1963). Vaginal smears were taken daily thereafter until the appearance of leucocytes (typically 3 days). Beginning on the first day of leucocytic vaginal smears, 100 µg oestrone plus 20 mg progesterone was administered between 10:00 and 12:00 h for nine consecutive days.

**Induction and assessment of decidualization**

On the fourth day of oestrone–progesterone treatment, the deciduogenic stimulus was applied between 11:00 and 12:00 h. Animals were anaesthetized with ether, a uterine horn was exposed via a small abdominal incision and 0.2 ml of 0.1 mol sodium phosphate buffer 1⁻¹ was injected into the uterine lumen at the tubal end of one horn using a 26-gauge hypodermic needle. On the ninth day of hormone treatment, animals were killed and the uterine horns removed, cleaned of adnexa and weighed to the nearest 0.1 mg. Uterine weight was used as an index of uterine sensitivity to deciduogenic stimuli and decidualoma growth.

**Experimental groups**

Animals were randomly assigned to one of four groups: (i) alcohol-treated days 1–4 (pre-induction = period of sensitivity); (ii) alcohol-treated days 5–9 (post-induction = period of growth); (iii) alcohol-treated days 1–9; (iv) non-alcohol-treated controls. Animals in each group received one of four doses: 0, 1, 2, or 4 g alcohol kg⁻¹ body weight.

**Alcohol treatments**

Alcohol (100% pure ethanol) was diluted in water: 15% v/v, 1 or 2 g alcohol kg⁻¹ body weight; or 30% v/v, 4 g alcohol kg⁻¹ body weight. Alcohol solution was administered via a stomach tube between 10:00 and 12:00 h on the days prescribed.

**Analysis of alcohol concentrations in blood**

Concentrations of alcohol were measured by head-space gas chromatography (Subramanian et al., 1990). After administration of 2 g ethanol kg⁻¹ via gavage, blood samples (100 µl) were collected from gently restrained rats by tail-tip sampling at 0, 30, 60, 90 and 120 min after alcohol administration and diluted in 1:0 ml 1% T-butanol solution.

**Statistical analysis**

Sample means from control and experimental groups were compared using analysis of variance and Duncan’s multiple-range test. A P value of ≤ 0.05 was assumed to indicate a statistically significant difference between groups.

**Results**

Daily administration of alcohol during the period of endometrial preparation for decidualization suppressed sensitivity to the deciduogenic action of phosphate buffer (Table 1). Doses of 2 and 4 g kg⁻¹ resulted in an approximately 30% decrease in decidualization, whereas 1 g kg⁻¹ was ineffective. By contrast, the 2 g kg⁻¹ dose significantly enhanced decidualoma growth when administered after delivery of the decidualizing stimulus. Exposure to alcohol before and after administration of phosphate buffer had no effect on the magnitude of decidualization, except at the intermediate dose (2 g kg⁻¹), which stimulated decidualoma growth. None of the alcohol treatments resulted in a significant increase in the weight of the non-decidualized horn; thus, alcohol treatment per se was not uterotrophic (data not shown).
All treatment groups were pair-fed to the 4 g kg\(^{-1}\) dose group, consumed equivalent amounts of food and did not differ in mean body weight (data not shown). The observed changes in decidualization were therefore not a result of altered caloric intake. Concentrations of alcohol in blood were greatest 30 min after treatment (133 mg%), remained high until 90 min (110 mg%) and declined to 82 mg% at 120 min.

### Discussion

In intact rats, uterine sensitivity to deciduogenic stimuli and subsequent decidua growth depend on a carefully regulated sequence of endocrine events in which the hypothalamic–pituitary–ovarian axis co-ordinates the timing and magnitude of oestrogen and progesterone secretion necessary to induce uterine sensitivity to decidualization and to sustain decidua growth (DeFeo, 1967; Glasser & Clark, 1975). In the present experiments, however, the endogenous secretion of oestrogen and progesterone was replaced by exogenous administration of ovarian steroids. Thus, the changes in uterine decidualization were not the result of altered ovarian steroid secretion induced by alcohol acting on the hypothalamic–pituitary–ovarian axis (Rettori et al., 1987). However, alcohol may have altered hepatic catabolism of steroids (Gordon et al., 1976) thereby changing the ratio of exogenous oestrogen and/or progesterone in plasma. Consequently, the time of greatest uterine sensitivity to deciduogenic stimuli may have shifted (Yochim & DeFeo, 1963).

Alcohol exerts a range of effects on cell function, which may adversely influence decidualization. Alcohol-induced alterations in cell membranes (Sun & Sun, 1975; Goldstein, 1985) may alter intracellular junction formation (Kleinfeld et al., 1976; Brökelmann & Biggers, 1979), impair the transduction of signals between epithelium and stroma (Renfree, 1980) or modify hormone receptor function (Cicero et al., 1979) necessary for induction of decidualization. As decidualization requires increased synthesis of DNA (Moulton & Koening, 1984) and protein (Bell et al., 1980) and enhanced carbohydrate metabolism (Yochim, 1975; Bell, 1983), alcohol impairment of amino acid transport (Heitman et al., 1987), DNA synthesis (Dreosti et al., 1981), protein synthesis (Rothschild et al., 1987) or glucose metabolism (Murdoch, 1987) could contribute to the observed changes in decidua induction and growth.

Among the earliest detectable responses to deciduogenic stimuli are alterations in concentrations of cyclic nucleotides (Rankin et al., 1977; Sanders et al., 1986). Thus, cyclic AMP (cAMP) is thought to play a role in the initiation of decidualization. Cyclic nucleotides may also be involved in decidua growth as cAMP concentrations influence duration of the cell cycle and tissue growth (Pastan et al., 1975). Alcohol alters cAMP concentrations (Weathersbee & Lodge, 1978) and binding activity (Pennington, 1988). Alcohol-induced alterations in the adenyl cyclase and protein kinase cascade have been implicated in retarded growth observed in infants exposed to alcohol in utero (Becker et al., 1988).

Other early events associated with induction of decidualization include altered concentrations of prostaglandins and increased endometrial vascular permeability. Uterine concentrations of
prostaglandins E and F increase within 15 min of decidualogenic stimulation (Kennedy, 1980) and are involved in subsequent enhanced vascular permeability (Kennedy, 1979) and in differentiation and growth of decidual cells (Kennedy, 1985). Alcohol alters tissue (Pennington et al., 1985; Randall et al., 1987) and vascular (Karanian et al., 1978; Randall et al., 1987) prostaglandin concentrations. Prostaglandins have been implicated in diverse aspects of fetal alcohol syndrome (Randall et al., 1987).

Alcohol markedly increases blood flow to sites of decidualization in rats (Mitchell & Goldman, 1991). One consequence of alcohol-induced enhancement of nutrient blood flow is a concomitant increase in intruterine oxygen tension (Mitchell & Van Kainen, 1990). The increase in oxygen availability is prompt and protracted. Such effects of alcohol on uterine physiology are consistent with the observed increase in deciduoma growth.

Clearly, alcohol may alter normal uterine function in a number of possible ways. Further experiments will be required to determine the primary means by which alcohol achieves its effects on endometrial physiology. However, the present studies clearly indicate that alcohol exerts diverse effects on decidualization, a key event in the establishment and maintenance of normal pregnancy.

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References


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