IMMATURE MOUSE UTERINE TISSUE IN ORGAN CULTURE: ESTROGEN-INDUCED GROWTH, MORPHOLOGY AND BIOCHEMICAL PARAMETERS

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(Received 12 March 1993; accepted 13 October 1993)

SUMMARY

Although estrogens have been shown to stimulate a variety of morphologic and biochemical changes in the uterus in vivo, no clear consistent demonstration of similar responses in vitro have been made; thus, a defined organ culture system using the immature mouse uterus was established to study the possibility of demonstrating estrogenic responses in vitro. Uterine tissue from immature outbred mice (17 to 24 days of age) were cut crosswise in 1-mm³ coins and cultured in a defined medium in the absence of serum, phenol red, or growth factor supplements. Diethylstilbestrol (DES), a synthetic estrogen, was added to the media at doses ranging from 1 to 100 ng/ml. The effect of DES on uterine cell proliferation was assessed by morphologic changes in uterine epithelial and stromal cells, increase in number of epithelial cells per unit basement membrane, increase in height of luminal epithelial cells, and [³H]thymidine incorporation. Functional changes were determined by measuring the amounts of the estrogen-inducible uterine protein, lactoferrin, that was localized in the epithelial cells and secreted into the media, and the localization of the estrogen receptor in the cultured tissues. Results indicate that under the described conditions of culture, estrogens like DES can induce morphologic and biochemical responses in the uterus that are similar to those seen in vivo. This organ culture system will aid in the investigation of various mechanisms involved in the hormonal regulation of growth and differentiation of estrogen target tissues.

Key words: uterus; estrogenic responses; lactoferrin; in vitro; organ culture; diethylstilbestrol; DES.

INTRODUCTION

Estrogens regulate growth and cell division in target tissues such as the uterus. The process of regulation is thought to involve the initial binding to high-affinity nuclear receptors which, in turn, bind to specific DNA sequences that regulate gene transcription (Yamamoto, 1985). However, as discussed in several reviews (Sonnensohn and Soto, 1980; Sirbasku and Benson, 1979; Cunha et al., 1983; Martin, 1980), the actual series of events that mediate the mitogenic effect of estrogen in the uterus remains unknown.

One theory suggests that estrogens act directly as a mitogen on target cells. Another hypothesis is that estrogens promote uterine growth indirectly via a local (paracrine, autocrine) or systemic (endocrine) regulation of levels of certain growth factors or their receptors or both. In fact, epidermal growth factor (EGF)-like and insulin-like peptides have been reported in the conditioned media of human breast cancer cells grown in the presence of estrogen (Lippman et al., 1986). Other studies (Mukku and Stancel, 1985a) have demonstrated EGF receptors in the rat uterus and have shown that EGF receptor levels increased threefold after estrogen administration (Mukku and Stancel, 1985b). In addition to modulation of the EGF receptor, estrogens stimulate a rapid increase in the uterine level of the EGF peptide (DiAugustine et al., 1985); these reports have further shown that EGF mRNA is detectable in uteri of ovariectomized mice, and its level of message is apparently stimulated by treatment with estrogen (DiAugustine et al., 1988).

Another theory of indirect estrogen action suggests that the removal of growth inhibitors will allow cell proliferation to occur (Soto and Sonnenschein, 1987). Support for this theory can be found in many studies suggesting little proliferative response to estrogens in vitro where the data are interpreted to be the ineffective removal of growth inhibitors by in vitro conditions (Soto and Sonnenschein, 1987). It can also be argued that suboptimal in vitro conditions cause the loss of a functioning estrogen receptor resulting in a decreased proliferative response to estrogens in culture.

For the most part, experiments designed to study the possible mechanisms of estrogen action have been carried out in isolated cell culture systems. Uterine endometrial cells have been grown in vitro by a number of laboratories (Gerschenson et al., 1974, 1979; Iuchi et al., 1985; Tomooka et al., 1986; Uchima et al., 1991), but the response of these cells to hormones is inconsistent, probably due to the varied culture conditions, media additives, growth factor supplements, and cell isolation methods that have been employed. For example, Fuchamachi and McLachlan (1991) have reported that estradiol suppresses uterine epithelial proliferation in vitro,
whereas other laboratories describe either no effect (Ghosh et al., 1991) or an enhanced effect (Gerschenson et al., 1977) of estradiol on proliferation.

Apparently, not only does the proliferative response vary in cultured uterine endometrial cells, but functional responses to estrogen also differ with culture conditions (Julian et al., 1992a,b). Using isolated uterine endometrial cells that were allowed to reestablish their polarized phenotype, functional responses to estradiol, such as the secretion of specific estrogen marker proteins, were demonstrated not to be estrogen dependent in vitro (Julian et al., 1992a,b). However, in this in vitro model system (Julian et al., 1992a, b), the cells were grown in media supplemented with a serum extender containing a variety of growth factors and hormones that may have masked the effects of estrogens. Thus, an in vitro model mimicking similar in vivo responses of the uterus to estrogens remains to be described. Furthermore, to study the role of growth factors and inhibitors in the response of tissues to hormones, an essential requirement of a model culture system would necessitate the addition of no growth factor supplements or serum additives.

Previously, we reported a serum-free organ culture system in which the biochemical and morphologic integrity of fetal mouse uterine tissues were maintained in vitro (Newbold et al., 1981; Carter et al., 1982; Maydl et al., 1983; Newbold et al., 1984). Using modifications of this organ culture system, including phenol red-free media, the present report describes morphologic and biochemical effects of estrogens on immature uterine tissue. These findings are significant because the literature contains no other example of a dose-related response of normal uterine tissues to estrogens in vitro in the absence of exogenous growth factors. The importance of maintaining stromal and epithelial cell interactions as well as the role of autocrine or paracrine regulatory factors, or both, is discussed.

**MATERIALS AND METHODS**

*Animals.* Immature (17 to 24 days of age) female CD-1 mice [Cr:CD-1(ICR)BR; Charles River Breeding Laboratories, Raleigh, NC] were housed under controlled lighting (12 h light and 12 h dark) and temperature (21° to 22° C) conditions in the animal facility at the National Institute of Environmental Health Sciences (NIH). Mice were provided with NIH 31 laboratory mouse chow and fresh water ad libitum. All animal procedures complied with NIH animal care guidelines.

*Organ culture conditions.* Mice were killed by cervical dislocation and the uterus aseptically removed, placed in Earle's phosphate buffered saline (pH 7.2), and cut crosswise into 1-mm² coins using cataract knives. The tissue was transferred to a strip of 1% agar (Difco Laboratories, Detroit,