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ABSTRACT

The effect of a combined dose of pregnant mares' serum (PMS) and human chorionic gonadotropin (HCG) on male and female rats 2-25 days old was studied. Groups of animals were given injections for three days, then sacrificed on the fourth day. All injections were begun on the 2nd, 7th, 12th, 17th, and 22nd day of life. Ovaries, uteri, seminal vesicles (SV), and ventral prostate (VP) were removed, dissected free of fat, and weighed. Because uterine weight increased earlier than either SV or VP weight, the ability of the uterus to respond to exogenous estradiol and of the SV and VP to respond to exogenous testosterone was examined. The injection schedule, age groups, and day of sacrifice were the same as above. The uterine response to estrogen was found to appear earlier than the VP-SV response to testosterone. The observations suggest that the receptor sites for estrogen may mature earlier than the receptor sites for testosterone.

INTRODUCTION

McQueen-Williams (1935) and Stein (1935) reported that peak ovarian weight response to a preparation of follicle stimulating hormone (FSH) and/or pregnant mares' serum (PMS) occurs at the end of the third week of life in the rat, rather than at some time during or after sexual maturity. Additional evidence that the ovaries undergo a period of increased responsiveness to gonadotropins was produced by Cole (1937), who observed both superovulation and superfecundity after injecting 22-day-old female rats with PMS. Cole believed that a response of any nature to gonadotropins does not occur with any consistency in females younger than 21-22 days of age. Greep (1961) concluded that ovaries in rats are refractory to exogenous gonadotropins until the 15th day of life.

It generally has been accepted that HCG will augment the action of either PMS or FSH on the ovary (Bates and Schooley 1942). Steelman and Pohley (1953) showed that combining HCG with small doses of FSH produced a marked and linear increase in ovarian weight. FSH alone in the same dose failed to elicit a significant weight response. These findings indicate that HCG makes intact ovaries of immature female rats extremely sensitive to exogenous FSH. This study, however, was conducted on 21-22-day-old rats, and no attempt was made to evaluate the effect of such an augmented gonadotropic stimulus on the ovaries of younger animals. Neither was the effect of combined gonadotropins on immature male sex organs (SV or VP) investigated.

The purpose of the present study was to investigate the effect of a combined dose of exogenous gonadotropins (PMS and HCG) on the ovaries, uteri, VP, and SV in rats that had not reached the 22nd day of life. An increase in uterine weight was considered the result of gonadotropin-mediated ovarian estrogen production and secretion; increased VP and SV weight was considered the result of gonadotropin-mediated testosterone production and secretion by the testes (Korenchevsky 1930, Sawin 1969, Velardo 1959). After delineation of these response patterns, estrogen and testosterone alone were administered exogenously to groups of rats younger than 22 days of age. Both studies were designed to show a graphic illustration of hormone receptor site maturation and resultant secondary sex organ response.

MATERIALS AND METHODS

Newborn Sprague-Dawley (Charles-River) rats were divided into five groups and each group was given injections of gonadotropins beginning on the 2nd, 7th, 12th, and 22nd day of life. The day of birth was considered to be day one. Each animal was given a total dose of five International Units (IU) of PMS (Nutritional Biochemicals) and 20 IU of HCG (Nutritional Biochemicals) dissolved in distilled water. Subcutaneous injections of the combined gonadotropins (0.1 ml) were given three times a day for three days. Control rats were injected concurrently with distilled water. On the fourth day (72 hours after the first injection) the animals were sacrificed by concussion; the organs were removed and trimmed free of excess tissue, and weighed to the nearest one-tenth of a milligram on a Roller-Smith torsion balance. The autopsies, therefore, were performed on the 5th, 10th, 15th, 20th, and 25th days of life. Actual organ weights were converted to mg of organ weight per 100 g of body weight (relative weight).

With the same age groups, injection schedule, and day of sacrifice, a total dose of three μg of 17-alpha estradiol (Nutritional Biochemicals) and 1500 μg of testosterone (Philadelphia Ampoule Laboratories) was given to females and males, respectively. The solvent for each was distilled water; control groups received injections of the solvent. Organs were trimmed, weighed to the nearest 0.1 mg, and converted to relative weight.

STATISTICS

The Student's *t*-test was used to compare mean organ relative weights of treated groups with mean organ relative weights of corresponding control groups. Calculations were based on $n = 12$ animals per group.

RESULTS

Rats injected with gonadotropins on days 22-24 showed a highly significant ($p < 0.01$) increase in ovarian weight (Fig. 1). This difference was also significant on the 15th and the 20th days (both $p < 0.01$), but the weights failed to vary from control weights on the 5th and 10th days.

Uterine weight increased rapidly between the 10th and 15th days of life (Fig. 1), and an increase was noted in both controls and in animals treated with gonadotropins. However, gonadotropin-treated animals had significantly higher relative weights on the 15th day than controls ($p < 0.01$).

In males, combining gonadotropins resulted in a gradual increase in the relative weights of both SV and VP which became significant by the 15th day of life ($p < 0.01$); the gain increased with the age of the rat (Fig. 1).

When female rats were given a total dose of 3 μg of 17-alpha estradiol over a three-day period, the uterus increased markedly in relative weight (Fig. 1). This increase was particularly noteworthy during the 5th through the 10th days of life. A large dose of 17-alpha estradiol (25 μg) elicited virtually the same response as did 3 μg. Such a response was unobtainable in the SV and VP by administering 1500 μg testosterone (Fig. 1).

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DISCUSSION

The findings indicate that the ovaries can be stimulated by combined exogenous gonadotropins to begin secreting estrogen shortly after the 10th day of life. This conclusion was reached because of the observed weight increase of the uterus which typifies this organ's unique response to estrogen. The binding of estrogen and resultant protein synthesis have been considered to be mediated by specific receptors in the nuclei of steroid target cells (Jensen and Jacobson 1962, Jensen and DeSombre 1972, O'Malley and Means 1974). These workers used both 17-beta estradiol and ultracentrifugation separation techniques to demonstrate both estradiol organ specificity and nuclear concentration. Toft and Gorski (1966) showed that the binding can be disrupted by proteolytic enzymes, a finding which suggests that the receptors are protein in nature. More recent studies have shown that these proteins also have binding specificity for entities other than estrogens, i.e. DNA (Yamamoto 1974a, b).

The results also suggest that the hormone receptor mechanism for estrogen in the uterus undergoes early maturation, enabling exogenous estrogen to induce significant uterine weight gain at four days of age. When combined gonadotropins (PMS and HCG) were given, the initial uterine weight increase occurred later (after 10 days), suggesting this to be the age in the rat at which the ovaries can release estrogen upon sufficient gonadotropin stimulation.

The SV and VP response indicates an absence of both testicular response to gonadotropins and secretion of testosterone until after the 10th day of life. Unlike the uterine response to estrogens, neither of these organs responds to exogenous testosterone until after the 10th day of life.

Presumably, the maturation process involves the receptor sites, although other biochemical entities (cyclic AMP, Sutherland et al 1965) should not be overlooked. Other studies to detect production of steroids within the cell could supply additional pertinent information. Histochemistry, for example, could be used to illustrate intracellular synthesis of steroids and to point out any age discrepancies in this process. Use of such an approach could provide a method for both extending and further clarifying the present findings.

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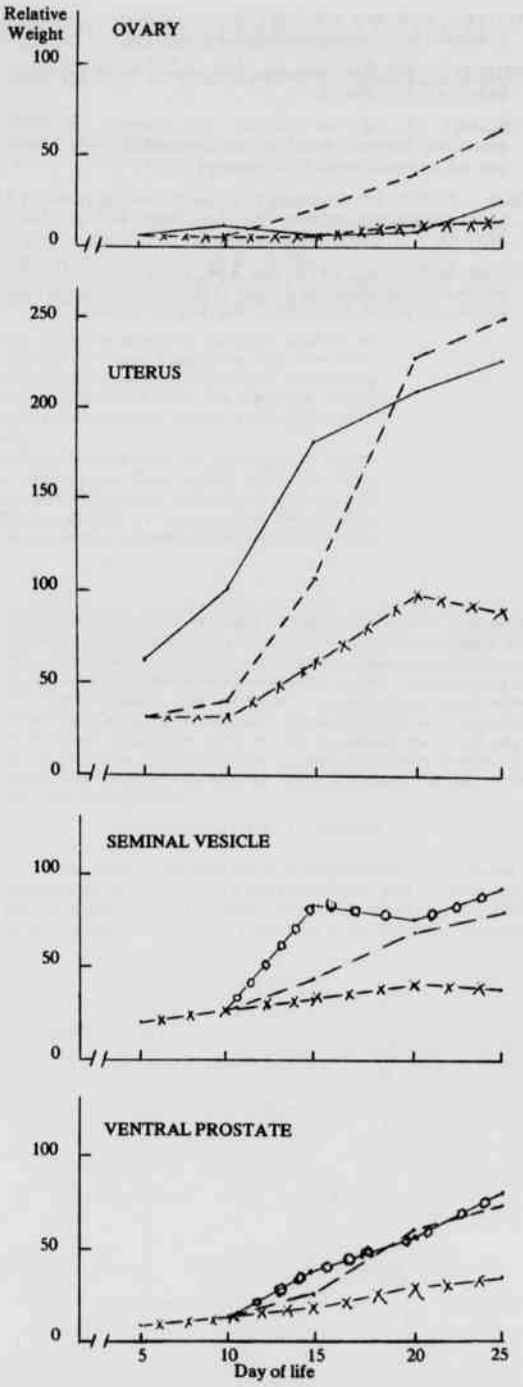

Figure 1. Change in relative weights of immature rat sex organs after treatment with various hormones. Control -x-x-x-; 17-alpha estradiol (3 μg) _____ ; testosterone (1500 μg) -o-o-o-; 5 IU PMS plus 20 IU HCG - - - - -.

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