Uterine glucose metabolism in the prepubertal rat treated neonatally with androgen, estrogen, and antihormones[1]

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Summary. Estrogen secretion during infancy may selectively enhance the phosphogluconate oxidative pathway in the rat uterus, for altered estrogen-stimulated glucose oxidation prepubertally is correlated (+0.91) with impaired ovarian development and not uterine estrogen receptor content.

Key words. Uterus, glucose; metabolism; ovary; neonatal; sex steroids.

Animals born in a very immature state, such as the rat, exhibit a number of reproductive anomalies as a consequence of exposure to sex steroid hormones during the infantile period[2-4]. The reproductive impairment in the adult is preceded by altered reproductive tissue function in the immature rat [5,6]. One aspect, impaired uterine growth responsivity evident in the androgenized or estrogenized prepubertal rat, is the consequence of decreased function of the hypothalamic-pituitary-ovarian axis [6]. The decreased uterine responsivity is due to independent, direct effects at both the hypothalamic and ovarian levels[7]. Furthermore, the degree to which uterine growth responses at 21 days of age are impaired by neonatal treatment with different doses of testosterone propionate (TP) or estradiol benzoate (EB) directly corresponds to the reduction in ovarian weights observed in such animals [6]. However, while neonatal EB treatment compromises later availability of uterine estrogen receptor, TP treatment neonatally does not. Neonatal ovariectomy also compromises the prepubertal uterine growth response to exogenous estradiol to the same extent as neonatal EB treatment [6], but neonatal ovariectomy does not decrease the production of estrogen receptor protein[8]. Thus, the objective of this study was to provide further insight into the influence of ovarian function upon the functional development of the uterus.

Materials and methods. Sprague-Dawley derived rats were obtained from Southern Animal Farms (Prattville, AL) and bred in the UAH animal facility. The animals were maintained in a controlled environment on a 13-h light-11-h dark cycle and provided commercially pelleted feed and water ad libitum. The morning on which pups were found was designated day 1 of life. Rats were injected with the different compounds utilized (see the caption to fig. 1) on day 3 of life when administered singly or on days 3 and 4 of life when administered in combination. Injections were administered subcutaneously using peanut oil as the vehicle. TP, EB and aromatase inhibitor (1,4,6-androstatrien-3, 17-dione) were purchased from Sigma Chemical Co. (St. Louis, MO) or Steraloids, Inc. (Wilton, NH). The antiandrogen, cyproterone acetate, and the antiestrogens, nafoxidine and CI-628, were generous gifts from Schering AG (Berlin, W. Germany), the Upjohn Co. (Kalamazoo, MI) and Warner-Lambert/Parke-Davis (Detroit, MI), respectively.

The oxidation of [U-^{14}C] glucose to $^{14}CO_2$ was determined by the method of Nicolette and Gorski[9]. Rats were injected s.c. on day 21–23 of life with 0.1 µg estradiol and killed 3 h later. Uteri were excised and placed into individual 25-ml Erlenmeyer flasks containing 2 ml Eagle's medium (Difco) and 10^{-6} M [U-^{14}C] glucose (0.5 µCi total activity). The radioactive glucose (SA 1.19–1.92 µCi/µg) was obtained from New England Nuclear Corp. and had a radiochemical purity of 99%. The flasks were aerated with a 95% O_2–5% CO_2 gas mixture and immediately sealed with rubber serum stoppers containing filter paper wicks soaked with 0.25 ml methyl benzonium hydroxide (Sigma Chemical Co.) to trap evolved $^{14}CO_2$. The flasks were incubated for 1 h at 37 °C. At the end of this incubation, 1.5 ml of 5 N H_2SO_4 was injected through the stopper of each flask to halt the reaction and promote release of $^{14}CO_2$ from the incubation medium. After an additional 2-h incubation at 37 °C to completely trap the $^{14}CO_2$, the filter paper wicks were removed and placed inside scintillation vials containing 10 ml Aquasol (NEN). The amount of glucose oxidized was expressed as µM x 10^4/µg glucose/uterus.

Estrogen receptor analysis was performed using the charcoal-dextran assay procedure of Korenman[10]. A single point assay utilizing 10 nM 6,7 [^{3}H]-estradiol (Sa 49–51 Ci/mmol; 98% radiochemical purity; NEN) plus or minus 100-fold excess diethylstilbestrol (DES, Sigma Chemical Co.) was employed. This hormone concentration is sufficient to occupy at least 95% of the specific estrogen binding sites[11]. Specific estrogen binding is determined by subtracting the radioacitivity in the assay tubes containing the labeled estrogen and excess DES (nonspecifically bound estradiol) from the radioactivity in the assay tube containing only [^{3}H]-estradiol (total bound estradiol). Bound [^{3}H]-estradiol was expressed at pM/uterus.

Results. As indicated in figure 1, there is a direct relationship between ovarian weight in the prepubertal rat treated neonatally with a variety of hormones and antihormones and the degree to which exogenous estradiol elicits stimulation of glucose metabolism by the uterus. This relationship between prepubertal ovarian weight and the ability of the uterus to metabolically respond to estradiol has a correlation coefficient of +0.91. Treatment with cyproterone acetate on day 3 of life does not impair ovarian development nor the capacity of the uterus to respond normally to estradiol at weaning. Neonatal exposure to 1250 µg TP results in a moderate reduction in ovarian weight, while sequential daily

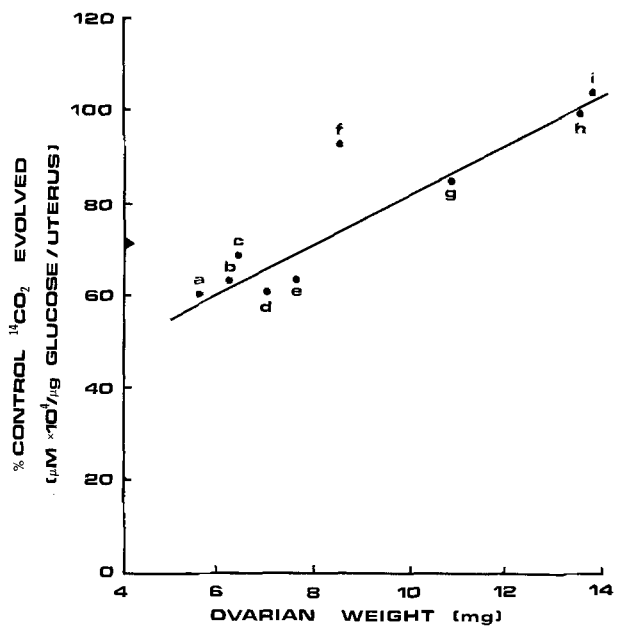

Figure 1. The relationship between ovarian weights in 21-day-old rats and estrogen-stimulated glucose oxidation in the uterus. Each point represents the mean of at least 8 determinations: a, 50 µg CI-628 (day 3) + 100 µg EB (day 4); b, 100 µg EB (day 3); c, 1250 µg TP (day 3) + 1250 µg TP (day 4); d, 50 µg nafoxidine (day 3); e, 50 µg nafoxidine (day 3) + 100 µg EB (day 4); f, 50 µg CI-628 (day 3); g, 1250 µg TP (day 3); h, peanut oil control (day 3); i, 2.0 mg cyproterone acetate (day 3). The point indicated on the y axis represents the effect of neonatal ovariectomy (day 3). The graph line was determined by performing a least-squares linear regression (y = mx + b). The data fit the linear curve with a correlation coefficient of +0.91.

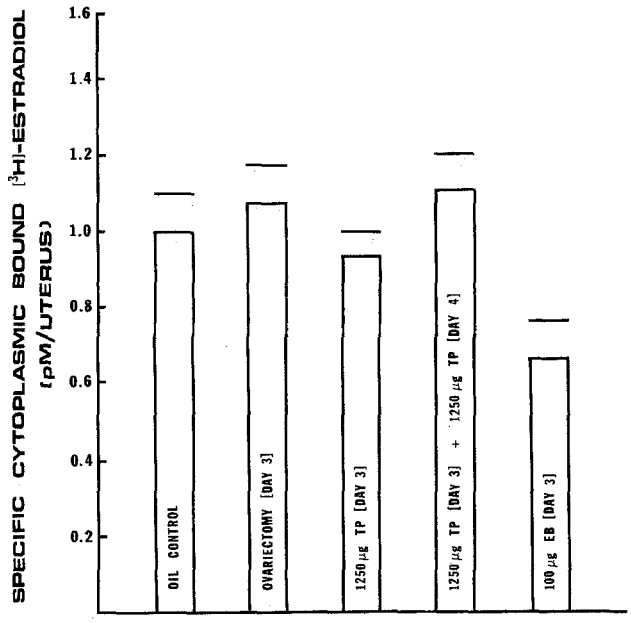

Figure 2. Cytoplasmic estrogen binding sites in the uterus of 21-day-old rats after neonatal hormone treatment or ovariectomy. Each bar represents the mean ±SEM of at least 8 experiments. Treatment with 100 µg EB on day 3 of life significantly ($p < 0.025$, Dunnett's t) reduced the content of the estrogen receptor in the prepubertal rat uterus.

administration of the same dose of TP on days 3 and 4 of life produces severe restriction in prepubertal ovarian growth similar to that produced by 100 µg EB administered on day 3. Likewise, both of the latter treatments result in a relatively severe restriction in the uterine metabolic response to estradiol at weaning in comparison to the neonatally administered single dose of TP. The metabolic impairment of the uterus appears to be a direct consequence of impaired ovarian development and not related to any altered availability of cytoplasmic estrogen binding sites in the prepubertal rat uterus. For example, neonatal treatment with 100 µg EB (day 3) or 1250 µg TP (day 3) + 1250 µg TP (day 4) produce approximately the same degree of reduction in prepubertal ovarian weights and affect the estrogen-stimulated oxidation of glucose similarly, but only EB treatment significantly ($p < 0.025$, Dunnett's t) reduces cytoplasmic estrogen receptor concentration. Additionally, neonatal ovariectomy substantially impairs the development of metabolic capacity in the uterus without an effect upon cytoplasmic estrogen receptor production (fig. 2).

Discussion. The above results suggest that normal ovarian function and endogenous estrogen secretion during the early prepubertal period of life may be instrumental in the development of the functional competency of the uterus. In fact, there is abundant evidence that neonatal androgenization or estrogenization alters ovarian development[12-14] and decreases circulating serum estrogen levels[15,16]. In this regard, previous results from our laboratory have indicated the importance of ovarian development and the extent of estradiol-stimulated uterine growth responses in the prepubertal rat [6]. Thus, it would appear that reduced endogenous estrogen secretion during infancy may limit the hormonal signal for the development of the uterine phosphogluconate oxidative pathway. However, it should be noted that the estrogen-stimulated glucose oxidation exhibited by the uteri of rats having ovarian weights below 8.0 mg is in the same range as the basal (saline-injected) uterine glucose metabolism. Thus, endogenous estrogen secretion during infancy does not induce this uterine metabolic pathway, but rather would appear to selectively enhance its development.

Our results also suggest that an obligatory relationship between impaired development of cytoplasmic estrogen receptor and impaired development of the uterine glucose metabolic pathway does not exist. Although neonatal treatments such as ovariectomy and sequential TP administration result in reduced metabolic responsivity in the estrogen-stimulated uterus which approximates that seen after neonatal EB treatment, cytoplasmic estrogen receptor production is reduced only by the neonatal exposure to EB. Hence, it would appear that the reduced uterine growth responses previously observed in neonatally ovariectomized, androgenized, or estrogenized rats[6] are more a consequence of a metabolic rather than a receptor deficit.

It should be noted that a neonatal treatment utilizing sequential administration of 1 mg of the aromatase inhibitor, 1,4,6-androstatrien-3,17-dione, and 1250 µg TP which was not used in establishing the relationship illustrated in figure 1 also provides good correspondence between actual glucose metabolism and predicted glucose metabolism based upon ovarian weight. These animals had weanling ovarian weights of 11.0 ± 0.5 mg. Using this number as the x value to predict the value of y in the regression curve of figure 1, the estrogen-stimulated uterus of these animals should have a $^{14}CO_2$ evolution which is 89% of the control. The actual level of glucose oxidation was 89.8% of control. Thus, the degree of ovarian development at weaning may be a good, reliable predictor of the metabolic competency of the uterus.

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