Effect of Insulin and Growth Hormone on Rat Uterine RNA Synthesis (34586)

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One of the early responses of the uterus of ovariectomized rats to 17β-estradiol administration is a stimulation of nuclear RNA synthesis (1–3). Since other hormones influence RNA and protein synthesis in various organs (4), the present study was undertaken to observe the effects of insulin and growth hormone on uterine RNA synthesis and to compare the results with those of 17β-estradiol.

Materials and Methods. Female ovariectomized (Ox) and hypophysectomized–ovariectomized (HxOx) Sprague-Dawley rats (200 g body wt) were purchased from Hormone Assay Co., Chicago, Ill. and used 2 to 3 weeks ater the surgical procedure. Uridine-5-^{3}H, 2.83 Ci/mmole, was purchased from New England Nuclear Corp., Boston, Mass.; 17β-estradiol from Ciba Pharmaceutical Products Inc., Summit, N.J.; bovine growth hormone from the Armour Lab., Chicago, Ill.; aqueous insulin (Iletin) and Testape from Eli Lilly and Co., Indianapolis, Ind.; Glytel reagent from Charles Pfiser and Co., Inc., New York, N.Y. and yeast RNA from Sigma Chemical Co., St. Louis, Mo.; alloxan monohydrate from Matheson, Coleman and Bell Lab., East Rutherford, N.J. and recrystalized from ethanol. HxOx rats were given 5% glucose as drinking water *ad libitum*. Diabetes was induced by the intravenous injection of alloxan monohydrate (100 mg/kg of body wt) to ovariectomized rats (DOx). Daily morning urine was tested for glucose with Testape. Animals excreting greater than 0.25% of glucose in the urine were used in this study. Blood glucose was determined at the time of sacrifice with the Glytel reagent.

The 17β-estradiol (10 μg dissolved in 1.0 ml of 1% ethanol–0.9% NaCl solution) was administered to the animals 30 min prior to sacrifice. Growth hormone (0.5 mg dissolved in 0.5 ml of slightly alkaline saline solution) was administered 18 and 2 hr prior to sacrifice. Insulin (8 IU/100 g of body wt) was administered at various time intervals up to 2 hr prior to sacrifice. Control animals received the same solutions without hormone. All hormones were administered intraperitoneally. Uridine-5-^{3}H (86.6 μCi dissolved in 0.5 ml of saline) was administered intraperitoneally to each rat 10 min prior to sacrifice. The animals were killed with a sharp blow to the head. Blood was collected from the cervical vein and allowed to clot. To the serum an equal volume of 6% TCA was added and centrifuged. The glucose and radioactivity of the deproteinized supernatant solution were determined. Uteri were removed, washed rapidly in saline, blotted, dissected free of fat and connective tissues, frozen on Dry Ice, crushed and homogenized individually in an all glass homogenizer with 10 vol of 0.32 *M* sucrose, 3 m*M* MgCl solution. The homogenizer was rinsed with the above solution and the washings were combined to give a total volume of 3 ml. The acid-soluble and RNA fractions were prepared as described by Schneider (5). Yeast RNA was used as standard. The radioactivity of the RNA, acid-soluble fraction, and sera was determined as described by Sunaga and Koide (6) except that Bray's solution was used. The average counting efficiency for tritium was found to be 18%.

The dry weight and water content were determined by homogenizing the uteri in a glass-homogenizer and lyophilizing the homogenates overnight. The lyophilized samples were placed in an oven and heated at 100° until constant weight was obtained. The drying time was about 5 hr.

Results. Figures 1 and 2 show the incorporation of tritiated uridine into the uterine acid-soluble fraction and RNA of DOx and

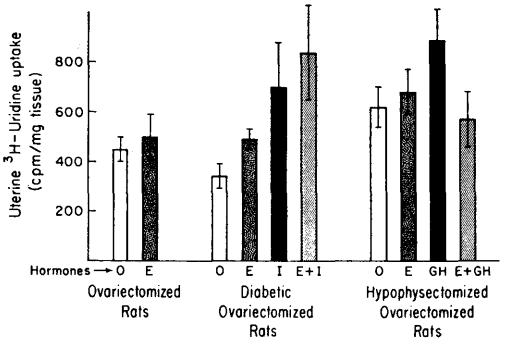

FIG. 1. Effect of 17β-estradiol (E) insulin (I) and growth hormone (GH) on the incorporation of tritiated uridine into the acid-soluble fraction of ovariectomized rat uterus. Values are means ± SE of 6 to 10 separate experiments.

HxOx rats following the administration of 17β-estradiol, insulin and growth hormone. 17β-Estradiol administered to Ox, DOx, and HxOx rats increased the incorporation of labeled uridine into the uterine acid-soluble fraction and RNA. Insulin and growth hormone administered to DOx and HxOx rats, respectively, stimulated the incorporation of uridine into both fractions. The combined administration of 17β-estradiol and insulin to DOx animals had a synergistic influence on the incorporation of uridine into uterine RNA (Fig. 2). The incorporation of labeled uridine into uterine RNA was slightly increased with the combined administration of 17β-estradiol and growth hormone to HxOx

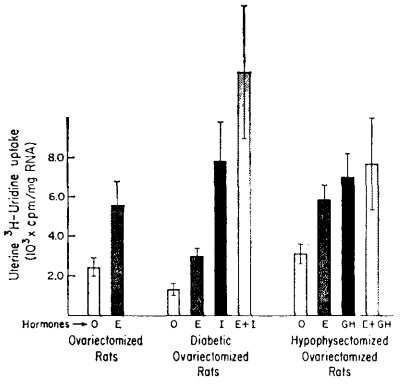

FIG. 2. Effect of 17β-estradiol (E), insulin (I), and growth hormone (GH) on the incorporation of tritiated uridine into RNA of ovariectomized rat uterus. Values are means ± SE of 6 to 10 separate experiments.

rats although the effect was less than additive. On the other hand, the incorporation into the uterine acid-soluble fraction was lower with the combined treatment than with each hormone separately (Figs. 1 and 2).

Figure 3 shows the incorporation of labeled uridine into the uterine acid-soluble fraction and RNA and the radioactivity level in blood serum during the initial 2-hr following the administration of insulin to DOx rats. A definite stimulatory influence on the incorporation of uridine into uterine acid soluble fraction and RNA was observed at 15 min following insulin treatment and a maximal effect at 30 min which was complemented with a decline in the blood radioactivity level.

Table I shows the weights and water content of uteri obtained from Ox, DOx, and HxOx rats treated with 17β-estradiol, insulin and growth hormone. The uterine weights of DOx and HxOx rats were lower than those of Ox rats. The decrease in uterine weights of DOx rats was reflected by a decrease in the water content. The decrease in uterine weight of HxOx rats was manifested in the dry weight and water content. A slight increase in the uterine weights of Ox and DOx rats was observed following the administration of 17β-estradiol which was reflected in changes in water content. In contrast treatment of HxOx rats with 17β-estradiol had no effect on the uterine weights. Insulin administered to DOx rats produced a slight increase in uterine weight.

When administered with 17β-estradiol, insulin induced a significant increase in the uterine weight which was due to changes in water content. Growth hormone administered to HxOx produced a significant increase in the uterine weight. Growth hormone combined with 17β-estradiol induced a greater increase in weight which was reflected in an increase of water content. In spite of the changes in water content the mean percentage water content of uteri obtained from Ox, DOx, and HxOx rats with and without hormonal treatment ranged between 78.1 to 81.9%.

Discussion. The results of the present study are in accord with the reports that one of the early responses of the uterus to 17β-

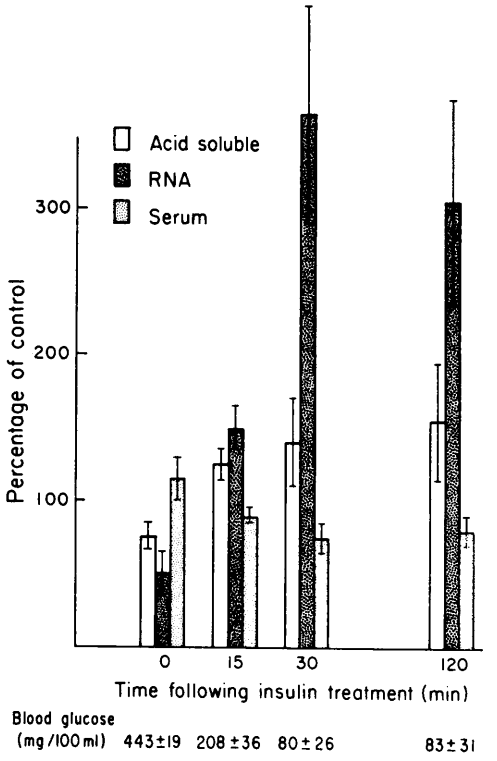

FIG. 3. Effect of insulin administration to ovariectomized-alloxan diabetic rats on the rate of incorporation of tritiated uridine into the uterine acid-soluble and RNA fractions and on the level in the serum. Values are means ± SE of 8 separate experiments. Uridine was administered intraperitoneally 10 min before each time interval following insulin administration. The mean values ± SE of uterine acid-soluble fractions obtained from ovariectomized rats without treatment was 450 ± 52 cpm/mg of tissue; RNA, 2.59 ± 0.46 × 10^3 cpm/mg of RNA; and serum, 1.41 ± 0.17 × 10^5 cpm/ml. These values were considered as 100%. The blood glucose level of the ovariectomized rats (control) was 119 ± 12 mg/100 ml.

estradiol is a stimulation of uterine RNA synthesis (1–3). This stimulatory influence of 17β-estradiol was observed with DOx and HxOx rats. It is noteworthy that uterine RNA synthesis is also influenced by insulin and growth hormone. The simultaneous administration of insulin and 17β-estradiol resulted in a synergistic effect on uterine RNA synthesis (Figs. 1 and 2). Since the administration of these hormones increase the incorporation of labeled uridine into the uterine acid-soluble and RNA fractions, the enhanced uptake may account for the increase of the labeled uridine in the RNA fraction. It remains to be established how these hormones increase RNA synthesis.

The demonstration of a diminished response to 17β-estradiol administration by the low incorporation of tritiated uridine into uterine RNA of DOx rats compared with Ox rats and the marked stimulation following insulin administration supports the views of Fowler *et al.* (7) that rat uterine responsiveness to estrogen is depressed in alloxan-diabetes and are reversed with insulin administration. The results of the present study are consistent with the findings of Lostroh (8, 9) that insulin is a potent stimulus of protein synthesis in sex accessory organs taken from mice and maintained as organ cultures. The influence of insulin on uterine metabolism was further demonstrated by Chieri and Fridhandler (10) who reported that acetate-1-^{14}C uptake into fatty acid fraction of rat uterus was increased by insulin administration.

Grattarola and Li (11) reported that the uterine weight was decreased in HxOx rats which responded to growth hormone administration. The decrease in uterine weight of HxOx rats was reflected in changes of dry weight and water content in contrast to the lower weights of DOx rats which was principally due to a decrease in water content (Table I). The increase in uterine weights induced by the administration of insulin, growth hormone, and 17β-estradiol was reflected in changes of water content. In the present study, the incorporation of tritiated uridine into uterine RNA of HxOx rats was increased following growth hormone administration and was greater when combined with 17β-estradiol. On the other hand, the incorporation of labeled uridine into the uterine acid-soluble fraction was depressed when growth hormone was administered simultaneously with 17β-estradiol. This apparent antagonism of growth hormone and 17β-estradiol is not clear.

Summary. 17β-Estradiol, growth hormone, and insulin were administered to ovariectomized (Ox), diabetic-ovariectomized (DOx)

TABLE I. The Effect of 17β-estradiol, Insulin, and Growth Hormone on the Uterine Weight of Alloxan-Diabetic and Hypophysectomized–Ovariectomized Rats.[a]

Rats	Hormone treatment	Estradiol	Wet wt (mg)	Dry wt (mg)	Water content (mg)	% Water
Ovariectomized	0	0	128.2 ± 3.0	25.0 ± 0.3	103.2 ± 3.0	80.4 ± 0.3
	0	+	134.3 ± 8.0	26.1 ± 1.3	108.2 ± 6.7	80.3 ± 0.4
Diabetic–ovariectomized	0	0	118.3 ± 6.7	25.5 ± 1.7	92.8 ± 4.8	78.2 ± 1.4
	0	+	119.3 ± 6.1	26.0 ± 1.2	93.3 ± 5.0	78.1 ± 0.3
	I	0	120.9 ± 3.9	25.3 ± 0.7	95.5 ± 3.5	79.0 ± 0.4
	I	+	130.8 ± 4.9	26.3 ± 1.0	104.5 ± 4.5	79.0 ± 0.5
Hypophysectomized–ovariectomized	0	0	91.4 ± 3.9	19.4 ± 0.6	72.0 ± 3.7	78.6 ± 0.5
	0	+	93.8 ± 1.7	19.2 ± 0.8	74.6 ± 1.0	79.5 ± 0.5
	GH	0	111.3 ± 3.0	20.2 ± 1.3	91.1 ± 2.1	81.9 ± 0.8
	GH	+	114.0 ± 4.9	20.5 ± 0.9	93.5 ± 4.2	81.8 ± 0.4

[a] Values are means ± SE; 6 to 7 rats were used in each group. The amount of hormones used are given in the text. The body weight of the rats were 200 g. The uteri were obtained 30 min following administration of 17β-estradiol, 2 hr after insulin and 18 hr after growth hormone. Abbreviations used: I, insulin; GH, growth hormone.

and hypophysectomized–ovariectomized (Hx-Ox) rats to observe their effects on the incorporation of tritiated uridine into the uterine RNA and acid-soluble fraction. Insulin administered to DOx rats stimulated the incorporation of tritiated uridine into uterine RNA and the acid-soluble fraction within 15 min. The stimulatory effects were potentiated with the combined administration of insulin and 17β-estradiol. The administration of growth hormone to HxOx rats resulted in an increase in the incorporation of labeled uridine into uterine RNA and acid-soluble fraction. The combined administration of 17β-estradiol and growth hormone caused a slight increase in the incorporation of uridine into uterine RNA which was less than additive and a decrease in the incorporation into the acid-soluble fraction. The results of the present study suggest that insulin and growth hormone in addition to 17β-estradiol influence uterine RNA synthesis.

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