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Effects of Aminoglutethimide on Cervical Dilatability and Serum Immunoreactive Relaxin in Pregnant Rats (41989)

B. G. STEINETZ, E. M. O'BYRNE, W. K. SAWYER, M. C. BUTLER, J. MUNIGLE, AND R. E. STEELE

Research and Development Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Ardsley, New York 10502

Abstract. Administration of aminoglutethimide (AG) at a daily sc dose of 20 mg/kg to rats from the 10th to 20th days of pregnancy resulted in fetal wastage, increased placental weights, and decreased placental alkaline phosphatase. In an effort to determine the endocrine problems relating to these abnormalities, serum relaxin (R) and progesterone (P) levels were determined by RIA and cervical distensibility was measured: All three parameters were subnormal in AG-treated rats. Therapy with estrogen (E) or (P) failed to correct any of the physiological problems associated with AG treatment, but a combination of these steroids increased fetal survival to control levels. However, cervical dilation in preparation for parturition failed to occur. Relaxin therapy alone increased cervical dilation but did not enhance fetal survival. A combination of E, P, and R maintained a normal complement of fetuses and provided normal cervical dilation. None of the treatments prevented placental enlargement but P treatment tended to maintain normal placental alkaline phosphatase.

Aminoglutethimide (AG) inhibits side chain cleavage of cholesterol and thus interferes with steroid hormone production by the adrenals, testes, and ovaries (1). AG reduced serum progesterone concentrations 80–90% and induced abortions when given to rats in large doses early in pregnancy (2). However, in our own laboratories, smaller daily doses of AG given over the second half of pregnancy have been found not to interfere with gestation but to cause placental enlargement and a reduction in the number of viable fetuses delivered at term. The mechanisms responsible for this effect of AG are the subject of the present investigation. Possible points of interference of AG with delivery were postulated to be low estrogen (E) or progesterone (P) levels or interference with relaxin (R) secretion. E and P are necessary for maintenance of pregnancy in rats, whereas R has previously been found responsible for cervical dilation necessary for parturition to this species (3). Alkaline phosphatase (AlP) is an important placental enzyme (4) and it was measured to provide a biochemical parameter associated with the AG-induced enlargement of the placenta.

Materials and Methods. Dated pregnant female rats (Sprague–Dawley derived) were purchased from Marland Farms (Hewitt, N.J.). The morning of sperm positive vaginal smears was considered Day 1 of pregnancy. AG (20 mg/kg), estradiol-17β (E, 0.1 μg) and/or P (1 mg), were injected daily sc in 0.2 ml sesame oil. R (0.1 mg = 300 units) was injected sc in 0.2 ml 5% beeswax in peanut oil Days 14–20. The rats were killed on Day 21 after taking a blood sample from the jugular vein under ether anesthesia, and living fetuses, placentas, ovaries, and uteri were quickly removed, freed of adhering tissues, and weighed. Placentas and uteri were frozen on dry ice for subsequent analysis. The dilatability of the uterine cervix was determined by passage of a tapered plastic rod, graduated in 0.5-mm concentric rings, through the cervical canal until firm resistance was encountered (5).

Serum P was determined by the RIA method of Abraham *et al.* (6) as modified in our laboratory (7). An homologous porcine relaxin RIA was used to estimate serum immunoreactive R (8). It is known that this RIA underestimates rat R but the *relative* values correlate well with those reported by Sherwood *et al.* using an homologous rat RIA (9). Placental AIP was determined according to Manning *et al.* (10).

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Statistical significance of differences between groups was determined by the Student *t* test.

Results. AG, injected at a dose of 20 mg/kg Days 10–20 of pregnancy, significantly retarded body weight gain, reduced the number of viable fetuses, increased the number of resorbing fetuses, increased placental weight, decreased placental (AlP), decreased immunoreactive serum R, and decreased cervical dilatability (Table I). AG exerted no significant effects on ovarian or uterine weights (data not shown). Serum P was 62 ± 9 ng/ml in AG-treated rats as compared with 95 ± 6 ng/ml in controls ($P < 0.01$).

The adverse effects of AG on intrauterine fetal mortality were reversed by a combination of E plus P, but not by either steroid alone (Table I). Placental enlargement and the decrease in placental AlP were not reversed by E, R, or their combinations (Table I). P appeared to prevent the AG-induced decrease in placental AlP although it did not inhibit placental enlargement (Table I).

The constricted cervix of AG-treated rats was correlated with a decreased level of serum immunoreactive R and neither parameter was corrected by exogenous E and/or P (Table I, Fig. 1). However, injections of porcine R not only elevated serum immunoreactive R levels but also specifically dilated the uterine cervix (Table I, Fig. 1).

Discussion. The reproductive abnormalities observed in AG-treated pregnant rats appear to be the result of specific hormonal deficiencies. The decreased number of viable fetuses and the increased number of resorptions in AG-treated animals seems to depend on reduced secretion of P and E. AG blocks the conversion of cholesterol to pregnenolone (the precursor of P) in the steroid synthetic chain (1), and in the present study serum P levels were found to be significantly reduced 24 hr after AG administration. We did not measure serum E, but AG is known to block the aromatization of testosterone to E (11). Thus it seems likely that E levels may also have been reduced in our animals. In any event, specific replacement therapy with a combination of E and P, but not with either hormone alone, prevented the fetal deaths associated with AG treatment. Our original observation that AG-treated rats failed to

TABLE I. EFFECTS OF AMINOGLUTETHIMIDE (AG) AND REPLACEMENT HORMONES ON PREGNANCY IN RATS

Treatment (sc daily) Days 10–20	No. rats	Fetus				Placenta		Cervical dilatability (mm)
		Final body wt (g)	Living	Dead and resorbing	Live fetus wt (g)	Weight (g)	AlP (u/g dry)[b]	
Control	22	381 ± 5	10.1 ± 0.6	0.6 ± 0.3	3.7 ± 0.2	0.60 ± 0.02	2273 ± 173	5.8 ± 0.2
AG 20 mg	22	344 ± 11[a]	6.5 ± 1.0[a]	4.7 ± 1.1[a]	4.1 ± 0.1	1.22 ± 0.10[a]	1420 ± 256[a]	2.6 ± 0.2[a]
+ Estradiol 0.1 μg	5	291 ± 15[a]	2.6 ± 1.1[a]	5.4 ± 1.1[a]	5.7 ± 1.1	1.24 ± 0.30[a]	1344 ± 139[a]	3.7 ± 0.3[a]
+ Progesterone 1 mg	5	359 ± 16	5.8 ± 1.9[a]	5.6 ± 1.7[a]	4.7 ± 0.1	1.00 ± 0.09[a]	2213 ± 570	2.3 ± 0.6[a]
+ Relaxin 0.1 mg	5	332 ± 14[a]	5.8 ± 2.3[a]	3.5 ± 0.9[a]	4.5 ± 0.3	1.27 ± 0.09[a]	1141 ± 219[a]	5.4 ± 0.4
+ Estradiol 0.1 μg + progesterone 1 mg	10	344 ± 15[a]	10.6 ± 0.5	0.2 ± 0.1	4.6 ± 0.1	1.03 ± 0.01[a]	1684 ± 165[a]	2.8 ± 0.2[a]
+ Estradiol 0.1 μg + relaxin 0.1 mg	5	307 ± 21[a]	7.3 ± 1.2	4.5 ± 1.8[a]	4.0 ± 0.1	1.20 ± 0.20[a]	880 ± 100[a]	4.2 ± 0.4
+ Estradiol 1 μg + progesterone 1 mg + relaxin 0.1 mg	10	363 ± 9	10.1 ± 1.1	1.4 ± 0.8	4.3 ± 0.1	1.17 ± 0.07[a]	1631 ± 249	6.2 ± 0.2

[a] Significantly different from control: $P < 0.05$–0.01.
[b] Ten units alkaline phosphatase activity liberate 1 mg phenolpthalein in 1 hr at pH 9.6 and 37°C from phenolpthalein diphosphate under standard conditions (10).

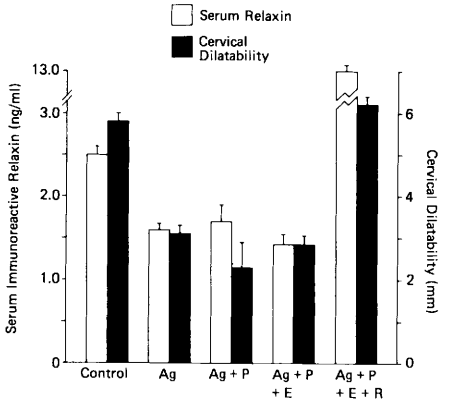

FIG. 1. Serum immunoreactive relaxin levels and cervical dilatability on Day 21 in pregnant rats treated with aminoglutethimide (AG) alone or in combination with progesterone (P), estradiol-17β (E), and/or relaxin (R). AG, E, and P were injected sc daily Days 10–20 of gestation. Relaxin was injected sc Days 14–20 of pregnancy. Serum immunoreactive relaxin and cervical dilatability were measured on Day 21, approximately 24 hr after the last injections.

deliver normal viable litters prompted the present study. We now suggest that parturition problems may arise because the uterine cervix does not soften in AG-treated pregnant rats. Thus, these rats may be mechanically prevented from normally delivering their young. Failure of cervical dilation in turn is likely related to the decreased circulating levels of R observed in AG-treated animals. This interpretation is supported by the restoration by injected R of cervical softening in AG-treated rats. Previous work (3, 4) showed that ovariectomy prevented cervical dilation in pregnant rats unless R was administered. R also facilitated delivery of young in ovariectomized rats in which pregnancy was maintained with E and P (3, 4).

The factor(s) responsible for placental enlargement and decreased AlP in AG-treated rats has not been elucidated. None of the various hormonal treatments restored placental weight to normal although P tended to restore AlP concentration to control level. Petroupolos and Cons (12) have pointed to the complex interaction of fetal metabolism, steroids, and hypophyseal gonadotrophins resulting in placental hypertrophy in ovariectomized pregnant rats. Leathem *et al.* (13) likewise had observed placental enlargement in ovariectomized rats in which pregnancy was maintained by P and E (estrone) and also in AG-treated pregnant rats. Thus, "chemical ovariectomy" by AG likely removes some negative feedback on the placenta in a fashion similar to that observed following surgical removal of the ovaries, and compensatory hypertrophy ensues. In any case, placental hypertrophy per se does not appear to be harmful to the fetuses, nor does reduced placental AlP. Replacement therapy with E and P provided complete support for litter survival in our AG-treated rats, although placental enlargement was not prevented. Higher doses of E and/or different ratios of E to P have been reported to resotre placental weight to normal in ovariectomized, fetectomized rats (13), and might well modulate placental weight in AG-treated pregnant rats. However, this does not appear to be critical to fetal survival.

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