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Androstenedione Synergizes with Stress or Prenatal Drug Exposure to Retard Fetal Growth: Role of IGF

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MCGIVERN, R. F., N. FATAYERJI AND R. J. HANDA. *Androstenedione synergizes with stress or prenatal drug exposure to retard fetal growth: Role of IGF.* PHARMACOL BIOCHEM BEHAV **55**(4) 549–557, 1996.—Pregnant Sprague-Dawley dams were implanted with a Silastic capsule (3 or 10 mm) containing androstenedione (AN) or cholesterol prior to being administered one of several treatments that create an endocrine profile of stress: maternal exposure to alcohol, pair feeding (PF), cocaine (COC), or restraint stress (RS). Controls (chow fed, CF) were left undisturbed during pregnancy. Treatments were administered from day 14 to day 22 of gestation. Fetuses were delivered by cesarean section on day 22. Results revealed that administration of AN to pregnant dams at a dose that does not influence fetal growth by itself can retard fetal growth in the presence of alcohol, PF, COC or RS. Data indicate that these effects are not directly attributable to changes in adrenocorticotropin (ACTH) or corticosterone levels. Preliminary results suggest a role for insulinlike growth factor (IGF) binding proteins (IGFBPs). Overall, these data demonstrate that AN can synergize with drugs and/or stress to enhance intrauterine growth retardation (IUGR). One underlying cause of this synergism between stress-related environmental events and androgenic actions on fetal growth may be increased expression of IGFBPs, which can sequester IGFs, thereby inhibiting their trophic actions on fetal and/or placental tissue.

Fetus Ethanol Cocaine Androstenedione Estrogen Birth weight Placenta Lung Liver Insulinlike growth factor Insulinlike growth factor binding proteins 1 and 2 Intrauterine growth retardation

ADVERSE effects of androgens on the maintenance of pregnancy in the rodent have long been recognized. The effects observed have included interference with implantation, fetal resorption or abortion and delay in parturition (12,13,20,21). These effects, which are produced by several aromatizable androgens including testosterone, androstenedione or dihydroepiandrosterone, are similar to those produced by estrogen administration, leading investigators to assume that the androgenic effects are mediated by aromatized estrogen metabolites (13,20,21,23).

In contrast with these effects of androgens on the maintanence or pregnancy during early embryonic periods of gestation, the consequences of prenatal androgen exposure on fetal growth has received little study. Limited exposure to exogenous androgens early in pregnancy had no adverse effect on postnatal body weight in two different strains of mice (12). However, with administration of testosterone during the last week of gestation, we observed retarded fetal growth in the presence of other environmental factors such as alcohol, restraint stress or restricted food intake at a dose that by itself had no effect on birth weight (37,39).

Because androstenedione is the primary androgen of importance during pregnancy and is the preferred substrate for aromatization in the human placenta (1), we did the present study to examine the inhibition of fetal growth produced by androstenedione when it is combined with ethanol or stress. Androstenedione has particular relevance to stress in humans because it is released into the general circulation from the adrenal gland in response to adrenocorticotropin (ACTH) stimulation (72). However, it is not stress responsive in the rat (3,15), necessitating exogenous androstenedione administration to model the primate.

Several studies have reported an inhibition of intrauterine implantation, fetal resorption or reduced litter size in rodents

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following ACTH administration (28,53,73), and stress has been associated with spontaneous abortion in humans (36). Therefore, we examined the role of hypothalamic–pituitary–adrenal (HPA) activation in androgen-related suppression of fetal growth by either adrenalectomizing or administering ACTH to pregnant dams.

We also examined the role of insulinlike growth factor mRNAs (IGF-I and IGF-II) or insulinlike growth factor binding protein mRNAs (IGFBPs 1 and 2) as potential mediators of androgen-related reductions in fetal growth. IGF-I and IGF-II have a potent mitogenic function in both peripheral and brain tissue (6,31), and their production can be inhibited by high levels of estrogen (63). IGFs are synthesized in a variety of fetal tissues and the placenta, with the highest production being in the liver (31,33). The liver is considered to be the primary source for the endocrine actions of the IGFs, whereas production in other tissues such as placenta or lung may reflect paracrine and/or autocrine actions (9,31). Both IGF-I and IGF-II are associated with six binding proteins that mediate the bioavailability and biological effects of the IGFs. IGFBP-1 and -2 are the primary IGFBPs, which are important during the fetal period (16,29). IGFBPs actions can increase or inhibit IGF actions, possibly through their actions at the IGF receptor and through their ability to increase the half-life of the peptide in plasma (31). IGFBP-1 production may also play a role in placental trophoblast invasion. Excessive production may inhibit vascularization, resulting in a restriction of uteroplacental blood flow and subsequent intrauterine growth retardation (23,24,25,61,69).

METHODS

Time-pregnant Sprague-Dawley from Charles River (Portage, MI) were used all experiments. All dams had to have given birth to at least one litter previously. The animals arrived in the laboratory on day 5 of gestation (day of mating = 0) and were housed in translucent plastic maternity cages in a separate vivarium room. Nesting material was provided to all dams on day 18 of gestation. The lighting schedule was 12-h on:12-h off with lights on at 0600 hours. On day 14 of pregnancy, dams were implanted subcutaneously under ether anesthesia with either a 3-mm or a 10-mm silastic capsule (inner diameter of 0.0625 inches × outer diamter of 0.125 inches) packed with the crystalline form of androstenedione (Sigma Chemical, St. Louis, MO). Control animals were implanted with similar 10-mm capsules containing cholesterol (Sigma Chemical) and remained on a diet of dry lab chow (chow fed CF). Animals not placed on liquid diets were allowed ad libitum access to Purina Lab Chow and tap water throughout gestation. Dams were weighed every other day during the period of treatment. On the morning of day 22 of gestation, dams were killed by cervical dislocation and the pups delivered by cesarean section. Two to three litter representatives of each sex were measured for anogenital distance (AGD) by using microcalipers. Distance was measured from the anus to the urethra and indexed to body weight as suggested by Graham and Gandelman (18) to equalize variations in fetal growth. Procedures for all experiments were approved by the Institutional Animal Care and Use Committee.

Prenatal Treatments

On day 14, weight matched dams were placed in one of the following treatment groups.

Ethanol. Dams administered alcohol were placed on a nutritionally fortified liquid diet containing 35% ethanol-derived calories beginning on day 15 of pregnancy by using previously reported procedures (41). Control dams were administered a similar diet in which sucrose was substituted isocalorically for ethanol. These dams were pair fed to weight-matched dams in the alcohol group. The diet consisted of chocolate-flavored Sustacal (Mead Johnson, Evansville, IN) supplemented with vitamins (Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals, Costa Mesa, CA; 1.3 g/12 oz. Sustacal) and salts (Salt Mixture IV; ICN Nutritional Biochemicals; 2.1 g/12 oz. Sustacal). Pair-fed (PF) dams were administered the same diet with sucrose (8.4 g/12 oz. Sustacal) isocalorically substituting for ethanol. The diets were made fresh daily and presented to the dams 2–3 h before lights off. PF dams were given the same amount of diet as that consumed by a weight-matched ethanol-fed dam during the previous evening. CF dams had access to dry food pellets and water ad libitum throughout pregnancy. Maternal and newborn blood were obtained from some animals following decapitation. Blood was collected in glass tubes containing aprotinen (250 kIU/ml) and ethylenediaminetetraacetic acid (EDTA) (0.75 mg), spun at 40°C for 20 min and plasma stored at −20°C until it was assayed for androstenedione by radioimmunoassay (RIA).

Stress. Another group of dams was subjected to light and restraint stress (RS) for 30 min twice a day from days 15 to 20 of pregnancy by using previously reported procedures (39). Briefly, the dams were placed in translucent plastic restraint tubes at 0900 and 1600 hours under bright lights (150-W bulbs) placed 20 inches above the tubes. On day 21 of pregnancy, the dams were exposed to stress only at 0900. Dams were returned to the home cage after each restraint period. Maternal and newborn blood were obtained from some animals following decapitation at the time of cesarean section.

Cocaine. In a separate experiment, another group of dams was administered cocaine hyrdochloride (10 mg/kg, sc; Sigma) at 0900 and 1430 hours from days 15 to 21 of gestation. The last injection was on the morning of day 21 of gestation. This dose regimen of cocaine by itself produces no effect on birth weight of the offspring (51). Controls were injected with the saline vehicle. An additional group of dams was exposed to prenatal stress as described above and a control group was included, which received no treatment during pregnancy. Half of the dams in each group were implanted with cholesterol capsules and half with androstenedione capsules (10 mm) on day 14 of pregnancy.

Role of the HPA Axis

Studies employing prenatal alcohol exposure were conducted to examine the involvement of HPA activation in the reduced birth weight of animals exposed to androgens. Dams were adrenalectomized (ADX) on day 8 of pregnancy or given sham surgery to examine the role of adrenal glucocorticoids. On day 15, these animals were implanted with the 3-mm capsules of androstenedione or cholesterol under ether anesthesia and placed on the ethanol diet. All ADX dams were given ad libitum access to 0.9% saline from the time of surgery. Work by others has shown that adrenalectomy of pregnant Sprague-Dawley dams on day 8 of pregnancy has no effect on birth weight (52). Therefore, we did not include an ADX alone group for study. Additional dams on the ethanol diet in this experiment were injected with the androgen antagonist flutamide (5 mg/kg, sc; gift from Schering Corp., Kenilworth, NJ) at 0800 and 1630 hours from days 15 to 21 of gestation. Flutamide crosses the placenta, and doses in this range have been shown to have potent anti-androgenic effects in rats and humans (4,27,67).

The role of ACTH was assessed by implanting dams with capsules (10 mm) containing androstenedione or cholesterol on the morning of day 15 of gestation. The animals were subsequently injected subcutaneously with ACTH (10 IU, ACTHAR Gel in 0.1 ml) at 0900 and 1430 hours from days 15 to 20 of gestation. The last injection was on the morning of day 21 of gestation. Controls were injected with a 5% gelatin solution in the same volume. A separate group of controls received no treatment.

Androstenedione radioimmunoassay. Androstenedione was measured by RIA by using ^{125}I-androstenedione and reagents supplied by ICN Nutritional Biomedicals. Samples (200 μl) were extracted with 6 ml of hexane:ethyl acetate (3:2) prior to being assayed in duplicate. Recovery averaged 92%. The intra-assay coefficient of variation was 3.1%. Cross reactivity of the antibody to other steroids was as follows: 4.4% for dehydroepiandrosterone (DHEA), 3.5% for DHEAS, 1.79% for estrone, 0.64% for testosterone, 0.07% for progesterone and 0.02% for estradiol-17b. Cross reaction with other steroids was less than 0.01%. All samples were within 20–80% B/B0. The lower sensitivity of the assay was 80 ng/ml.

Northern blot analysis. Fetal lung, liver tissue and placenta from pups exposed to alcohol while in utero (ETOH) and PF and CF litter representatives at 22 days of gestation were measured for IGF-1, IGF-2 mRNAs or IGFBP-I or -II mRNAs using Northern blot hybridization analysis. Total RNA was prepared by the method of Chomzynski and Sacchi (8) and stored at −80°C until processed. Twenty micrograms of total RNA were loaded onto 1.5% agarose formaldehyde gels and electrophoresed at 28 V for 18–20 h. RNA was transferred by capillary action onto nylon membranes. After transfer, filters were exposed to ultraviolet light to fix RNA onto filters. Filters were prehybridized overnight in hybridization buffer (50% formamide, 1 M sodium phosphate, 1% sodium dodecyl sulfate (SDS), 2× Denhart's, 250 μg/ml herring sperm DNA and 200 μg/ml yeast RNA). Hybridization was performed in the same buffer overnight at 45°C with 5×10^6 cpm/ml of radiolabeled cDNA probe. Probes used were a 422-bp cDNA for IGF-1 and a 780-bp cDNA for IGF-II (American Type Culture Collection, Rockville, MD) and a 407-bp cDNA for IGFBP-1 and a 397-bp cDNA for IGFBP-2 (both a gift from Dr. S. Shimasaki). All cDNAs were labeled to a specific activity of approximately 1 × 108 cpm/μg with ^{32}P by random priming. After hybridization, filters were washed with 2× standard saline citrate (SSC)/0.5% SDS for 5 min RT, followed by two 30-min washes with 0.5× SSC/0.5% SDS at 45°C. Filters were exposed to Hyperfilm (Amersham, Lake Forest, IL) with intensifying screens for 2–6 days at −70°C.

Data analysis. The litter mean of each sex was used as the unit of analysis in all experiments involving body weights. All data were analyzed by analysis of variance (ANOVA) with BMDP software. Post hoc tests were conducted using Newman-Keuls or Tukey's *t* test.

RESULTS

Birth Weights (Stress, Alcohol, Pair feeding)

Birth weights were reduced in a dose-dependent fashion in offspring from dams that were exposed to stress or alcohol and implanted with 3-mm or 10-mm androstenedione capsules. The threeway ANOVA revealed main effects for sex, $F(1,160) = 10.98$, $p < 0.0001$, prenatal treatment, $F(3,160) = 23.17$, $p < 0.0001$, and hormone, $F(2,160) = 47.10$, $p < 0.0001$, and for prenatal treatment × hormone, $F(6,160) = 6.23$, $p < 0.005$. Because there were no interactions involving sex, data were collapsed for presentation (Fig. 1).

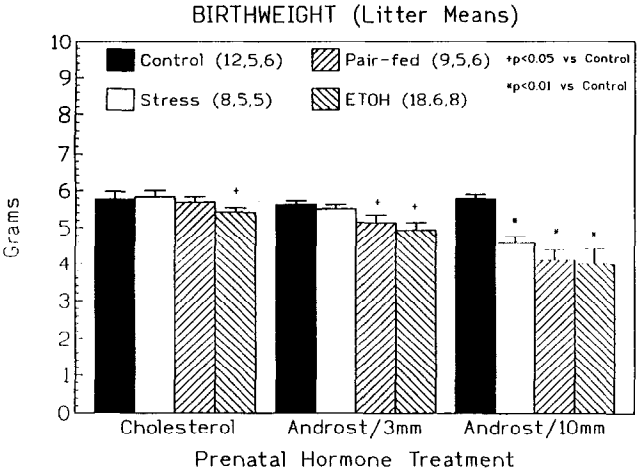

FIG. 1. Birth weights of dams implanted with capsules on day 14 of gestation; these capsules contained cholesterol or androstenedione. Data are litter means (±SEM).

Neither the 3-mm nor 10-mm capsule of androstenedione significantly influenced birth weights of pups from CF control dams. However, the 3-mm capsule significantly decreased birth weights for the PF (10%) and ETOH (10%) groups compared with the cholesterol-implanted PF and ETOH controls. The weight of RS animals in this group decreased by 6% (*ns*). The 10-mm androstenedione capsule significantly deceased birth weight by 29% in PF, 27% in ETOH and 22% in RS animals compared with their cholesterol-exposed counterparts. Thus, these decreases in birth weights add to the effect of the treatment on birth weight.

Maternal Weight Gain

Analysis of weight gain during pregnancy was conducted with a repeated measures ANOVA. Main effects were detected for prenatal treatment, $F(2,81) = 11.45$, $p < 0.0001$, day, $F(3,243) = 975.86$, $p < 0.0001$) and for interactions between day and hormone, $F(6,243) = 6.07$, $p < 0.0001$, day and prenatal treatment, $F(9,243) = 18.75$, $p < 0.0001$, and day × hormone × prenatal treatment, $F(18,243) = 1.82$, $p < 0.03$. No significant changes in maternal weight gain were observed in the PF and RS groups across the 3-mm and 10-mm androstenedione conditions as compared with the control dams, whereas weight gain of ETOH dams treated with 10-mm capsules was significantly lower than that for all other groups ($p < 0.05$) at each gestational time point (Fig. 2, right panel).

Androstenedione Levels

Maternal plasma concentrations of androstenedione were elevated by approximately 22% by the 3-mm capsule over control plasma levels, which was not significantly different from that of the control. The 10 mm elevated plasma levels in the dams by approximately 115% over that for control levels, $F(4,24) = 3.30$, $p < 0.05$ (Fig. 3).

AGD

The effect of androstenedione treatment on AGD was variable. The three-way ANOVA revealed main effects for sex, $F(1,304) = 593.25$, $p < 0.0001$, androstenedione, $F(2,304) =$

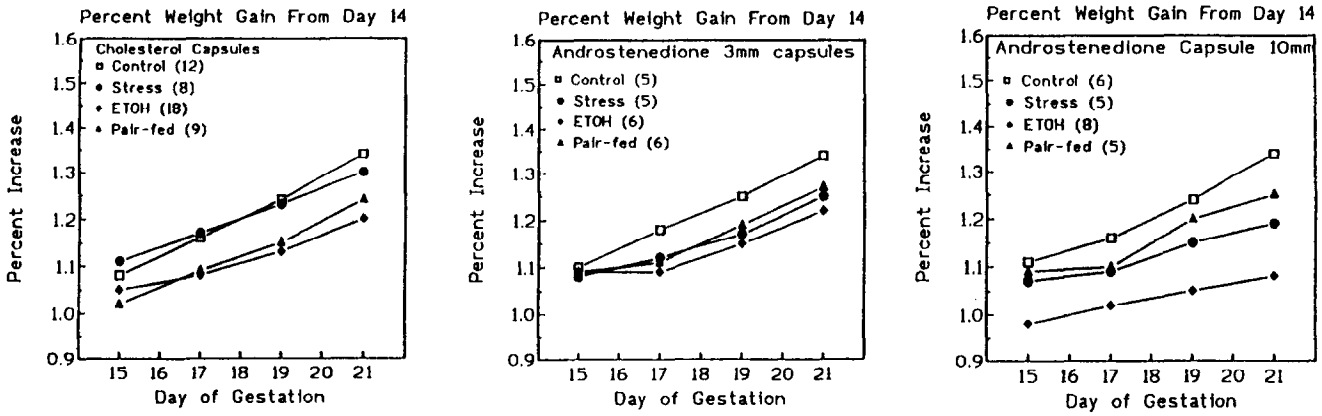

FIG. 2. Maternal weight gain during the last week of gestation. Weight gain is expressed as the percentage of increase on each day from the day preceding. Standard errors are smaller than the symbols. The number of dams is shown in parentheses. Dams are those used to obtain birth weight data in Fig. 1.

10.75, $p < 0.0001$, and prenatal treatment, $F(3,304) = 6.19$, $p < 0.001$. Post hoc comparisons within sex were conducted by using the Tukey–Kramer multiple comparisons test to compare all groups with cholesterol controls. AGD distance in the 3-mm androstenedione group exposed to no stress and in the 10-mm androstenedione ethanol-exposed males was significantly greater than that for cholesterol controls. In females exposed to 10-mm androstenedione plus pair feeding or no stress, AGD distance was increased significantly ($p < 0.05$) compared with cholesterol controls (Fig. 4). Similar to previous reports (37,40,42), AGD in ETOH animals was not influenced by prenatal alcohol exposure when indexed to body weight.

Cocaine Treatment

Cocaine administration decreased birth weight significantly when it was combined with androstenedione (Fig. 5), but it had no effect by itself. The decrease was similar to that of stress and androstenedione. The ANOVA yielded only a significant effect of hormone, $F(1,33) = 34.62$, $p < 0.0001$. However, a priori planned comparisons using Student's t tests revealed that androstenedione treatment significantly decreased birth weight in pups from cocaine and stress dams compared with dams given androstenedione alone ($p < 0.05$). Androstenedione treatment alone also produced a significant decrease in birth weight compared with cholesterol treatment alone ($p < 0.05$).

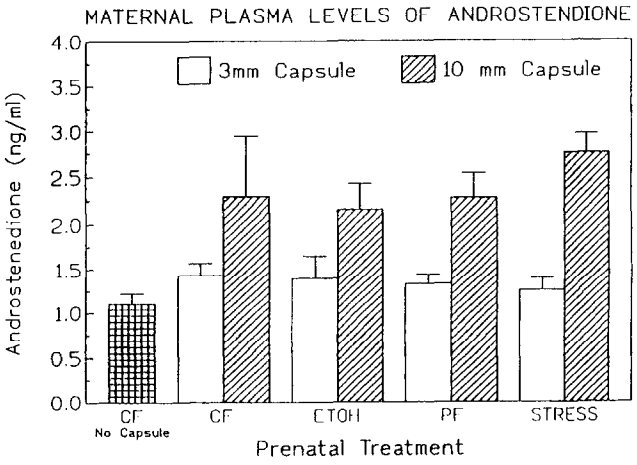

FIG. 3. Androstenedione levels in plasma of dams. Data are means ± SEM from 4–7 dams/treatment group.

ACTH Treatment

ACTH administration to pregnant dams had no effect on birth weight as compared with no treatment (Fig. 6). When ACTH treatment was compared with vehicle treatment in weights of pups from dams treated with androstenedione, no significant effect of ACTH was detected. However, both ACTH and vehicle treatments resulted in a significant decrease in birth weight as compared with controls, $F(4,17) = 4.27$, $p < 0.02$, in the presence of androstenedione treatment. The birth weight of pups from dams implanted with 10-mm androstenedione capsules, but which were uninjected, did not differ from controls.

Adrenalectomy

Significant decreases in birth weight were observed when dams were administered ethanol plus flutamide, androstenedione or adrenalectomy, $F(3,14) = 10.39$, $p < 0.001$. The decrease in birth weight induced by the combined ethanol and androstenedione treatments was not reversed by concomitant flutamide administration (Fig. 7). Flutamide administration combined with ethanol and cholesterol treatment produced the same approximate decrease in birth weight as did the combination of flutamide, ethanol and androstenedione (35–40%). Adrenalectomy potentiated the effects of a low dose of androstenedione (3-mm capsule) in the presence of ethanol by inhibiting birth weight by more than 50% of the control weight. In intact dams, this same dose of androstenedione in the presence of ethanol produced only a 10–15% decrease in birth weight (Fig. 1).

IGF mRNAs and IGFBP mRNAs

By using Northern blot analysis, we detected a 7.7-Kb and 1.7-Kb message for IGF1; a 3.7- and 1.0-Kb transcript for IGF-II, a 1.8-Kb transcript for IGFBP-1 and a 1.6-Kb transcript

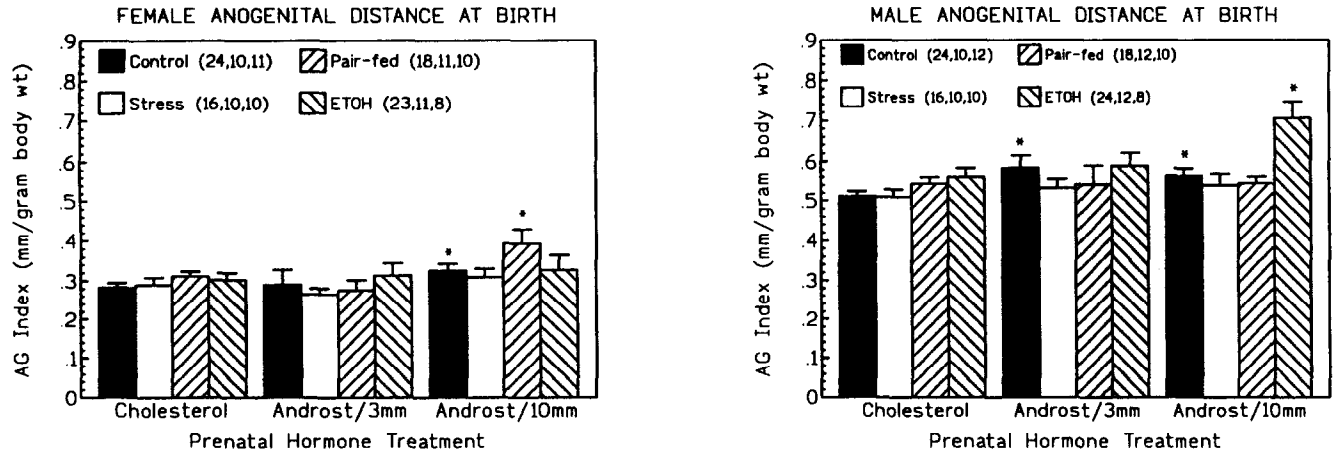

FIG. 4. Anogenital distance in 1–2 litter representatives of each sex. Number of litters are shown in parentheses. Data are indexed to body weight. Asterisks designate significant ($p < 0.05$) differences from the corresponding prenatal treatment group implanted with cholesterol capsules (bars 1–4).

for IGFBP-2. Androstenedione treatment combined with ethanol or pair feeding produced apparent increases in IGFBP-1 and -2 mRNAs in fetal lung and fetal liver as compared with these treatments in the presence of cholesterol or incomparison to androstenedione treatment combined with no stress (CF) (Fig. 8). Androstenedione treatment produced apparent increases in placental IGFBP-1 and -2 mRNAs in CF, PF and ETOH animals. There was no apparent change in mRNA levels for IGF-I or -II in any of these tissues induced by androstenedione (data not shown). All effects were replicated two to three times.

DISCUSSION

Our results demonstrate a significant enhancement in the ability of androstenedione to inhibit fetal growth in the presence of a variety of stressful environmental factors. These factors include exposing the dam to ethanol, pair feeding (food restriction) and restraint stress. A similar but less robust effect was also observed with cocaine. Overall, these results parallel those obtained with testosterone administration (38,39). Exogenous administration of testosterone at a dose that had no effect on birth weight reduced birth weight by up to 40% when combined with alcohol administration or restraint stress during the last week of gestation.

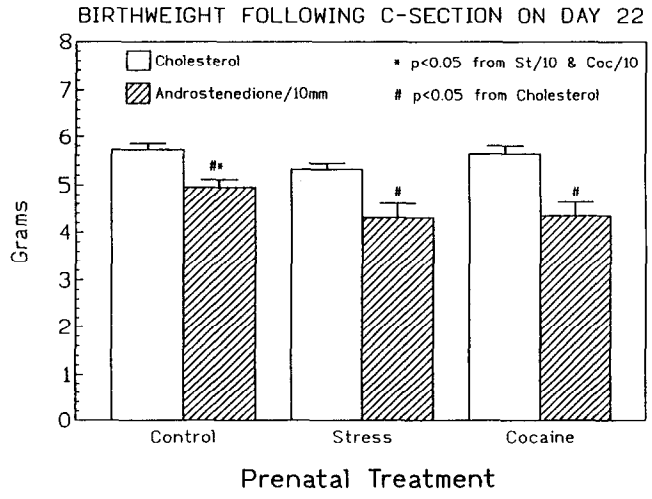

FIG. 5. Birth weights of offspring from dams exposed to restraint stress or administered cocaine during the last week of gestation. Data are litter means ± SEM of pups born to 5–7 dams/treatment group.

The reductions in birth weight in the present study had only a modest association with maternal weight gain over the treatment period. For instance, little change in maternal weight gain was observed in the PF and RS groups across the 3-mm and 10-mm androstenedione conditions, whereas birth weights of offspring were significantly reduced. This discrepancy suggests a direct effect of the treatment on the placenta or fetus as opposed to an indirect effect mediated by maternal nutrition or wasting. Placentas from fetuses exposed to androstenedione plus ethanol, stress or cocaine were notable for their greenish brown appearance, suggestive of increased iron deposition. This effect was not detected in animals that were not treated with androstenedione and was most pronounced in animals exposed to androstenedione plus stress, alcohol, pair feeding or cocaine. Stress, alcohol and cocaine are all factors that constrict blood flow to the fetus (26,42), but it is not clear whether or how this effect might be magnified by androstenedione. However, increased plasma levels of estrogen have been shown to greatly increase iron absorption in ovariectomized and pregnant rats (19,58), suggesting that aromatization of androstenedione may be involved in this apparent increase in placental iron deposition.

Rats, like humans, have hemochorial placentas, in which there is an uphill maternal/fetal iron concentration gradient (44). Iron is accumulated by placental cells by an energy-dependent active process involving transferrin receptors. The accumulated iron is moved across the trophoblast cytosol while bound to these receptors and subsequently released. Increases in the activity of the placental iron transport system of the rat are linear with growth during the last week of gestation (34). Under the conditions of the present study, the apparent accumulation of iron in the placentas of fetuses suggests the possibility of increased binding affinity of iron to the transferrin receptor or a disruption of release in these

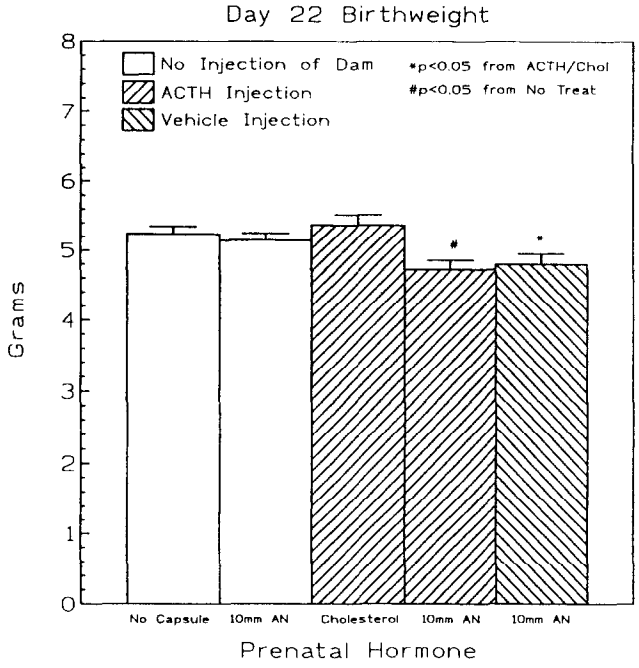

FIG. 6. Birth weights of offspring from dams treated with ACTH during the last week of gestation. Data represent litter means ± SEM of pups born to 4–6dams/treatment group.

placentas. Such effects would be expected to lead to a hypoxic condition in the fetus.

Previous investigators have established that elevations in androgen levels during gestation of the rat will interfere with implantation, suggesting a causal role for aromatized estrogens (13,20,21,23). To what degree similar mechanisms are involved in retarding fetal growth later in gestation remains to be established. Studies in human placental tissue fragments have demonstrated an inhibitory effect of androgens on placental progesterone formation through a suppression of 3b-HSD activity (17,62), but the physiological role of androgens in placental regulation of progesterone production is not yet established (1). Similar studies in rodents have not been conducted to our knowledge. With respect to the present study, results from studies comparing the effectiveness of a nonaromatizable androgen such as dihyrotestosterone with diethylstilbesterol, which does not bind to alpha-fetoprotein, could be helpful in determining the importance of androgenic versus estrogenic actions in mediating the effects of androgens on fetal growth.

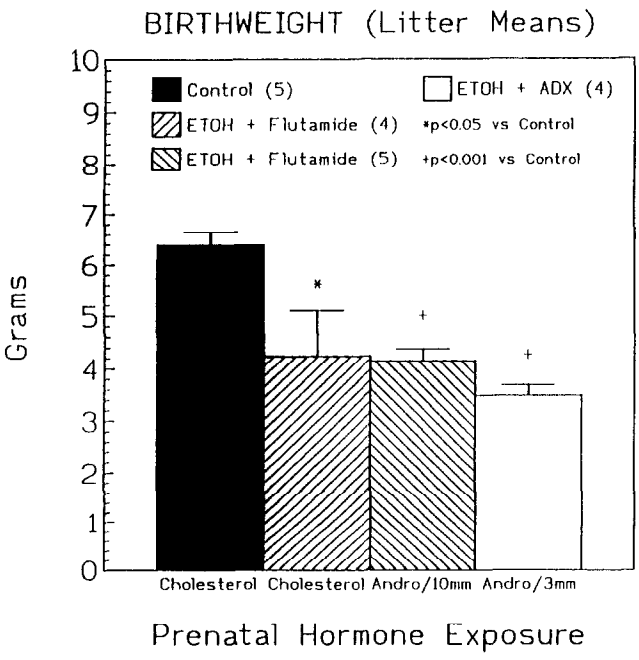

FIG. 7. Birth weights of offspring from dams treated with the androgen receptor blocker flutamide. Number of litters is shown in parentheses.

Significant effects on fetal growth were observed in the presence of relatively small changes in the blood levels of androstenedione in the androstenedione-treated dams compared with controls. The 3-mm androstenedione capsule did not significantly elevate blood levels over controls but did significantly inhibit fetal growth in combination with stressful manipulations in the mother. The 10-mm androstenedione capsule significantly elevated androstenedione levels by twofold, which was associated with significantly larger decreases than the 3-mm androstenedione on fetal growth in the presence of stressful manipulations in the mother. These data indicate a dose–response relationship with androgens and stress. However, the relation of maternal plasma androstenedione levels to steroid action on fetal growth is difficult to infer from our results because maternal blood levels do not provide substantive information regarding placental steroid conversion, nor do they reflect accurate fetal plasma concentrations.

Our results appear to rule out a significant role for HPA activation in the fetal growth reduction. ACTH treatment of intact dams failed to decrease birth weight significantly in the presence of androstenedione beyond the small degree observed with the gel vehicle and androstenedione treatment. Elimination of maternal glucocorticoids by ADX appeared to enhance the ability of a low dose of androstenedione (3 mm) to inhibit fetal growth in the presence of ethanol treatment.

Corticosterone might be expected to inhibit fetal growth based on results in rodents showing that it can inhibit uterine growth and physiological preparation for implantation (5,55,60,66). In addition, stress disrupts implantation in the rodent (14,71). However, de Cataranzo et al. (12) suggested that estrogen rather than corticosterone is responsible for stress-induced blockade of pregnancy. In their studies, corticosterone administration had no effect on birth weight or live births in two strains of mice treated with 100 or 500 μg of corticosterone from days 1 through 6 of pregnancy. In contrast to the lack of effect of corticosterone, significant reductions in the number of dams bearing litters were observed following administration of estradiol-17b, dihydroepiandrosterone, or androstenedione. There were no effects on birth weight of pups that were born to steroid-treated dams during this period of implantation/early gestation.

Harper (20) reported protective effects of cortisol acetate on the ability of androstenedione to block pregnancy in the hamster. However, there was no effect of adrenalectomy on the effect of androstenedione in blocking pregnancy. Subsequent work in the rat found a similar protective glucocorticoid effect against androstenedione that was absent in partially hepatectomized rats, suggesting that increased liver metabolism of androstenedione to nonestrogenic steroids might be the mediating factor (21). Studies showing an ability of cortico-

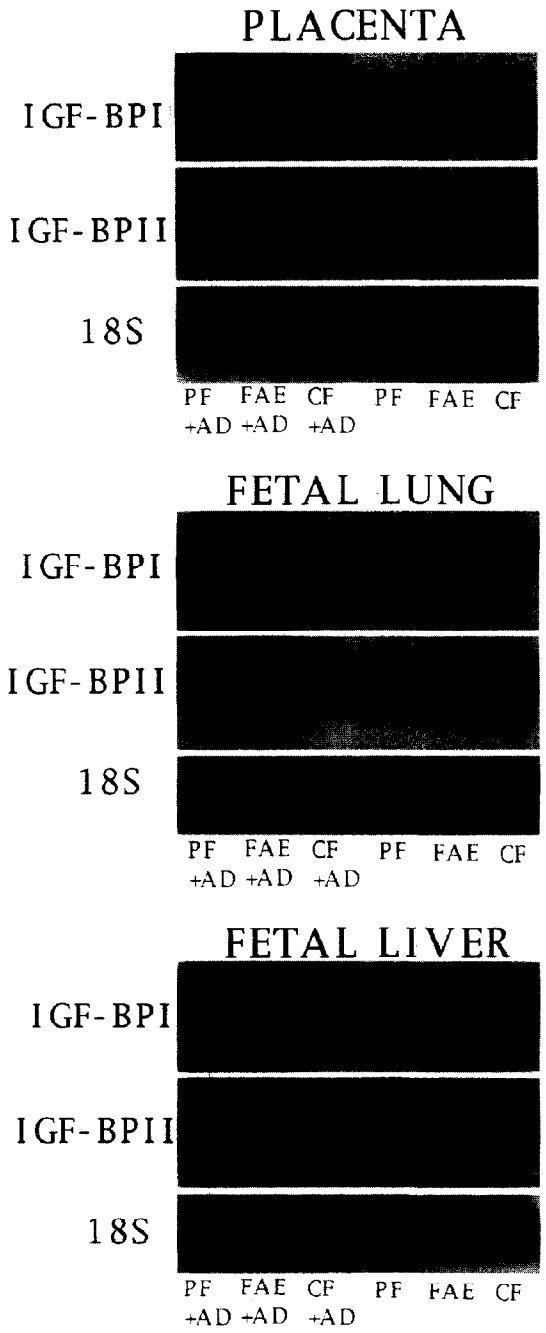

FIG. 8. Northern blot hybridization of total RNA from placenta, fetal lung and fetal liver. Membranes were probed, stripped and reprobed with cDNA probes for IGFBP-1 (m.w. = 1.8 kb) and IGFBP-2 (m.w. = 1.6 kb). An oligonucleotide probe directed against 18s ribosomal RNA was used as an internal control to determine the equality of loading. PF = pair-fed, CF = chow fed, FAE = fetal alcohol exposed, AD = androstenedione.

sterone to inhibit alphafetoprotein in vivo (48) and in fetal hepatocytes in vitro (32) raise the possibility that corticosterone serves to increase estrogen availability during critical periods of fetal development. This idea is consistent with the rise in corticosterone levels late in rat gestation (70). Our results also suggest a protective effect of glucocorticoids because adrenalectomy enhanced the inhibitory effect of androstenedione on fetal growth in the presence of ethanol. However, this possibility is tempered by the fact that during the last few days of gestation, when fetal growth is most pronounced, the fetal adrenal can compensate for maternal adrenal deficiency (7).

In the presence of androstenedione treatment, both pair-feeding and prenatal ethanol exposure were observed to induce apparent increases in the expression of IGFBP-1 and -2 mRNAs in fetal lung and liver when compared with CF controls. Because these are the conditions under which we observed fetal growth inhibition, these preliminary data indicate an involvement of IGFBPs in the growth reduction induced by the synergism between androstenedione and maternal stressors. In placenta, there was a large increase induced by androstenedione alone that was not specific to the prenatal treatment.

Hypoxic conditions have been the primary environmental factor studied in the developmental regulation of IGF, and little is known about developmental regulation by androgens. Recent studies of hypoxia have identified a role for IGF and IGFBPs in the regulation of growth. However, several different approaches to inhibit fetal growth have been employed in these studies, and a clear pattern for the role of each IGF and IGFBP has yet to emerge. Unterman et al. (65) used bilateral uterine arterial ligation to restrict fetoplacental blood flow in the rat on day 19 of gestation. Twentyfour hours later, the fetuses were found to be small for gestational age (SGA), with body weight and liver weight reduced by 20% as opposed to levels observed in fetuses from dams given sham surgery. Serum levels of both IGF-I and insulin were reduced by approximately 50%, whereas IGF-II was unchanged. Western ligand blotting, combined with immunoprecipitation studies, revealed a fourfold increase in IGFBP-1, with little change in IGFBP-2. Thus, hypoxia-induced alterations in IGF-1 availability and bioactivity appear to be important mediators of the reduced growth in these fetuses. The observed increase in IGFBP-1 levels confirmed an previous observation by this group in the SGA rat (64). These investigators have suggested that increased levels of circulating IGFBP-1 may be a marker for intrauterine growth retardation of various unknown causes. Similar reductions of IGF-I in the SGA fetal rat, but not IGF-II, were also reported by Davenport et al. (10) following 48 h of maternal fasting. In that study, IGF-I mRNA in liver and lung were lower in the dam but not in the fetus, suggesting that fasting influenced posttranslational events in the fetus. IGFs have not been studied in the rat following prenatal stress, but restraint stress similar to that which we used has been found to reduce fetal pH, indicating a hypoxic response in the fetus (49). With respect to the effects of HPA activation on fetal IGFs or IGFBP, little is known. Pharmacological levels of corticosteroid exposure in utero reduce birth weight. Dexamethasone (100 μg/injection) administered daily from days 15 to 19 of gestation significantly retarded intrauterine growth and increased IGFBP-1 and -2 in fetal liver and decreased IGFBP-3 (50).

In general, the primate is protected from elevated androgen levels by the high rate of aromatization in the placenta (57). However, little is known about the effects of drugs or stress on placental aromatase activity. In the primate model, induction of fetal hypoxic conditions through arterial ligation induced a dramatic rise in plasma androgens and in estrogen metabolites (56). Thus, fetal stress and maternal stress are capable of inducing significant rises in androgen levels. It is not known whether changes in sex hormone binding globulin

(SHBG) mirror these elevations or are influenced differentially by these conditions. The overall results from animal models appear to correlate well with those from studies of the SGA human in which IGFBP-1 serum levels have been consistently found to be elevated (30,68,69).

The demonstration of changes in IGFBPs, which correlate with fetal growth retardation, provides a potential mechanism whereby steroids may interact with stressful factors to cause intrauterine growth retardation. Intrauterine growth retardation is the major factor associated with infant mortality and morbidity (35). Risk of neonatal death increases by 40 times for infants born <2500 g and 200 times for infants <1500 g (2). Intrauterine growth retardation occurring in the absence of medical complications and genetic disorders is usually associated with environmental factors such as maternal alcohol consumption, smoking, stress, and malnutrition (11,47,54,59). Other nonpathological factors associated with low birth weight include race, age and socioeconomic status (43,45,46). All of these nonpathological factors are associated only with risk, and many pregnancies involving one or more of these factors give rise to a normal weight infant at term. Consequently, other unknown elements must play a significant role in causing intrauterine growth retardation in the presence of risk factors.

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