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ESTROGEN-LIKE COMPOUNDS AND PROGESTERONE IN MALE AND FEMALE RATS BEFORE PUBERTY—II.

EFFECTS OF ADMINISTRATION OF ACTH, STEROIDOGENESIS INHIBITORS, DEXAMETHASONE AND hCG

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SUMMARY

Short-acting ACTH was administered to 14 day old male and female rats, at the time of the plasma peak concentration of those estrogen-like compounds, principally of adrenal origin. Neither plasma concentration nor adrenal content were modified by this treatment while progesterone and corticosterone productions were stimulated. In contrast, plasma and adrenal concentrations of estrogen-like compounds significantly decreased after the administration of steroidogenesis inhibitors: cycloheximide and aminoglutethimide. After administration of long-acting ACTH, dexamethasone or aminoglutethimide during the 3 day period preceding the peak, the estrogen-like compounds were dramatically decreased in both plasma and adrenals; furthermore, the adrenal content of estrone-like compounds decreased more significantly than that of estradiol-like compounds after ACTH. The pattern of plasma and adrenal steroids on day 15 after a single injection of ACTH on day 7, 9 or 11 was similar to that observed in non-treated rats after weaning. This suggests that ACTH could induce maturation of adrenal steroidogenesis. HCG administration stimulated the gonadal secretions of progesterone and estrogens but did not modify the adrenal production of estrogen-like compounds.

Prepubertal rats of both sexes show very high concentrations of estrogens [1–5]. We have shown that this plasma peak of estrogens, which occurs in 13–14 day old rats was related to adrenal steroidogenesis, and that most of these estrogenic compounds were neither estrone nor estradiol [3, 4]. The evolution of the plasma concentrations of progesterone, estrone-like, and estradiol-like compounds, and their pattern of adrenal and gonadal content between the fifth day of life and puberty raises the question of the hormonal control of their secretions.

We present in this paper the effects observed in plasma, adrenal, and gonadal concentrations of estrogen-like compounds, corticosterone, and progesterone after the administration of ACTH, glucocorticoids, hCG, and some steroidogenesis inhibitors.

* The following abbreviations are used: "Estrone-like" ("E_1") for estrone measured with anti-estradiol-17β-hemisuccinate bovine serum albumin (SLC-X, gift of Dr. Caldwell). "Estradiol-like" ("E_2") for 17β-estradiol measured with the same antibody. E_1 for estrone measured with antibodies obtained after injection of estrone-6-carboxymethyloxime-bovine serum albumin (Pasteur Institute). E_2 for 17β-estradiol measured with antibodies obtained after injection of 17β-estradiol-6-carboxymethyloxime-bovine serum albumin (Pasteur Institute). Dexamethasone: 9α-fluoro-11β,17α,21-trihydroxy-16-methyl-pregna-1,4-diene-3,20-dione. ACTH-Retard: Long-acting ACTH.

MATERIAL AND METHODS

$ACTH_{1-24}$ (Synacthen®), long-acting $ACTH_{1-24}$ (Synacthene-Retard®) and aminoglutethimide (Elipten®) were purchased from CIBA (Basel); Cycloheximide from Sigma; Dexamethasone* (Soludecadron®) from MSD; and hCG (Pregnyl®) from Organon.

Sprague–Dawley rats, age 14 days, were studied according to the following experimental protocol: (1) $ACTH_{1-24}$, 12.5 μg in 0.1 ml of saline (sc) administered. Animals were killed 90 min later. (2) Cycloheximide, 2 mg in 0.1 ml of 10% ethanol (sc). Animals were killed 90 min later. (3) Cycloheximide plus $ACTH_{1-24}$: cycloheximide was injected 10 min before $ACTH_{1-24}$. Animals were killed 1 h after $ACTH_{1-24}$ administration. (4) Long-acting $ACTH_{1-24}$, 50 μg per day (sc) either for 3 days (11th to 14th day) or for 15 days (11th to 25th day). The animals were killed 5 h after the last injection. (5) Dexamethasone 25 μg (ip) every 6 h for 3 days (11th to 14th day). Animals were killed 3 h after the last injection. (6) Aminoglutethimide, 2.5 mg (sc) in 0.1 ml buffer pH 3, administered twice per day for 3 days (11th to 14th day). The animals were killed 12 h after the last injection. (7) hCG, 50 IU (sc) twice per day for 3 days (11th to 14th day). The animals were killed 8 h after the

last injection. (8) A single dose of long-acting ACTH (25 μg in 0.1 ml of saline) was injected on day 7, 9 or 11. Animals were killed on day 15.

For each experimental protocol, a control group received only the vehicle. Every experimental point in control and treated groups corresponds to a pool of at least 10 rats. All animals were injected for the first time at 9.00 a.m. The animals were killed by decapitation without anesthesia.

Progesterone (P) and estrogen concentrations in plasma and tissue homogenates were measured by the method indicated in the accompanying paper [4]. Corticosterone (B) in plasma and adrenal homogenates was extracted with ethanol and then measured by radioimmunoassay with a specific antibody (gift of Dr. A. Kowarski). Proteins were measured according to Lowry[6]. DNA to the method of Burton[7]. hCG (15,700 IU/mg, Organon) was labelled with (^{125}I) (The Radiochemical Centre, Amersham) by the lactoperoxidase method [8]. Statistical analysis was performed by means of Student's *t*-test.

RESULTS

1. Short-term effects of $ACTH_{1-24}$ and cycloheximide on plasma concentrations and adrenal contents of corticosterone (B), progesterone (P), and estrogen-like substances

After the administration of $ACTH_{1-24}$ (Fig. 1) there is a marked increase of plasma progesterone and corticosterone concentrations. The concentration of "E_1" is moderately but significantly elevated, but that of "E_2" is not significantly changed. In the adrenals, the concentrations of corticosterone and progesterone are increased, those of "E_2" being slightly but not significantly elevated, while those of "E_1" remain unchanged.

Cycloheximide leads, in 90 min, to a marked decrease of plasma levels and adrenal content of all steroids except plasma "E_2" concentrations (Fig. 1). The effects of cycloheximide plus ACTH on plasma and adrenal corticosterone, progesterone and "E_2" levels are similar to those of cycloheximide. However, plasma and adrenal "E_1" levels are decreased more by cycloheximide alone than by cycloheximide plus ACTH.

2. Effects of the administration of long-acting ACTH, dexamethasone, and aminoglutethimide during three days on plasma and adrenal contents of corticosterone, progesterone, "E_1" and "E_2" (Fig. 2)

ACTH administration is followed by a marked elevation of corticosterone and progesterone concentrations and also by a dramatic drop in "E_1" and "E_2" levels in the plasma. In the adrenals there is a significant increase of corticosterone, while "E_2" shows a marked decrease, and "E_1" almost totally disappears.

Dexamethasone produces a significant decline in plasma as well as adrenal concentrations of the 4 compounds; however, the fall of the "E_1" level in the adrenals is less marked than that observed after ACTH administration.

Aminoglutethimide has similar effects on plasma concentrations of "E_1" and "E_2" and on adrenal levels of corticosterone as does dexamethasone but the decrease of plasma and adrenal progesterone is less marked.

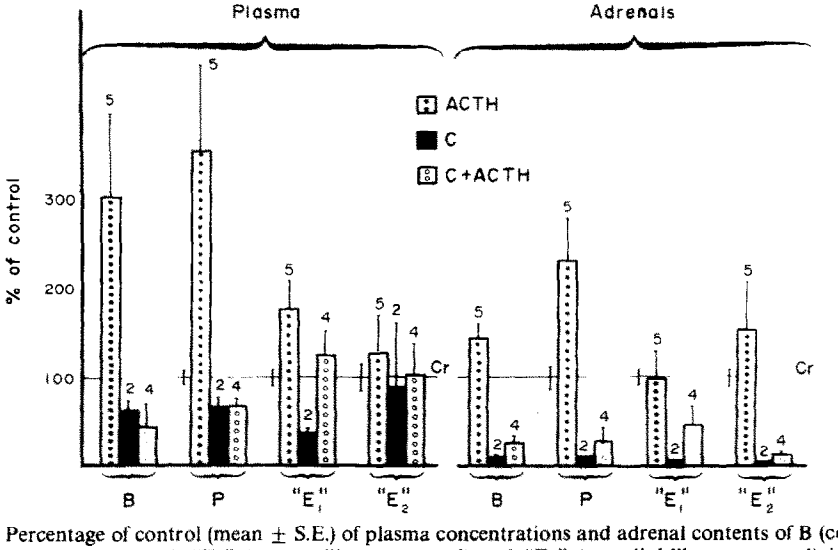

Fig. 1. Percentage of control (mean ± S.E.) of plasma concentrations and adrenal contents of B (corticosterone), P (progesterone), "E_1" (estrone-like compound) and "E_2" (estradiol-like compound) in rats, 90 min after administration of ACTH, or cycloheximide (C) or the two simultaneously (C + ACTH). The results of treated groups are expressed as percentage of the control group treated with 0.1 ml of vehicle (control = 100%). The numbers at the top of each column indicate the number of experiments. The vertical bars on the 100% line represent the S.E. of percentage of variation observed in controls.

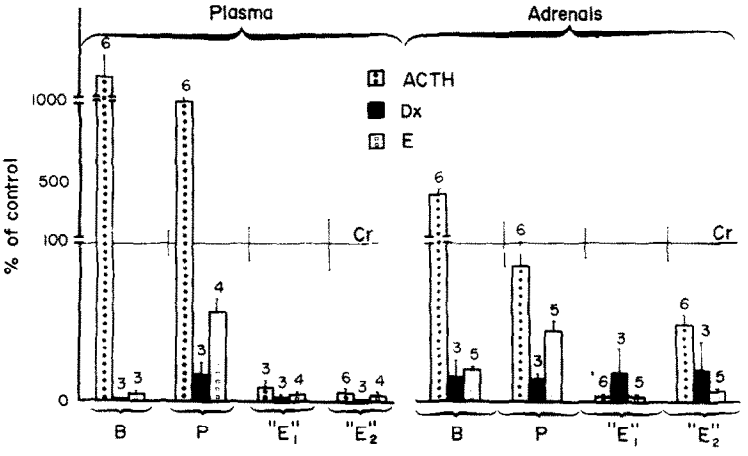

Fig. 2. Percentage of control (mean ± S.E.) of plasma concentrations and adrenal contents of B (corticosterone), P (progesterone), "E_1" (estrone-like compounds), and "E_2" (estradiol-like compounds) after three days of treatment (11th to 14th days) by ACTH Retard (ACTH) or by dexamethasone (Dx) or by aminoglutethimide (E). The results of treated groups are expressed as percentage of control group treated with vehicle (control = 100%). The numbers at the top of each column indicate the number of experiments. The vertical bars on the 100% line represent the SE of percentage of variation observed in controls.

The plasma peak of "E_1" and "E_2" disappears after daily administration of ACTH-Retard between the 11th and 24th days. Similarly, their adrenal contents as expressed in pg per μg of DNA are decreased (Fig. 3). However, since the weight and DNA content of the adrenals increase after ACTH administration (data not shown), the absolute adrenal content of "E_2" after the 19th day is similar in both control and treated animals. Progesterone contents in the adrenals parallel those of "E_2" (data not shown).

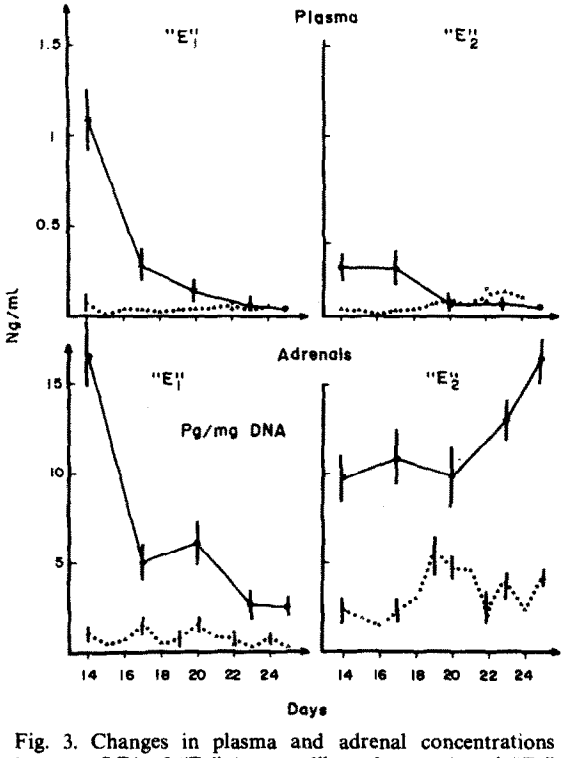

Fig. 3. Changes in plasma and adrenal concentrations (mean ± S.E.) of "E_1" (estrone-like substances) and "E_2" (estradiol-like compounds) between the 14th and 25th days in male rats, both controls (●) and those treated by ACTH-Retard (50 μg/day) from the 11th day (△).

3. *Long-term effects of a single injection of long-acting ACTH on plasma and adrenal contents of corticosterone, "E_1" and "E_2"* (Fig. 4)

After a single injection of 25 μg of ACTH-Retard on days 7, 9 or 11, the plasma levels of "E_1" and "E_2" were significantly decreased on the 15th day of life. The adrenal content of "E_1" was also diminished but that of "E_2" was significantly lowered only in animals injected on the 11th day. Plasma cortico-

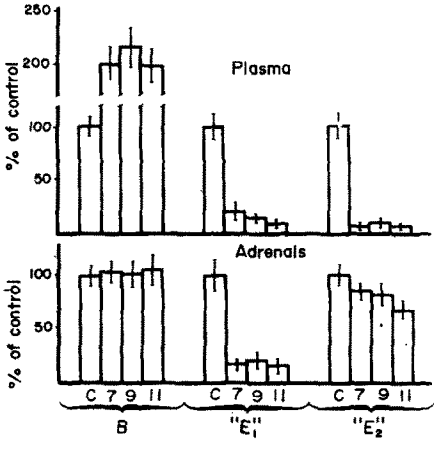

Fig. 4. Percentage of control of plasma concentrations and adrenal contents on the 15th day of B (corticosterone), "E_1" (estrone-like compounds), "E_2" (estradiol-like compounds) in rats treated on day 7, 9, or 11 by a single injection of ACTH-Retard (25 μg). The results of treated groups are expressed as percentage of the control group treated with vehicle (control = 100%).

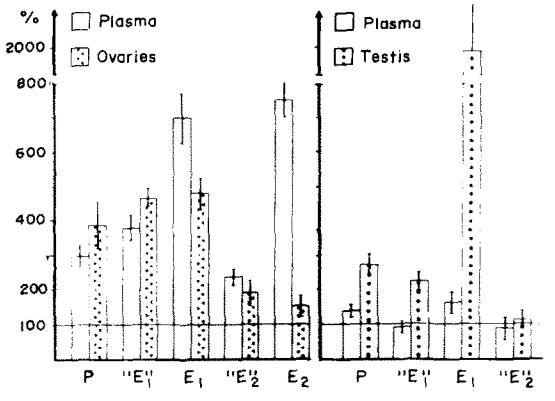

Fig. 5. Percentage of control of plasma concentrations and gonadal contents of P (progesterone), "E_1" (estrone-like compounds), E_1 (estrone measured with the anti-E_1-C_6), "E_2" (estradiol-like compounds), E_2 (estradiol measured with the anti-E_2-C_6) in male and female rats treated for three days (11th to 14th day) by hCG (50 IU) twice a day. The results of treated groups are expressed as percentage of the control groups treated with vehicle (control = 100%).

sterone levels of control rats (6.5 ± 1.2 μg/ml) were similar to those reported by Ramaley[9], whereas the values obtained in ACTH-treated rats were about two times higher. This increase is probably not due to the ACTH which was injected 4 to 8 days before since the steroidogenic action of higher dose (on body weight basis) of long-acting ACTH in 25 to 30-day old rats lasted only for 24 h [10].

4. *Effects of hCG on plasma concentrations and gonadal contents of progesterone, "E_1", "E_2", E_1 and E_2* (Fig. 5)

Administration of hCG to male rats is followed by a significant increase of plasma concentrations of progesterone and E_1 and by an increase of testicular concentrations of all steroids measured, E_1 being the most affected.

In female rats, hCG administration leads to an increase of plasma concentrations of all measured steroids but the increase is greater for E_1 and E_2. A significant increase of the contents of all these steroids was also found in the ovaries.

We did not observe any effects of hCG on adrenal contents of steroids in the same male rats and female rats as above, nor in male rats castrated one day before the beginning of treatment (data not shown).

DISCUSSION

A plasma peak of immunoreactive estrogens is observed in male and female rats towards the end of the second week of life [3–5]. The adrenal origin of these estrogenic compounds is suggested by the fact that adrenalectomy is more effective than gonadectomy in decreasing their plasma concentrations [3, 4]. In addition, in 14-day old animals the contents of estrogen-like compounds are several times higher in the adrenals than in the gonads [4].

Steroidogenesis seems to be necessary for the synthesis of these compounds since the administration of steroidogenesis inhibitors, such as cycloheximide and aminoglutethimide, inhibited this synthesis. However, short-acting ACTH did not significantly modify plasma and adrenal concentrations of "E_1" and "E_2". These results could be explained either by the existence of a large pool of these compounds and their relatively long half life in young rats or by the fact that the biosynthesis of those compounds is not under ACTH positive control. The latter hypothesis is consistent with the finding that daily injection of long-acting ACTH prior to the expected peak of estrogen-like compounds completely blocked its onset in the plasma and markedly reduced the adrenal contents of estrogen-like compounds. The mechanism by which long-term ACTH administration inhibits the synthesis of estrogen-like compounds in the adrenal is not known.

The pattern of steroids levels on day 15 in both plasma and adrenals after a single injection of ACTH on days 7, 9 or 11, is similar to that of animals after weaning. This suggests that exogenous ACTH might have accelerated the maturation of adrenal steroidogenesis which is normally under endogenous ACTH control. Indeed, Ramaley's data [9] suggest that ACTH elevates corticosteroid production only after the 13th day.

ACTH and dexamethasone had similar effects on plasma and adrenals levels of spurious estrogens. Therefore the effects of ACTH could be indirectly due to an increase of glucocorticoid production. Indeed these steroids inhibit the *in vivo* hepatic synthesis of α-fetoprotein [11] and this would in turn decrease the half-life of plasma estrogen-like compounds, since α-fetoprotein binds these compounds [4]. However, the decrease of the adrenal content of these compounds could not be explained by this hypothesis.

The more likely explanation is that exogenous ACTH accelerates the normal maturation of adrenal steroidogenesis normally induced by endogenous ACTH later. Change in ACTH secretion would be responsible for the modification of plasma steroid pattern after weaning. The same pattern was observed in the 15-day old rats when they were injected by ACTH at 7, 9 or 11 days of life.

Although it has been shown that prolactin binds to adrenal plasma membranes [12, 13] it is not very likely that this hormone controls the estrogen-like compound's synthesis as its plasma concentration is low at the time of estrogen-like compounds [14–17]. In addition, in preliminary experiments in this laboratory, attempts to reduce or to stimulate the prolactin secretion were not followed by any change in the plasma and adrenal concentrations of the estrogen-like compounds (data not shown).

Since autoradiographic studies support the existence of an hCG-receptor in the adrenal gland [18], and since the rats present simultaneously to the peak of estrogen-like compounds, high concentrations of plasma LH and/or FSH, we were led to study the effects of hCG administration. Our results did not show evidence for any effects of hCG on the adrenal content of estrogen-like compounds in male and female rats, nor any change in the plasma levels in castrated male rats. Indeed, the increase of plasma estrogens levels after hCG administration to intact rats of both sexes was due exclusively to an increased production of estrogens by the gonads. Furthermore, [^{125}I]-hCG was not able to bind to adrenal plasma membranes, while it bound to testicle and ovary plasma membranes from the same 14-day old rats (data not shown).

The exact nature and mechanisms controlling the biosynthesis of those adrenal estrogen-like compounds before puberty and the eventual role of these compounds on the hypophyso-gonadal axis before puberty pose numerous unresolved questions for future research.

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