Pergamon

Life Sciences, Vol. 57, No. 9, pp. 833-837, 1995

0024-3205/95 $9.50 + .00

0024-3205-(95)02015-B

EFFECTS OF SEX HORMONES ON THE STEROIDOGENIC ACTIVITY OF DISPERSED ADRENOCORTICAL CELLS OF THE RAT ADRENAL CORTEX

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(Received in final form June 2, 1995)

Summary

The effect of 17β-estradiol and testosterone on glucocorticoid secretion were studied *in vitro* by using dispersed inner adrenocortical cells obtained from gonadectomized female and male rats. Independently of the sex of animals, estradiol enhanced basal, but not ACTH-stimulated corticosterone (B) secretion; conversely, testosterone inhibited ACTH-stimulated, but not basal B output. HPLC analysis of steroid secreted demonstrated that estradiol induced comparable rises (53-62%) in basal pregnenolone (PREG) and total post-PREG secretion (progesterone, 11-deoxycorticosterone and B). Testosterone inhibited by about 30% ACTH-stimulated PREG production and by about 54% total post-PREG secretion (B was decreased to 56% of the control value, and other steroid hormones were below the limit of sensitivity of our assay system). These findings indicate that sex hormones directly affect rat adrenocortical secretion, mainly by acting on the rate-limiting step of steroidogenesis (i.e. the conversion of cholesterol to PREG); moreover, they suggest that testosterone is also able depress the activity of the enzymes operating distally to cholesterol side-chain cleavage.

Key Words: estradiol, testosterone, adrenal steroidogenesis

It is well known that several mammalian species display clearcut sex-related differences in glucocorticoid secretion (for review, see 1,2). As far as the rat is concerned, under basal conditions females possess higher output and blood concentration of corticosterone (B) than age-matched males, and this clearly depends on the secretion of sex hormones, since any difference disappears after gonadectomy. Compelling evidence indicates that the action of sex hormones on adrenal cortex is mainly indirect and involves the modulation of pituitary ACTH release. However, this does not seem to be the only mechanism involved, inasmuch as the inhibition of the hypothalamo-pituitary-adrenal axis by dexamethasone does not completely abolish sex-related differences in glucocorticoid secretion.

Although the presence of receptors for sex hormones in adrenocortical cells is well documented (for review, see 2), only scanty and rather old data are available suggesting a direct effect of estrogens and androgens on rat adrenal steroidogenesis (see Discussion). Moreover, these in vitro studies employed adrenal slices and the effect of sex hormones was assessed by means of non-specific methods of steroid-hormone quantification (e.g. sulfuric acid fluorescence for B assay).

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It therefore seemed wothwhile to re-investigate the direct effect of estradiol and testosterone on adrenocortical steroidogenesis, by employing dispersed zona fasciculata and zona reticularis (ZF/ZR)-cell preparations and using more uptodated methods of steroid-hormone assay (HPLC and RIA).

Materials and Methods

Adult female and male rats of the Wistar strain were bilaterally gonadectomized, and maintained under standardized conditions of lighting (14:10 h light-dark cycle) and temperature (23 ± 1 °C), with free access to laboratory pellets and tap water. The rats were decapitated 8 days after surgery and adrenal glands promptly removed and freed of pericapsular fat.

Preparation of dispersed adrenocortical cells. Adrenal glands were gently decapsulated to separate zona glomerulosa from ZF/ZR, and then hemisected; decapsulated adrenal halves were enucleated to remove zona medullaris. Dispersed inner (ZF/ZR) cells were obtained by collagenase digestion and mechanical disaggregation (3). The viability of isolated cells was checked by the trypan-blue exclusion test and found to be higher than 90%. Dispersed cells obtained from six rats were pooled to obtain a single cell suspension, and six cell preparations for each incubation experiment were employed. Dispersed cells were put into Medium 199 (DIFCO, Detroit, MI) and potassium-free Krebs-Ringer bicarbonate buffer with 0.2% glucose (2:1 vol/vol), containing 5 mg/ml human serum albumin.

Incubation procedures. Dispersed cells were incubated ($3x10^5$ cells/ml) with increasing concentration (from 10^{-10} to 10^{-6} M) of 17β-estradiol or testosterone (hydrosoluble form; Sigma, St. Louis, MO), in the presence or absence of 10 nM ACTH (Synacthen; Ciba, Milan, Italy). For estimation of pregnenolone (PREG) production, dispersed cells were incubated as described above in the presence of 10 μM cyanoketone (WIN 24540; Sterling-Winthrop, Guilford, U.K.); according to Aguilera *et al.* (4), this concentration of cyanoketone is able to prevent further metabolism of PREG. The incubation was carried out for 90 min in a shaking bath at 37°C in an atmosphere of 95% O_2 and 5% CO_2.

Hormonal assays. B was extracted from incubation media and purified by HPLC, and its concentration was measured by RIA, using [1α, 2α (n)-^{3}H]-B (1.96 TBq/mmol) (Amersham Int., Amersham, U.K.), and a B antiserum developed in rabbit (Sigma). Intra- and interassay variations were 7% and 9% respectively. The concentrations of PREG, progesterone (PROG), 11-deoxycorticosterone (DOC), B, 18-hydroxy-11-deoxycorticosterone (18OH-DOC), 18-hydroxycorticosterone (18OH-B) and aldosterone (ALDO) were measured by quantitative HPLC, as described elsewhere (5), in the incubation media of cell preparations obtained from male rats.

Statistics. The statistical comparison of the data was performed by ANOVA, followed by the Multiple Range Test of Duncan.

Results

Estradiol stimulated basal B secretion by dispersed inner adrenocortical cells of female rats, but the effect was significant only at a concentration of 10^{-6} M (90%); conversely, it did not affect ACTH-stimulated B output. The reverse was true for testosterone: basal B secretion was not changed, while ACTH-stimulated one was decreased; the minimal and maximal effective concentrations were 10^{-9} M (-15%) and 10^{-6} M (-25%). Superposable results were obtained by using cell preparations from male rats: 10^{-6} M estradiol and testosterone elicited a 2-fold rise in basal B secretion and a -32% decrease in ACTH-stimulated one respectively; these effects were already significant at a concentration of 10^{-7} M.

HPLC assay revealed that estradiol enhanced basal PREG synthesis (10^{-7} M, 55%; 10^{-6} M, 62%) and total post-PREG secretion (10^{-7} M, 46%; 10^{-6} M, 53%). It raised PROG (10^{-6} M, 67%), DOC (10^{-7} and 10^{-6} M, 2-fold) and B outputs (10^{-7} M, 44%; 10^{-6} M, 63%), without affecting the secretion of 18-hydroxylated steroids (Table 1). Testosterone, at a concentration 10^{-6} M, inhibited ACTH-stimulated PREG synthesis (-30%) and total post-PREG secretion (-54%); B output underwent a 44% decrease, and other steroid hormones were below the limit of sensitivity of our assay system. The inhibitory effect

TABLE I

Effect of 17β-estradiol (E) on basal and 10nM ACTH-stimulated steroid secretion of dispersed inner adrenocortical cells of male gonadectomized rats.

pmol/10^6 cells·h	Controls	E 10^{-8} M	E 10^{-7} M	E 10^{-6} M
Basal secretion				
PREG	320 ± 47	375 ± 64	495 ± 87$^+$	518 ± 71*
PROG	30 ± 6	42 ± 5	35 ± 6	50 ± 7$^+$
DOC	25 ± 3	30 ± 3	50 ± 8*	48 ± 9*
B	135 ± 15	120 ± 15	195 ± 23$^+$	220 ± 28*
18OH-DOC	10 ± 1	8 ± 2	15 ± 1	—
18OH-B	15 ± 3	13 ± 1	18 ± 3	10 ± 2
ALDO	—	—	—	—
Total post-PREG secretion	215 ± 26	213 ± 29	313 ± 42$^+$	328 ± 43$^+$
ACTH-stimulated secretion				
PREG	2140 ± 351	2089 ± 306	2210 ± 413	2305 ± 389
PROG	150 ± 23	195 ± 20	148 ± 15	205 ± 39
DOC	108 ± 19	99 ± 15	120 ± 20	109 ± 15
B	1198 ± 143	1210 ± 126	1089 ± 183	1307 ± 202
18OH-DOC	21 ± 4	22 ± 2	30 ± 4	25 ± 7
18OH-B	62 ± 6	51 ± 5	70 ± 6	60 ± 10
ALDO	—	—	—	—
Total post-PREG secretion	1539 ± 190	1577 ± 172	1457 ± 234	1706 ± 280

Data are means ± (n = 6). $^+P<0.05$ and $^*P<0.01$ from control group.

TABLE II

Effect of testosterone (T) on basal and 10 nM ACTH-stimulated steroid secretion of dispersed inner adrenocortical cells of male gonadectomized rats.

pmol/10^6 cells·h	Controls	E 10^{-8} M	E 10^{-7} M	E 10^{-6} M
Basal secretion				
PREG	407 ± 61	382 ± 55	392 ± 55	450 ± 82
PROG	40 ± 6	35 ± 5	48 ± 7	30 ± 7
DOC	20 ± 3	25 ± 4	31 ± 3	19 ± 4
B	150 ± 22	141 ± 19	170 ± 29	139 ± 24
18OH-DOC	16 ± 4	25 ± 5	10 ± 3	31 ± 5
18OH-B	10 ± 3	15 ± 4	8 ± 3	16 ± 4
ALDO	—	—	—	—
Total post-PREG secretion	236 ± 40	241 ± 36	267 ± 46	235 ± 44
ACTH-stimulated secretion				
PREG	2201 ± 242	2318 ± 213	2095 ± 203	1541 ± 163$^+$
PROG	142 ± 28	150 ± 20	138 ± 16	—
DOC	78 ± 9	68 ± 9	73 ± 9	—
B	1261 ± 166	1308 ± 229	889 ± 113$^+$	709 ± 90*
18OH-DOC	35 ± 6	29 ± 4	—	—
10OH-B	31 ± 4	35 ± 7	6 ± 1*	—
ALDO	—	—	—	—
Total post-PREG secretion	1547 ± 210	1590 ± 272	1106 ± 142$^+$	709 ± 90*

Data are means ± S.E. (n = 6). $^+P<0.05$ and $^*P<0.01$ from control group.

of testosterone on total post-PREG and B secretions was already significant at a concentration 10^{-7} M (Table 2). As expected, estradiol and testosterone did not modify ACTH-stimulated and basal steroid synthesis, respectively (Tables 1 and 2).

Discussion

Scanty and rather conflicting data are available on the direct effect of sex hormones on corticosteroid secretion. Fukui *et al.* (6) and Kitay (7,8) reported that 17β-estradiol enhances basal corticosteroid secretion by rat adrenal slices, while methyltestosterone or testosterone are ineffective. Yudaev and Mikosha (9) described an estrone-stimulated cortisol output by adrenal quarters of male and female guinea pigs. Conversely, McKerns (10) found no effect of estradiol and an inhibitory influence of stilbestrol and ethinylestradiol on B secretion *in vitro*.

Our present findings clearly show a direct stimulatory effect of 17β-estradiol on basal glucocorticoid secretion of dispersed rat adrenocortical cells. HPLC analysis of secreted steroids suggests that the increase in B output is due to the enhanced conversion of cholesterol to PREG, whose increased delivery may be responsible *per se* for the raised production of DOC and B. Available literature data do not support a stimulatory action of estrogens distal to PREG formation. In acetone powder of the bovine adrenal gland, Kowal *et al.* (11,12) showed an inhibitory effect of estradiol on 3β-hydroxysteroid dehydrogenase activity, and similar results were obtained in the microsomal fraction of mature human adrenals (13). Studies of Provencher *et al.* (14) did not reveal any effect of estradiol on 21-hydroxylase activity in primary cultures of glomerulosa-fasciculata cells of guinea-pig adrenals, while in the microsomal fraction of the human fetal adrenals estrone, estradiol and estriol were reported to competitively inhibit this enzyme (15). Crivello *et al.* (16) did not observe any effect of estradiol on 11β-hydroxylase activity in primary cultures of bovine adrenocortical cells. Hence, on the ground of the above reviewed data and of our findings it seems legitimate to suggest that the mechanism whereby 17β-estradiol enhances basal B secretion of dispersed rat adrenocortical cells involves the stimulation of cholesterol side-chain cleavage, the rate-limiting step of steroidogenesis (17,18). This contention may also explain why the secretagogue effect of estradiol does not manifest itself in the presence of a maximal effective concentration of ACTH, the main agonist of inner adrenocortical cells: in fact, it is well demonstrated that the main locus of action of ACTH on steroidogenesis is the conversion of cholesterol to PREG (for review, see 17,18).

An opposite effect on steroidogenesis is exerted by testosterone. Our HPLC findings indicate that 10^{-6} M testosterone reduces ACTH-stimulated PREG output by 30% and total post-PREG steroid production by 54%; moreover, 10^{-7} M testosterone, though not affecting PREG production, decreases by about 28% total post-PREG hormonal output. These results suggest that testosterone inhibits not only the conversion of cholesterol to PREG, but also the activity of the enzymes operating distally to the rate limiting step of adrenal steroidogenesis. This contention appears to be supported by the following lines of evidence: 5α-dihydrotestosterone was found to competitively inhibit 3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$ isomerase system (13), and androgens depress the activity of 21-hydroxylase and 11 β-hydroxylase (14,16,19,20). It remains to be elucidated why testosterone affects only ACTH-stimulated steroidogenesis and not the basal one. At a first glance, this finding would suggest that testosterone acts indirectly on adrenocortical cells by interfering with the mechanism(s) involved in the intracellular transduction of the ACTH secretory signal. An alternative, but not conflicting explanation could be that the inhibitory effect of testosterone is so moderate that requires an enhanced activity of steroidogenic enzymes to become manifest. As far as molecular mechanism(s) underlying the action of sex hormones on adrenocortical cells is (are) concerned, it must be recalled that, according to Wehling (21), there is evidence for acute nongenomic effects of virtually every steroid hormone and for the presence of membrane binding sites for all steroids, including estrogens. Hence, the possibility cannot be disregarded that the effects of estrogens and androgens on adrenocortical steroidogenic activity may be coupled with and, at least partly, due to *de novo* protein synthesis. Further studies are needed to address this important issue.

Before concluding, we want to stress that no significant differences either in the basal and stimulated glucocorticoid secretion or in the response to sex hormones were observed between dispersed adrenocortical cells of male and female rats. However, earlier studies reported that sex-related differences in glucocorticoid output are maintained by dispersed ZF/ZR cells. These discrepancies can be easily explained by considering that cell-suspensions employed by previous investigators were

obtained from intact rats, while our preparations are from animals 8 days after gonadectomy. Thus, our present investigation allows us to conclude that, in the rat, the sex-linked differences in the secretory activity of the adrenal glands are maintained by circulating gonadal hormones, which act not only at the hypothalamo-pituitary level, but also directly on adrenocortical cells.

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